

## Special Feature

# Modulating immune responses with probiotic bacteria

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**Summary** For many years, probiotic bacteria have been known to confer health benefits to the consumer. One possible mechanism for this may be the ability of probiotic bacteria to modulate immune responses. Oral administration of *Lactobacillus casei* strain Shirota (LcS) has been found to enhance innate immunity by stimulating the activity of splenic NK cells. Oral feeding with killed LcS was able to stimulate the production of Th1 cytokines, resulting in repressed production of IgE antibodies against Ovalbumin in experimental mice. The ability to switch mucosal immune responses towards Th1 with probiotic bacteria provides a strategy for treatment of allergic disorders. Growth of Meth A tumour cells in the lungs was also inhibited by intrapleural injection of LcS. Oral administration of other probiotic bacteria, such as *Streptococcus thermophilus* (St), *Lactobacillus fermentum* (Lf) and yeast (Y), elicited different immune responses. Mice that were prefed yeast or Lf followed by feeding with ovalbumin (OVA) responded better to vaccination with OVA than mice not given either probiotic or OVA or mice that had been prefed only OVA. However, antibody responses were significantly suppressed in response to vaccination with OVA in mice that had been prefed yeast followed by yeast and OVA as well as mice prefed Lf followed by Lf and OVA. Prefeeding St followed by OVA feeding enhanced cellular immune responses against ovalbumin. In contrast, mice prefed St followed by St + OVA were hyporesponsive against OVA. While antigen feeding alone appears to prime for an immune response, cofeeding antigen with probiotic bacteria can suppress both antibody and cellular immune responses and may provide an efficacious protocol to attenuate autoimmune diseases, such as experimental allergic encephalomyelitis, by jointly dosing with myelin basic protein and probiotic bacteria.

**Key words:** autoimmunity, dietary antigens, gut immunity, lymphoproliferation, mucosal immunity, oral tolerance.

## Introduction

It has been known for years that commensal bacteria in our intestines play an important role metabolizing digesta in the lumen and in so doing provide an additional source of nutrients. The composition of commensal bacteria is highly stable and characteristic for an individual over time but does appear to vary considerably between individuals.<sup>1</sup> Dietary changes, stress and consumption of antibiotics can disturb the balance of commensal bacteria and a healthy balance can be re-established with probiotic supplementation. In recent years, lactic acid bacteria (LAB) have been marketed by the dairy industry as an important natural bacterium for the maintenance of gut health. Other Gram-positive bacteria, such as *Streptococcus thermophilus* and *S. faecalis*, have been used as probiotics for farm animals.<sup>2</sup> Although not officially recognized as probiotic bacteria, *S. thermophilus* and *Staphylococcus carnosus* are often used as starter cultures in the production of cheeses and salami and are normally consumed in very large numbers in our diets. These bacteria may also have a biological impact on the consumer.<sup>3</sup>

To qualify as a probiotic, bacterial strains must satisfy a number of criteria, including the ability to survive in the

gastrointestinal tract.<sup>4</sup> *Bifidobacterium* species and LAB are naturally commensal bacteria in the small and large intestines. These bacteria protect the host against potential pathogens by competitive exclusion and also by the production of antibacterial agents known as bacteriocins. It has only been recently shown that another mechanism whereby probiotic bacteria may provide a health benefit is by modulating immune responses. The immune-stimulating effect of LAB has been investigated using several animal experimental models. *Lactobacillus casei* strain Shirota (LcS) is a strain of LAB that was selected for its specific biological activity in man and animals. LcS is widely used in the production of probiotic dairy products and also as a food ingredient in Japan and in Europe, because of its proven safety and functional characteristics. To examine a role for probiotic bacteria in the modulation of immune responses, we describe, in the present paper, recent studies that have been carried out to demonstrate that some probiotic bacteria appear to have the ability to affect innate, humoral and cellular arms of the immune system.

## Effect on innate immunity

Natural killer (NK) cells are large granular lymphocytes derived from bone marrow and these cells display non-MHC-restricted cytotoxicity against a variety of tumours. It is well recognized that NK cells act as cytolytic effector cells of the innate immune system. Recent studies have revealed that NK cells, including gamma/delta T cells, also regulate the

