Enhanced Immunological Memory Responses to *Listeria monocytogenes* in Rodents, as Measured by Delayed-Type Hypersensitivity (DTH), Adoptive Transfer of DTH, and Protective Immunity, following *Lactobacillus casei* Shirota Ingestion

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We have investigated the effect of orally administered *Lactobacillus casei* Shirota (*L. casei*) on immunological memory, as measured by delayed-type hypersensitivity (DTH) and acquired cellular resistance (ACR). The studies were performed in animal models in which the animals were rendered immune by a primary *Listeria monocytogenes* infection. It was shown that orally administered viable *L. casei*, and not heat-killed *L. casei*, enhanced significantly the antigen-specific DTH at 24 and 48 h in Wistar rats, Brown Norway rats, and BALB/c mice in a time- and dose-dependent fashion. *L. casei* had to be administered at least 3 days prior to the DTH assay at a daily dose of $10^9$ CFU in order to induce significant effects. Long-term administration of $10^5$ CFU of viable *L. casei* resulted in enhanced ACR, as demonstrated by reduced *L. monocytogenes* counts in the spleen and liver and diminished serum alanine aminotransferase activity after reinfection. Enhancement of cell-mediated immunological immune responses by *L. casei* was further established in an adoptive transfer study. Naïve recipient BALB/c mice, which were infused with nonadherent, immunized spleen cells from *L. casei*-fed donor BALB/c mice, showed significantly enhanced DTH responses at 24 and 48 h compared to recipient mice which received spleen cells from control donor mice. In conclusion, orally administered *L. casei* enhanced cell-mediated immunological memory responses. The effects relied on lactobacillus dose and viability as well as timing of supplementation and, further, appeared to be independent of host species or genetic background.

Various cells of the innate and acquired immune system interact in a concerted fashion to constitute a sophisticated set of responses to protect the physiological integrity of the host against infectious agents and tumors. Inappropriate orchestrated immune responses lead to, e.g., autoimmune and allergic disease states as well as enhanced susceptibility to infections. Different compounds are known which have the capacity to enhance or decrease biological responses of the immune system in order to restore disturbed immune responses or to elevate resistance to infectious agents. These compounds can be classified as so-called immunomodulators or biologic response modifiers (23).

Lactobacilli form a source of potential modulators of the immune system. It has been demonstrated that specific *Lactobacillus* strains can modulate host immunity, which positively correlates with enhanced resistance to various viral and bacterial infections (9, 20, 24). The immunomodulating effects are dependent on various factors, such as intrinsic adjuvanticity properties (21), dose (13, 31), viability (16, 20, 30), route and timing of administration of the specific *Lactobacillus* strains (i.e., oral versus parenteral), and the physiological state and genetic background of the host (20).

Previous results from our group established that $10^9$ orally administered viable *Lactobacillus casei* Shirota strain YIT9029 (*L. casei*) bacteria given daily enhanced *Trichinella spiralis* antigen-specific immunoglobulin G2b (IgG2b) and delayed-type hypersensitivity (DTH) responses in Brown Norway and Wistar rats in an oral infection model of *T. spiralis* (8). The immunostimulating activity of *Lactobacillus casei* Shirota was also observed in a rat model of food-borne *Listeria monocytogenes* infection. It was shown in orally *Listeria*-infected rats that ingestion of *L. casei* enhanced the DTH reaction (7). From these studies, and based on the described immune cell populations involved in DTH responses (29), it was asserted that orally supplemented *L. casei* could exert a direct effect on T-helper-1-cell immunity by modulating the amount and activity of antigen-specific T lymphocytes and/or could exert an indirect effect on T-lymphocyte activity through stimulation of other cell types, such as phagocytes.

Collectively our studies prompted further examination of the effect of orally administered *Lactobacillus casei* Shirota strain YIT9029 on cell-mediated immunity in an animal model of systemic *L. monocytogenes* infection. Rats and mice, which are sublethally infected with viable *L. monocytogenes*, generate DTH and acquired cellular resistance (ACR) during the course of the infection (29, 36). ACR provides long-lasting,
protective immunity to reinfection and can be measured as the pathogenic load after reinfection. Although the correlation between DTH and ACR remains controversial, they are both inversely related to the immune status of the host. Measurement of DTH and ACR forms a reliable method for examining the effects of orally administered lactobacilli on immunological memory.