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**Table 1** Cytolytic NK activity of MLN, PP and SC from newborn mice orally administered LcS

| Group   | Effector to target ratio |            |            |
|---------|--------------------------|------------|------------|
|         | 200:1                    | 100:1      | 50:1       |
| MLN     |                          |            |            |
| Control | 3.1 ± 0.9                | 4.1 ± 0.6  | 1.6 ± 0.5  |
| LcS     | 2.6 ± 0.5                | 4.7 ± 0.6  | 3.0 ± 0.8  |
| PP      |                          |            |            |
| Control | -1.9 ± 1.6               | -1.8 ± 0.4 | Not done   |
| LcS     | -1.4 ± 1.4               | -1.5 ± 0.6 | Not done   |
| SC      |                          |            |            |
| Control | 1.3 ± 0.9                | 1.8 ± 0.2  | 1.7 ± 0.4  |
| LcS     | 3.7 ± 0.7*               | 7.1 ± 0.3† | 6.4 ± 0.4† |

*Lactobacillus casei* strain Shirota (LcS; 250 µg/50 µL distilled water per mouse per day) was orally administered to the newborn C57BL/6 mice ( $n = 10$ ) for 14 consecutive days from the second day after birth. The NK activity of mesenteric lymph node cells (MLN), Peyer's patch cells (PP) and spleen cells (SC) was measured by [ $^{51}$ Cr]-release assay. \* $P < 0.05$ , † $P < 0.01$  compared with the control.

development of allergic airway disease.<sup>5</sup> These findings indicate that host innate immunity may be important in relation to the development of some diseases.

It has been previously shown that intravenous, intraperitoneal and intrapleural injection of LcS into mice augmented the NK activity of spleen cells, peritoneal exudate cells and thoracic exudate cells, respectively.<sup>6-8</sup> It was unclear at the time whether oral administration of LcS to mice activated NK cells to become tumoricidal. We have performed several experiments to examine this possibility. In the first experiment, LcS was orally administered to newborn mice. This was done on the second day after birth and LcS was administered at a dose of 250 µg in 50 µL distilled water per mouse to newborn mice for 14 consecutive days. The NK activity of mesenteric lymph node cells (MLN), Peyer's patch cells (PP) and spleen cells (SC) were measured at this time. As shown in Table 1, the NK activity of SC was significantly augmented after oral administration of LcS but not so for MLN and PP, which may be lacking NK cell populations. The NK activity in adult mice (7 weeks of age) was also examined after they had been fed LcS (100 µg/200 µL distilled water per mouse) for 5 consecutive days. The NK activity of spleen cells from these mice was examined 3 days after the last dose. As shown in Fig. 1, the NK activity of spleen cells from mice fed LcS was significantly greater than that of controls not fed the probiotic. These observations confirmed that orally administered LcS enhanced the innate immune response.

### Effects on cellular immunity

Murine helper T cells are mainly classified into two categories according to the pattern of cytokine production displayed. Type 1 helper T cells, Th1 cells, produce IFN-γ, IL-2 and TNF-β, whereas Type 2 helper T cells, Th2 cells, produce IL-4, IL-5, IL-6 and IL-10. The Th1 cytokines augment cellular immunity and Th2 cytokines augment humoral immunity.<sup>9,10</sup> The balance of the two cell populations is

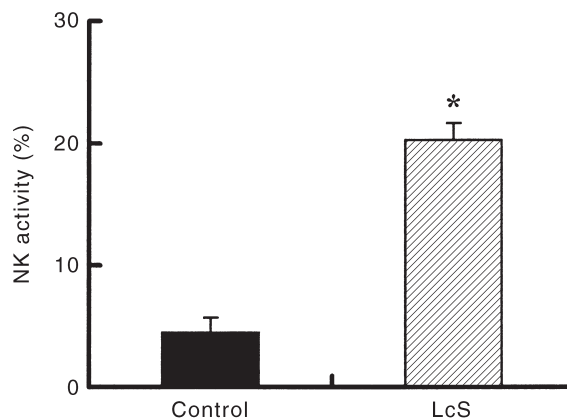


**Figure 1** Augmentation of NK activity by oral administration of *Lactobacillus casei* strain Shirota (LcS). The LcS (100 µg/200 µL distilled water/mouse per day) was orally administered to 7-week-old male BALB/c ( $n = 5$ ) mice for 5 consecutive days and the NK activity of spleen cells from these mice was examined 3 days after the final administration by [ $^{51}$ Cr]-release assay. Bars represent mean values ( $\pm$  SD) for triplicate cultures. \* $P < 0.01$  compared with the control.

believed to be important for the maintenance of homeostasis in the host. Once this balance becomes disturbed, various immunological diseases, such as allergies and infections, can occur through the evasion of host defence mechanisms. In recent years, beneficial effects of probiotics on the immune-mediated diseases, such as allergy and asthma, have been documented.<sup>11</sup> It has therefore been suggested that probiotic bacteria can also be used in the treatment of food allergies.

One possible mechanism for the beneficial effect of probiotics on allergic responses is to exert an inhibitory influence on the production of IgE.<sup>12</sup> It is not clear whether oral administration of these probiotics can inhibit IgE production *in vivo*. This has been investigated by feeding heat-killed LcS to BALB/c mice. The mice were then immunized by intraperitoneal injection of ovalbumin (OVA) and Al(OH)<sub>3</sub> on days 0 and 14. Seven days after this, blood was collected from all mice and assayed for OVA-specific serum IgE, while spleen cells were prepared for assays of OVA-specific IgE production and OVA-induced cytokine production. The level of OVA-specific IgE in serum in each group is shown in Fig. 2. Mice fed a diet containing 0.05% (w/w) LcS had a significantly reduced level of OVA-specific IgE compared with non-probiotic controls. In addition, in mice fed LcS, the level of production of Th1-associated cytokines, such as IFN-γ and IL-2, by spleen cells was higher than that in the control group (Fig. 3a). In contrast, the level of production of Th2-associated cytokines, such as IL-4, IL-5, and IL-6, by spleen cells from the mice fed LcS was lower than that of the control group (Fig. 3b). Furthermore, levels of IL-12, which augments IFN-γ production by spleen cells from the mice fed LcS, were also higher than those of the control group (Fig. 3c). These results indicate that LcS feeding induces a Th1 rather than a Th2 response. Therefore, we propose that augmentation of Th1 cells by feeding LcS provides a way to inhibit Th2 cells and thereby reduce the production of IgE.

As mentioned earlier, some probiotics may have the potential to ameliorate host immune system disorders via

regulation of the Th1 and Th2 balance. We believe that it is now time for researchers to focus more research on probiotics for the treatment of immune-mediated diseases, including allergies.

### Animal disease models to study action of probiotics

Lactic acid bacteria are Gram-positive non-pathogenic bacteria that are widely distributed in nature. These bacteria inhabit the intestines of various animals and have long been used in food processing. In 1981, Matsuzaki *et al.*<sup>13</sup> screened 26 strains of 14 species of lactic acid bacteria for *in vivo* antitumour activity against sarcoma 180, a transplantable mouse

tumour, and found that some had potent antitumour activity (Fig. 4). Among these, heat-killed cells of LcS (LC 9018) showed especially high potency. This finding stimulated further studies of the antitumour and immunoregulatory action of LC 9018 in various experimental models.<sup>13–15</sup>

It has been reported that some bacteria, such as Bacille-Calmette-Guérin (BCG), OK432 or a preparation of *Streptococcus pyogenes*, have been used in treatment of lung cancer patients with malignant pleurisy.<sup>16,17</sup> Subsequently, a system for the evaluation of the antitumour effect of bacterial preparations against pleural effusion has been developed and used to compare the antitumour effects of several bacterial preparations.<sup>18</sup> BALB/c mice were inoculated with Meth A tumour cells ( $1 \times 10^5$  cells/mouse) intrapleurally (i.pl.) on day 0. Then, LcS (100 µg/100 µL sterile saline per mouse) was injected i.pl. on days 3 and 6 and the subsequent survival of the mice was monitored. The i.pl. injection of LcS into tumour-bearing mice was shown to effectively inhibit the growth of tumour cells in the thoracic cavity and to significantly prolong the survival of the mice in comparison with other bacterial preparations. It was also shown that i.pl. injection of LcS stimulated the NK cell activity of thoracic exudate cells and augmented the phagocytic activity of thoracic macrophages against sheep red blood cells. We have found that the effect of LcS on the survival of Meth A-bearing mice was in part dependent on T cells, because there was no effect in either nude mice or severe combined immunodeficient (SCID) mice. We have also demonstrated that anti-IFN-γ or anti-IL-12 monoclonal antibody partially abolishes the effect of LcS, whereas anti-TNF-α monoclonal antibody completely abolishes its effect (Fig. 5). These results show that cytokines induced by i.pl. injection of LcS may play an important role in the antitumour action of LcS.