In a parenteral Listeria monocytogenes infection model we studied the effects of orally administered Lactobacillus casei Shirota strain YIT9029 on T-cell-dependent immune responses by measurement of Listeria-specific DTH reaction and ACR in rechallenge and adoptive transfer studies. Since immune-modulating effects may rely on a range of factors, we investigated whether the effects induced by L. casei ingestion were dependent on the lactobacillus dose, viability, and administration timing as well as host genetics. In all experiments with actively infected Wistar rats, Brown Norway rats, and BALB/c mice, it was shown that ingestion of viable L. casei enhanced immunological memory, as determined by DTH and ACR, in a dose-, viability-, and timing-dependent fashion. Since DTH, ACR, and transfer of DTH are T-cell-dependent phenomena, it is suggested that L. casei-induced immunomodulation could be attributed, at least partly, to T memory cells.

MATERIALS AND METHODS

**Animals.** Male specific-pathogen-free Wistar (U:Wu) rats, Brown Norway (Rij/Hsd) rats, and BALB/c mice were obtained from the animal facility of the Utrecht University (Utrecht, The Netherlands), Harlan Netherlands B.V. (Horst, The Netherlands), and the National Institute of Public Health and the Environment (Bilthoven, The Netherlands), respectively. The rats and mice, 6 to 7 weeks of age, were randomly allocated and kept in filter-topped cages (two animals per cage). The animals were provided with autoclaved food pellets and drinking water ad libitum. The animals were housed in the barrier under standard conditions (50 to 60% humidity, 12-h dark-12-h light cycle). All animal studies were approved by an independent ethical committee of the Utrecht Faculty of Veterinary Medicine, as required by Dutch law.

**Bacteria.** Lactobacillus casei Shirota strain YIT9029 (L. casei) was obtained from the Yulaku Central Institute for Microbiological Research (Tokyo, Japan). L. casei was grown overnight at 37°C in MRS broth (CM359; Oxoid, Haarlem, The Netherlands) and resuspended in sterile saline (0.9% NaCl) to a final concentration of 2 × 10⁸, 2 × 10⁹, or 2 × 10¹⁰ CFU/ml, respectively. The number and viability of the lactobacilli were determined by aerobic culturing on MRS plates (Oxoid CM361). Heat-killed L. casei was obtained by heating a viable L. casei suspension of 2 × 10⁷ CFU for 15 min at 65°C. The animals received 0.5 ml of saline (control) or bacterial suspensions in saline (experimental groups) per day by a feeding tube placed in the esophagus.

Lactobacillus monocytogenes strain L24273 type 4b was obtained from the National Institute of Public Health and the Environment (Bilthoven, The Netherlands). To prepare activated L. monocytogenes for oral infections, an egg passage was performed as described by Ruitenberg et al. (32). The stimulated bacteria were maintained in brain heart infusion BHI broth (Oxoid CM225) at ~80°C. In each experiment, an aliquot was thawed and cultured on BH (Oxoid CM375) agar plates. Inocula of L. monocytogenes were grown for 18 h in BHI broth and resuspended in sterile saline to the appropriate concentrations. The L. monocytogenes concentration was confirmed by aerobic culturing on both BHI and modified Oxford agar (MOA) plates. The MOA medium was modified from a selective medium for L. monocytogenes as described elsewhere (35).

**Experimental design.** In various independent experiments, the effect of orally administered Lactobacillus casei Shirota strain YIT9029 on L. monocytogenes-specific DTH responses and ACR in mice and rats was studied. The studies were designed to study whether the immune effects induced by oral L. casei intake are dependent on lactobacillus dose, viability, and administration timing and host genetics. In all experiments, the moment of L. monocytogenes infection was defined as time zero (t = 0).

**Host genetics.** In separate experiments, the effect of L. casei on the DTH response in Wistar rats, Brown Norway rats, and BALB/c mice was studied. Starting 10 days (t = −10) before the subcutaneous L. monocytogenes infection, the animals (n = 6 per group) were daily administered in 0.5-ml suspensions, which contained 10⁷ CFU of viable L. casei. Control animals received saline instead of lactobacilli. At day 10 post infection (t = 0), the L. monocytogenes-specific DTH was measured.