In clinical trials, it has been demonstrated that i.pl. administration of LcS significantly prolongs the survival of lung cancer patients with malignant pleural effusion in a phase III randomized controlled study in Japan. Furthermore, it has been reported that the LcS-treated patients had higher Quality of Life (QOL) scores in an evaluation that included

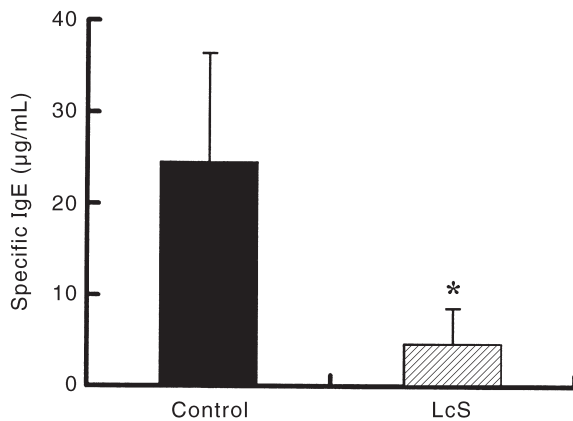


**Figure 2** Effects of *Lactobacillus casei* strain Shirota (LcS) on ovalbumin (OVA)-specific IgE production. BALB/c mice ( $n = 5$ ) were injected i.p. on day 0 and 14 with 20 µg OVA and 2 mg  $\text{Al}(\text{OH})_3$  at a total volume of 0.2 mL. The mice were then fed a diet containing (w/w) 0.05% LcS for 21 days. Control mice were injected with OVA only. Sera were collected on day 21 and the level of OVA-specific IgE was determined by passive cutaneous anaphylaxis reaction. Bars represent mean values ( $\pm$  SD) for five mice. \* $P < 0.01$  compared with the control.

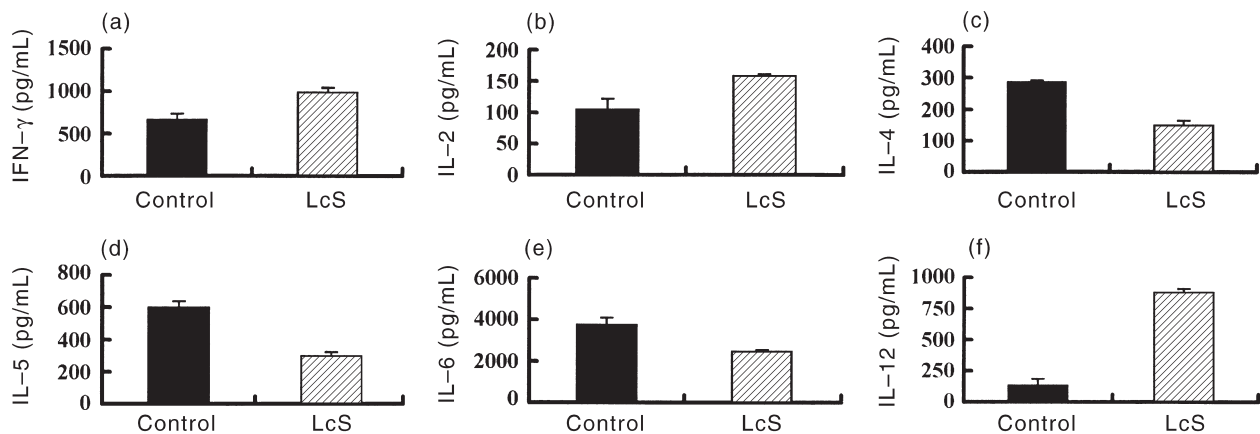


**Figure 3** Production of interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-5, IL-6 and IL-12 by spleen cells in vitro. BALB/c mice ( $n = 6$ ) were injected i.p. on day 0 and 14 with 20 µg ovalbumin (OVA) and 2 mg  $\text{Al}(\text{OH})_3$  at a total volume of 0.2 mL. The mice were then fed a diet containing (w/w) 0.05% *Lactobacillus casei* strain Shirota (LcS) for 21 days. Spleen cells were collected on day 21 and were co-cultured with OVA (final concentration 100 µg/mL) for 24 h. The amounts of IFN-γ, IL-2, IL-4, IL-5, IL-6 and IL-12 in the supernatant were measured by ELISA. The data shown are representative of one of three independent experiments. Bars represent mean values ( $\pm$  SD) for triplicate cultures.

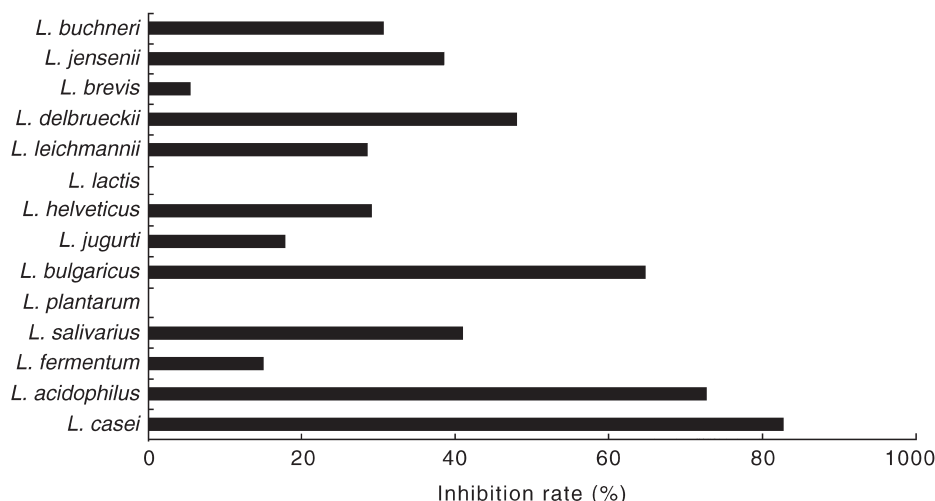


**Figure 4** Anti-tumour effects of *Lactobacillus* lactic acid bacteria in mice. Sarcoma 180 cells ( $1 \times 10^6$  cells/mouse) were inoculated subcutaneously into ICR mice ( $n = 10$ ) on day 0. Each preparation (250  $\mu\text{g}$ /mouse per day), suspended in 0.1 mL sterile saline, was injected i.v. on days 1–5 after tumour inoculation. Tumours were weighed on day 21 and the inhibition rate was calculated as follows: Inhibition rate (%) =  $(1 - \text{average tumour weight of treated group} / \text{average tumour weight of control group}) \times 100$ .

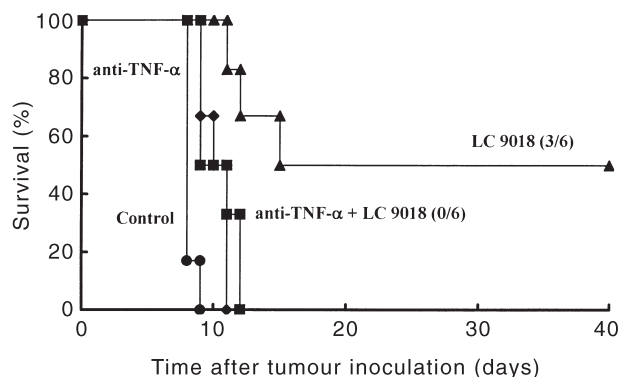


**Figure 5** Effect of anti-tumour necrosis factor (TNF- $\alpha$ ) monoclonal antibody (mAb) on the anti-tumor effect of a heat-killed cell preparation of *Lactobacillus casei* strain Shirota (LC 9018) in the murine pleurisy model. Meth A cells ( $1 \times 10^5$  cells/mouse) were inoculated i.p. into BALB/c mice ( $n = 6$ /group) on day 0 and anti-TNF- $\alpha$  mAb (25  $\mu\text{g}$ /mouse per day) dissolved in 0.1 mL of sterile saline was injected i.p. on days 2 and 4. The LC 9018 (100  $\mu\text{g}$ /mouse per day) suspended in 0.1 mL sterile saline was injected i.p. on days 3 and 6 and the survival was monitored. Numbers in parentheses indicate tumour-free mice/tested mice. (●), Control; (◆), anti-TNF- $\alpha$  antibody; (▲), LC 9018; (■), anti-TNF- $\alpha$  antibody + LC 9018.

health parameters, such as chest pain, insomnia, nausea/vomiting, chest discomfort and anorexia. Studies to date have demonstrated the potential of various LcS preparations in counteracting tumour formation.<sup>18,19</sup> Therefore, we expect that LcS, particularly LC 9018, a heat-killed preparation of LcS, will be used in further trials not only in Japan but throughout the world.

#### Effects of probiotic feeding on immune responses against a co-fed marker antigen

A series of experiments was conducted to investigate the effects of feeding *Streptococcus thermophilus* (St), yeast (Y) and *Lactobacillus fermentum* (Lf) on humoral- and cell-mediated immune responses in C57Bl mice. Three groups of mice ( $n = 12$  per group), designated St, Y and Lf, were fed daily doses of each bacterium (20 mg) for 4 days (days -4,