**Administration timing.** Starting 10 days before (t = −10) or 7 days after (t = 7) the subcutaneous L. monocytogenes infection, rats were daily administered 0.5-ml suspensions with 10⁸ CFU of viable L. casei. Control animals received saline instead of lactobacilli (n = 6 rats per group). At t = 7, the host has completely cleared the bacterial pathogen (data not shown), which means that the control and experimental groups have been exposed to the same amounts of Listeria antigen, since the animals were not L. casei treated before day 7 post infection. Differences found in DTH responses at day 10 would be due only to ingestion of L. casei from day 7 till day 10 postinfection. At day 10 post infection (t = 10), L. monocytogenes-specific cell-mediated immunity was measured using the DTH assay.

**Lactobacillus dose, viability, and administration timing.** Starting 7 (t = 7), 8 (t = 8), or 9 (t = 9) days after the subcutaneous L. monocytogenes infection, rats were daily administered 0.5-ml suspensions containing 10⁷ CFU of viable L. casei. Additionally, the effects of orally administered 10⁶ heat-killed, 10⁸, and 10⁹ CFU of viable L. casei on the DTH response were analyzed. Control animals received saline instead of lactic acid bacteria (n = 4 rats per group). At day 10 postinfection (t = 10), L. monocytogenes-specific cell-mediated immunity was measured using the DTH assay.

Lactobacillus strains were grown for 18 h in BHI broth and resuspended in sterile saline to the appropriate concentrations. The number and viability of the lactobacilli were determined by aerobic culturing on MRS plates (Oxoid CM361). Heat-killed L. casei was obtained by heating a viable L. casei suspension of 2 × 10⁷ CFU for 15 min at 65°C. The animals received 0.5 ml of saline (control) or bacterial suspensions in saline (experimental groups) per day by a feeding tube placed in the esophagus.

**Primary and challenge infections.** Rats and mice were subcutaneously infected at day zero with 5 × 10⁵ or 2 × 10⁶, respectively, viable L. monocytogenes cells at five different sites, 0.1 ml at two sites in the left and right thighs, 0.1 ml at two sites in the left and right forelimbs, and 0.1 ml in the neck region. Challenge infections were performed by intraperitoneal infusion of 5 × 10⁵ viable L. monocytogenes cells in both rats and mice.

**Adoptive transfer of immunity.** Starting 10 days previously (t = −10), subcutaneously L. monocytogenes-infected donor mice received orally 10⁵ CFU of L. casei or saline per day (n = 10 mice per group). Seven days (t = 7) after the primary L. monocytogenes infection, single-cell suspensions were prepared from the spleens of donor mice. After passage over a column packed with glass wool fiber, 10⁶ wool-nonadherent spleen cells of five mice of each group were infused via the orbital plexus into syngeneic naive recipients. The recipient mice received no lactobacilli. Twenty-four hours after the transfer (t = 8), the DTH was measured in the recipient mice and the remaining five donor mice of each group. Twenty-four and forty-eight hours after the challenge, the increase in ear thickness was measured. Five days after setting the DTH assay (t = 13), all mice were challenged with live L. monocytogenes. The mice were sacrificed 2 days later and analyzed for L. monocytogenes counts in the liver and spleen. ACR was determined by the capacity of the rats to eliminate L. monocytogenes cells from the spleen and liver after the challenge.

**DTH assay.** Animals in the experimental and control groups were challenged in left and right ear pinnas with 25 μl (10⁷ CFU) (for rats) 10 μl (10⁶ CFU) of heat-killed L. monocytogenes (HKLM) under light ether anesthesia. The application and preparation of HKLM in DTH assays have been described in previous studies (1, 12, 14). The absolute increase in ear thickness was measured with a digital engineer’s micrometer (Mitutoyo, Veenendaal, The Netherlands) 24 and 48 h after challenge. The swelling was calculated according to the following equation: net swelling = (S24 – S0) – (P24 – P0). S0 is thickness of ear before phosphate-buffered saline injection, and P0 is thickness of ear 24 h after phosphate-buffered saline injection. The same type of equation for DTH measurements at 48 h after challenge was performed. It was shown that challenging the ear pinnas in noninfected L. casei-fed rats with heat-killed L. monocytogenes or challenging the ear pinnas of L. monocytogenes-immunized rats with heat-killed L. casei did not result in a significant net swelling response. This excludes possible cross-reactivity of L. monocytogenes antigens with L. casei antigens.