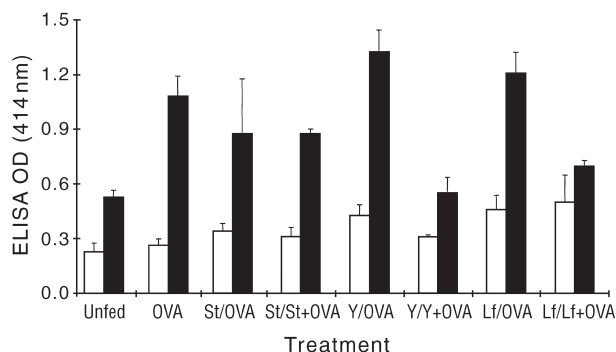


**Figure 6** Effects of feeding probiotic bacteria on the serum IgG response against the co-fed marker antigen ovalbumin (OVA). Three groups of mice ( $n = 12$  per group) were fed daily doses of 20 mg of yeast (Y), *Streptococcus thermophilus* (St) or *Lactobacillus fermentum* (Lf) for 4 days (days -4, -3, -2 and -1). On day 0, half of the mice in each group were then fed with a single 25 mg dose of OVA, while the rest were given OVA with the respective test probiotic. Thus, mice previously fed St and then given OVA were identified as St/OVA group, while those previously fed St and then given OVA with St were designated St/St+OVA. Groups of mice designated as Y/OVA and Y/Y+OVA, as well as Lf/OVA and Lf/Lf+OVA groups, were generated in this manner. Another group of six mice, which had not been previously given any probiotic, were fed on day 0 with only OVA (OVA group). Seven days later (day 7), all mice were vaccinated subcutaneously with OVA (100  $\mu\text{g}$ ) formulated in a final volume of 100  $\mu\text{L}$  in an oil-based adjuvant (Emulsigen). An unfed control group (Unfed group), which had not been given either probiotic or OVA, was vaccinated in the same way. A booster injection was given on day 14 and blood was collected by retro-orbital bleeding 4 days later (day 18 or 4 days post-vaccination; □). All mice were killed on day 22 (8 days post-vaccination; ■). ELISA assays were conducted as described previously.<sup>2</sup>

-3, -2 and -1). On day 0, half of the mice in each group were then fed with a single 25 mg dose of OVA, while the rest were given OVA with the respective test probiotic. Thus, mice previously fed St and then given OVA were identified as the St/OVA group, while those previously fed St and then given OVA with St were designated St/St + OVA. Groups of mice

designated as Y/OVA and Y/Y + OVA, as well as the Lf/OVA and Lf/Lf + OVA groups, were generated in this way. Another group of six mice that had not been previously given any probiotic were fed on day 0 with only OVA (OVA group). Seven days later (day 7), all mice were vaccinated subcutaneously with OVA (100 µg) formulated in a final volume of 100 µL in an oil-based adjuvant (Emulsigen; Ausvac, Bendigo, Vic., Australia). An unfed control group (Unfed group) that had not been given either probiotic or OVA was vaccinated in the same way. A booster injection was given on day 14 and blood was collected by retro-orbital bleeding 4 days later (day 18). All mice were killed on day 22. Blood was collected at this time to determine antibody levels against OVA and probiotic bacteria, while spleen cells were obtained for lymphoproliferation analysis.<sup>3</sup>

The effects of probiotic feeding on the antibody response against OVA are shown in Fig. 6. Mice fed with a single dose of OVA (25 mg; OVA group) mounted a significantly higher serum IgG response ( $P = 0.002$ ) against OVA compared with unfed mice after subcutaneous vaccination with OVA emulsified in an oil-based adjuvant. Pre-feeding mice with yeast or Lf followed by OVA (i.e. Y/OVA and Lf/OVA mice, respectively) stimulated a higher IgG response against OVA compared with mice given a single OVA feed, but this was not significant at the 5% level. However, antibody responses against OVA in mice fed Y/Y + OVA ( $P = 0.003$ ) or Lf/Lf + OVA ( $P = 0.04$ ) were significantly suppressed compared with OVA-fed mice. The antibody responses in Y/Y + OVA- or Lf/Lf + OVA-fed mice were also significantly suppressed when compared, respectively, with Y/OVA-fed ( $P < 0.001$ ) and Lf/OVA-fed ( $P = 0.013$ ) mice. Feeding St/OVA or St/St + OVA did not significantly alter antibody responses against OVA compared with OVA-fed mice. These results demonstrate that a single dose of OVA feeding was successful in priming the immune system to respond more vigorously to peripheral vaccination than in unfed controls. Although tolerance against OVA was not initiated by OVA feeding alone, the oral administration of yeast with OVA or Lf with OVA suppressed the immune response relative to OVA-fed mice. Tolerance induction did not occur in mice that had been previously fed Y or Lf and were then given a single dose of OVA. To exclude the possibility that probiotic feeding may have induced tolerance against Y and Lf and that bystander suppression of antibody responses may have occurred following feeding with probiotic and OVA, serum samples were analysed by ELISA for antibody responses against Y, St and Lf, respectively (Fig. 7). The absence of detectable antibody responses against any one of the three orally fed bacteria in the relevant groups of mice indicates that suppression was not due to immune deviation against a bystander antigen.

The effects of probiotic feeding on lymphoproliferative (LP) responses of splenocytes in various treatment groups are depicted in Fig. 8. The stimulation index (SI) in mice pre-fed with St followed by OVA was significantly higher than in unfed mice ( $P = 0.011$ ) or in mice given a single dose of OVA ( $P = 0.011$ ) when assayed against 25 µg OVA. However, feeding of St/St + OVA significantly suppressed the LP response compared with mice given St/OVA ( $P = 0.001$ ). The LP responses in mice fed Y/Y + OVA were also significantly suppressed relative to Unfed ( $P = 0.014$ ) and OVA-fed ( $P = 0.015$ ) mice. Although the SI in Y/OVA mice was also suppressed compared with Unfed and OVA fed mice,

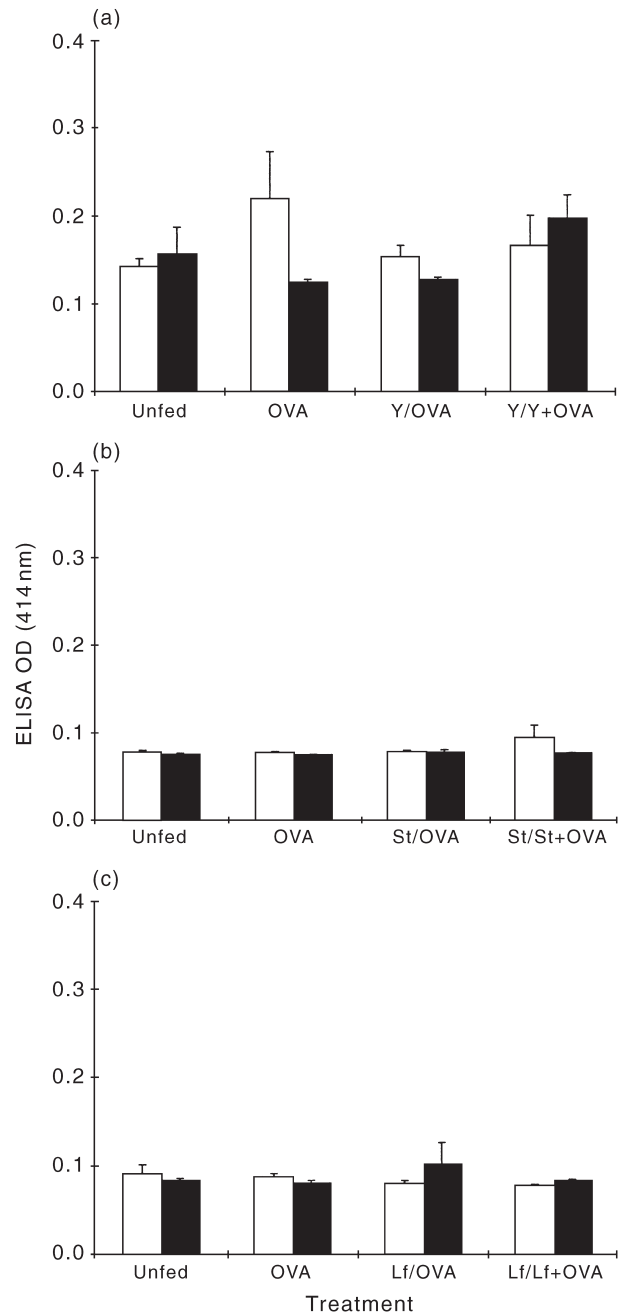


**Figure 7** Effects of feeding probiotic/ovalbumin (OVA) regimes on the serum IgG response against the probiotic bacterium. (□), Four days; (■), 8 days. (a) Serum antibody response in mice fed yeast (Y) OVA and Y/Y+OVA against yeast antigen. (b) Serum antibody response in mice fed *Streptococcus thermophilus* (St)/OVA and St/St+OVA against *S. thermophilus* antigen. (c) Antibody response in mice fed *Lactobacillus fermentum* (Lf)/OVA and Lf/Lf+OVA against *L. fermentum* antigen. Bacterial antigens used for ELISA assays were produced by heat denaturation in the presence of 2-mercaptoethanol and diluted in bicarbonated buffer for coupling to microtiter plates.

differences were not significant at the 5% level ( $P = 0.053$  and  $0.056$ , respectively). These results show that prefeeding certain probiotics, such as *Streptococcus thermophilus*, is able to augment cellular immune responses against an orally