**Bacteriological analysis.** Spleens and livers were removed aseptically from the animals. The numbers of viable L. monocytogenes were determined by culturing 100 μl of serial 10-fold dilutions of organ homogenates in saline on MOA plates. The numbers of L. monocytogenes bacteria were expressed as CFU per gram of spleen or liver. Detection limit was 100 CFU of livers and 100 CFU of stools.

ALT. ALT levels in rat serum were analyzed with an automated chemistry analyzer (Beckman Synchrone CX7; Beckman-Coulter Ned., Mijdrecht, The Netherlands).
RESULTS

Host genetics and the effects of oral L. casei administration on DTH. Figure 1 shows that L. casei, following oral delivery, significantly enhanced the L. monocytogenes-specific DTH response in Wistar rats at 24 h (P < 0.05) and 48 h (P < 0.05), compared to results for control animals. In a parallel study it was shown that orally administered L. casei enhanced significantly the expression of DTH at 24 h (P < 0.05) and 48 h (P < 0.05) in the Brown Norway rats (Fig. 1). Further, in two experiments with L. monocytogenes-immunized BALB/c mice, it was demonstrated that L. casei ingestion enhanced the antigen-specific DTH in rats. In the first experiment the DTH was significantly enhanced at 24 h (P < 0.01) and 48 h (P < 0.01) after challenging the ear pinna (Fig. 1). In the second experiment DTH values in lactobacillus-treated mice were significantly higher at 24 h (P < 0.001) and 48 h (P < 0.05) after challenging the ear pinna (Fig. 1). In the second experiment DTH values in lactobacillus-treated mice were significantly higher at 24 h (P < 0.001) and 48 h (P < 0.05) after challenging the ear pinna (Fig. 1). In the second experiment DTH values in lactobacillus-treated mice were significantly higher at 24 h (P < 0.001) and 48 h (P < 0.05) after challenging the ear pinna (Fig. 1).

The effect of L. casei administration timing on DTH. Starting daily, oral L. casei administration at a dose of 10^7 CFU, 10 days prior to as well as 7 days after the primary Listeria monocytogenes infection in Wistar rats, resulted in significantly enhanced antigen-specific DTH expression, as shown in Fig. 2. The magnitude of the DTH responses at 24 and 48 h was comparable for the two groups of rats which received L. casei 20 or 3 consecutive days prior to the DTH assay.

The effect of oral administration of 10^9 CFU of viable L. casei 3 days prior to the DTH assay in Wistar rats was reproduced, as shown in Fig. 3. A significantly enhanced Listeria-specific DTH reaction was measured at 24 h (P < 0.05) and 48 h (P < 0.01) in animals which received L. casei starting 3 days before the DTH assay. DTH values at 24 and 48 h after ingestion of 10^9 CFU of lactobacilli at 2 days or 1 day prior to

TABLE 1. DTH and adoptive transfer of ACR with splenocytes from L. casei Shirota-fed donor mice infected subcutaneously with viable L. monocytogenes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean result for indicated group ± SD</th>
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<tbody>
<tr>
<td>inf</td>
<td>Cells transferred</td>
</tr>
<tr>
<td>s.c., saline</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>s.c., L. casei</td>
<td>10.3 ± 1.0***</td>
</tr>
<tr>
<td>s.c., saline</td>
<td>3.6 ± 1.8</td>
</tr>
<tr>
<td>s.c., L. casei</td>
<td>8.9 ± 2.6**</td>
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a BALB/c donor mice were infected (inf) subcutaneously (s.c.) with 2 x 10^9 viable L. monocytogenes bacteria. Starting 10 days before infection, the mice received a daily dose of L. casei (n = 10) or saline (n = 10). Nonadherent (10^8) spleen cells (Inf Spl) from five BALB/c mice were infused into syngeneic naive recipients 7 days (t = 7) after infection. Twenty-four hours after the transfer (t = 8), the L. monocytogenes-specific DTH was set and was determined 24 and 48 h later for the five recipient mice and the five remaining donor mice of the respective experimental and control groups. Five days after setting of the DTH assay (t = 13), all mice were challenged intraperitoneally with 5 x 10^7 viable L. monocytogenes bacteria. The mice were killed 24 h later, and counts of viable L. monocytogenes in the spleen were determined. Listeria counts are expressed as geometric means.

b Asterisks indicate significant difference (*, P < 0.05; **, P < 0.01; ****, P < 0.001) from the respective control group as calculated with Student's t test.
the DTH assay did not significantly differ from those for control rats.

Decreasing the number of lactobacillus applications prior to the DTH assay resulted in a strong, but not significant, tendency to correlate with decreasing DTH values at 24 h. This correlation was significant at 48 h (r = 0.55; P < 0.05).

The effect of L. casei viability on DTH. Ingestion of heat-killed L. casei did not affect DTH expression. As shown in Fig. 3, DTH values at 24 and 48 h were not significantly different from control values after oral administration of 10^9 CFU of heat-killed L. casei 3 days prior to the DTH assay.

Effect of L. casei dose on DTH. DTH values at 24 and 48 h were not significantly different from control values after oral administration of 10^8 and 10^9 viable L. casei bacteria 3 days prior to the DTH assay, as denoted in Fig. 3. A significant correlation was observed between lactobacillus dose, administered 3 days prior to the DTH assay, and DTH values, measured at 24 and 48 h after challenge (r = 0.61 and P < 0.05 [24 h]; and r = 0.71 and P < 0.01 [48 h]).

Effects of long-term oral administration of viable L. casei on ACR. Long-term (i.e., approximately 8 weeks) oral supplementation of viable L. casei at a dose of 10^9 CFU, but not 10^7 or 10^8 CFU, enhanced ACR as measured by reduced counts of viable L. monocytogenes in the spleen and liver at 36 h after reinfection compared to results for control animals (Table 2). Immunologically primed animals responded to reinfection in a more vigorous and effective manner, as shown in Table 2. Unprimed control animals (control-1) had approximately 10 times higher Listeria counts in the visceral organs, spleen (P < 0.01), and liver (P < 0.01), as well as significantly higher serum ALT activity (P < 0.01) than primed control animals (control-2).

Further, L. monocytogenes growth was not affected by long-term oral administration of heat-killed L. casei. Serum ALT activity was significantly lower in two (out of three) groups of animals which were fed viable L. casei Shirota at a dose of 10^9 CFU per day for 63 consecutive days, compared to the control group (control-2), as shown in Table 2. Other experimental
groups did not affect significantly the ALT activity. In spite of that, ALT values were significantly correlated with bacterial load in the liver ($r = 0.75; P < 0.01$).

**Adoptive transfer of immunity.** It was shown that expression of antigen-specific DTH was significantly enhanced in recipient BALB/c mice which received nonadherent spleen cells from *L. casei*-fed donor BALB/c mice. In Fig. 4, it is shown that DTH was enhanced at 24 h ($P < 0.01$) and 48 h ($P < 0.05$). The magnitude of the DTH values of recipient mice, as depicted in Fig. 4, was comparable to DTH values of the remaining, corresponding groups of mice (Table 1) that were assayed parallel to the spleen cell-infused recipient mice, following 7 days after the primary infection with *L. monocytogenes*. Ten days after the DTH assay, all groups of mice were reinfected with viable *L. monocytogenes*. Numbers of *Listeria* bacteria in the spleen appeared reduced, but not significantly, in normal *L. casei*-fed mice as well as in reconstituted recipients which received spleen cells from *L. casei*-supplemented donor animals, compared to the respective controls.

**DISCUSSION**

The immune system can be optimized through oral supplementation of specific *Lactobacillus* strains (2, 9). Modulation of B-cell-mediated immune responses can be monitored relatively simply by, for instance, measurement of titers of antigen-specific antibody in serum. In vivo measures of T-cell-mediated immunity comprise delayed-type hypersensitivity responses and reinfections with intracellular pathogens, such as *L. monocytogenes* (11, 36).

In the present study it is shown that ingestion of a daily dose of $10^8$ CFU of live, *L. casei* Shirota strain YIT9029, starting at least 3 days prior to infection, the mice received a daily dose of *L. casei* or saline. Nonadherent spleen cells from *L. casei* and control-fed BALB/c mice were infused into syngeneic naive recipients 7 days after infection. Twenty-four hours after the transfer, the *L. monocytogenes*-specific DTH was set, and it was determined 24 and 48 h later for the recipient mice. Bars represent the means ± standard deviations for five animals per group. Asterisks indicate significant differences ($*, P < 0.05; **, P < 0.01$) from the control group as calculated with Student’s *t* test.
tation of L. casei leads to a prominent state of cellular innate immunity via phagocyte activation, which subsequently enhances Th1 cell activity. The results from this and other laboratories strongly suggest that L. casei has a genuine effect on Th1-cell-mediated immunological memory.