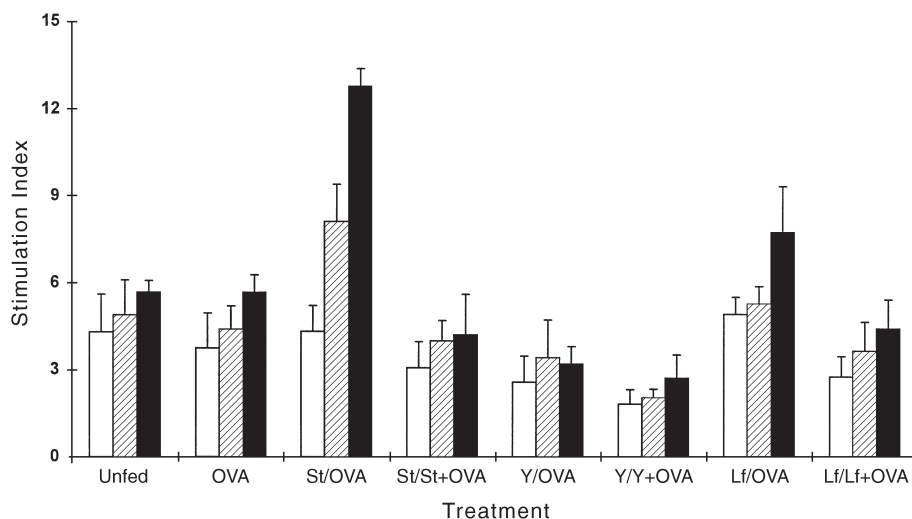


**Figure 8** Effects of feeding probiotic bacteria on the lymphoproliferative response of spleen cells against the co-fed marker antigen ovalbumin (OVA). (□), 5 µg; (▨), 10 µg; (■), 25 µg OVA. (Experimental groups of mice were identical to those described in Fig. 6. Conditions for lymphoproliferation assays were as described previously.<sup>3</sup> St, *Streptococcus thermophilus*; Y, yeast; Lf, *Lactobacillus fermentum*).

fed antigen only if that antigen is delivered singly and in a pure state. Codelivery of the antigen with St resulted in significant tolerance induction. This is noticeable even in Y/Y + OVA and Lf/Lf + OVA mice.

## Discussion

Health benefits emanating from the consumption of probiotics have in recent years been used as a strong marketing tool, even though supportive data has not been available. The health effects of probiotic bacteria have already been identified (see Kailasapathy and Chin<sup>4</sup>), but little attention has been given to their role in manipulating the immune system. We have now shown that probiotics such as LcS exert potent antitumour and antimetastatic effects in rodents. In particular, intrapleural injection of LcS effectively inhibits tumour growth in the thoracic cavity, not only in mice, but also in humans, with extension of survival periods. Part of the mechanism of action of LcS appears to be its ability to stimulate production of IFN-γ, IL-12 and TNF-α. By promoting a bias towards a Th1 response, it is possible to inhibit OVA-specific IgE production by treatment with LcS and this suggests that there is some merit in exploring the potential of probiotics in the treatment of allergic disorders.

Another action of LcS appears to be directed towards the stimulation of innate immune responses. Stimulation of NK activity is probably associated with the bias towards a Th1 response and it appears that consumption of LcS has the ability to augment natural resistance levels in the host animal. Other bacteria used in food preparations, such as *Streptococcus thermophilus*, although not formally recognised as probiotic, also have the ability to modulate immune responses. While feeding a single pure marker antigen, such as OVA, appears to prime for an enhanced antibody response to subsequent peripheral vaccination with adjuvanted OVA, cofeeding OVA with probiotic bacteria such as yeast or *Lactobacillus fermentum* has provided a source of tolerogenic signals. This finding suggests that the use of oral antigen feeding to attenuate autoimmune diseases may be considerably enhanced if proteins such as myelin antigen and retinal S-antigen were to be

delivered in conjunction with probiotic bacteria for the alleviation of clinical states associated with multiple sclerosis<sup>20</sup> and experimental autoimmune uveoretinitis.<sup>21</sup>

Recent trends in the marketing of food products have clearly demonstrated a tendency towards claiming specific or general health benefits from the consumption of special food formulations, probiotic bacteria or medicinal plants.<sup>22</sup> This new field of nutraceuticals and health benefits that may ensue from their consumption can now be subjected to rigorous immunological analysis. It may now be timely to determine whether immunological parameters can be used as indicators of health and well-being. Certainly, there is available a range of immunological tools and techniques, as well as animal models, to study the effects of various dietary supplements on the immune response.

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