Adoptive transfer studies with mice increasingly confirmed the involvement of T memory cells in the enhanced DTH response after L. casei application. Ample evidence exists that DTH to heat-killed L. monocytogenes or soluble Listeria antigens is transferred by spleen-derived CD4+ T-helper cells (1, 35, 39). The in vivo effector assay comprised the transfer of nonadherent spleen cells from L. monocytogenes-infected donor BALB/c mice, which were fed L. casei or control saline, to naive recipients, which were challenged 24 h later in the ear pinna. The results demonstrated that naive recipient mice, which received nonadherent spleen cells from L. casei-fed donor mice, had enhanced DTH responses compared to control animals. This finding supports our view that L. casei ingestion enhances the activity and/or proliferation of T-helper-1 memory cells.

Although the association of DTH and acquired cellular immunity remains controversial, they both are manifestations of T-cell-mediated immunological memory (1, 6, 27, 35, 39). The kinetics of development and endurance of both responses have been reported to be similar (22). Presumably, different subsets of T cells are involved in DTH and ACR (1, 26, 39). Despite the uncertain association between DTH and ACR, it was shown that long-term, i.e., for approximately 8 weeks, oral administration of a daily dose of 107 CFU of L. casei enhanced acquired cellular resistance against a reinfection with L. monocytogenes as measured by reduced numbers of bacterial pathogen in the spleen and liver as well as reduced ALT levels in serum, next to the enhancement of DTH. In the transfer studies with mice, the bacterial counts in the spleen also appeared to be decreased after reinfecion, albeit this was statistically not significant.

Further, it was shown that the effects of L. casei on immunological memory responses were dose dependent. Incrementally increasing doses of L. casei significantly conveyed higher levels of enhancement of DTH and ACR. A dose of 107 L. casei bacteria was shown to be ineffective in modulating DTH or ACR. It is ambiguous whether the effective dose of L. casei is associated with a disparity in sensitivity of the specific cells involved in the measured immune parameters. It has been shown that a specific Lactobacillus rhamnosus strain was able to enhance the phagocytic capacity of white blood cells at a dose of 107, while a 100-times-higher dose of lactobacilli was needed to increase the phagocytic capacity of peritoneal cell preparations, following oral delivery (13).

In our specific experimental setup with L. monocytogenes-infected rats, it is possible that viable and killed lactobacilli exert different effects, for example, through differential stimulation of dendritic cell subsets (DC1 versus DC2), which control the directional development of T-helper-cell phenotypes (4, 19). Another explanation for differential effects of viable and dead L. casei, as shown in our studies, could be that immune stimulation by L. casei is mediated by heat-sensitive structures on the bacterial surface. Data obtained by Haller et al. showed that higher concentrations of heat-killed lactobacilli were needed to induce the same tumor necrosis factor alpha production by monocytes as was induced by live lactobacilli (16). It was speculated from these studies that heat-killed lactobacilli express less undisturbed surface molecules (i.e., peptidoglycans or lipoteichoic acids) which can be recognized by pattern recognition receptors (e.g., CD14 or toll-like receptors) on various antigen-presenting cells (10, 34).

In summary, the present study endorses the idea that orally administered L. casei Shirota strain YIT9029 enhances cell-mediated immunological memory responses, as determined by DTH, ACR, and adoptive transfer of DTH in an L. monocytogenes infection model. Since DTH, ACR, and transfer of DTH are T-cell-dependent phenomena, it is suggested L. casei-induced immunomodulation is mediated, at least partly, by memory T cells. Still, the nature of the cells, in terms of cytokine profiles and cell surface markers, in the observed effects remains to be established. Nevertheless, the effect of L. casei on cell-mediated memory responses relied on the timing of administration, was dose dependent, and possibly was imparted through a heat-sensitive constituent of L. casei. The effects of viable L. casei appeared to be independent of the responding host species and genetic background.

REFERENCES


