Lactobacillus gasseri suppresses Th17 pro-inflammatory response and attenuates allergen-induced airway inflammation in a mouse model of allergic asthma

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Abstract

Probiotics are normal inhabitants of the gastrointestinal tract of man and are widely considered to exert a number of beneficial effects in many diseases. But the mechanism by which they modulate the immune system is poorly understood. The present study was planned to explore the anti-allergic effect of *Lactobacillus gasseri* on a mouse model of allergic asthma. *Dermatophoides pteronyssinus* (Der p) sensitised and challenged BALB/c mice were orally administered via oral administration with three different doses of *L. gasseri* (low, 1×10^6 colony-forming units (CFU); medium, 2×10^6 CFU; high, 4×10^6 CFU), in 700 µl of PBS daily, starting from 2 weeks before Der p sensitisation for 4 weeks. After the allergen challenge, airway responsiveness to methacholine, influx of inflammatory cells to the lung, and cytokine levels in bronchoalveolar lavage (BAL) fluids and splenocytes culture were assessed. Our results showed that oral administration of a high dose of *L. gasseri* (4×10^6 CFU) decreased airway responsiveness to methacholine, attenuated the influx of inflammatory cells to the airways and reduced the levels of TNF-α, thymus and activation-regulated chemokine (TARC) and IL-17A in BAL fluids of Der p-sensitised and -challenged mice. Moreover, *L. gasseri* decreased IL-17A production in transforming growth factor-α and IL-6 stimulated splenocytes and cell numbers of IL-17 producing alveolar macrophages in *L. gasseri*-treated mice as compared to non-treated, Der p-sensitised and -challenged mice. In conclusion, oral administration with *L. gasseri* can attenuate major characteristics of allergen-induced airway inflammation and IL-17 pro-inflammatory immune response in a mouse model of allergic asthma, which may have clinical implication in the preventive or therapeutic potential in allergic asthma.

Key words: *Lactobacillus*: Allergic asthma: Th17: Mite allergen

One of the explanations for the increased prevalence of allergic diseases lies in the well-known 'hygiene hypothesis', which postulates that a lack of opportunities for exposure to immunostimulating pathogens during early childhood increases the prevalence of allergic diseases[1]. For example, allergic children in Estonia and Sweden were found to be colonised with lactobacilli less often than non-allergic children[2]. Lactic acid bacteria (LAB) are widely considered to exert a number of beneficial effects including immunomodulation, interference with enteric pathogens and maintenance of a healthy intestinal microflora[3,4]. The disturbance in the gut flora induced by the ingestion of live microbiota could favour appropriate maturation of the immune system[5,6]. In particular, lactobacilli are considered to induce reactions involving Th1 cells and to improve allergic diseases[7]. Orally administered heat-treated *Lactobacillus casei* (strain *Shirota*) was found to inhibit IgE production induced by ovalbumin in mouse serum[8]. Moreover, heat-treated *L. plantarum* L-137 injected intraperitoneally was demonstrated to suppress IgE production in response to casein allergy in mice[9].

In human subjects, *L. rhamnosus* strain GG administered perinatally reduced the incidence of atopic eczema in children at risk during the first 2 years of life[10] and beyond infancy[11]. *L. rhamnosus* and *L. reuteri* administration moderately improved the clinical severity of eczema in children with atopic dermatitis[12]. Our double-blinded, randomised, controlled clinical trial for the effect of *L. gasseri* on asthmatic children with allergic rhinitis also shows significantly improved mucosal symptoms and pulmonary function in the treated subjects as compared to placebo controls[13].

Allergic asthma is characterised by chronic airway inflammation with intense eosinophil and lymphocyte infiltration, mucus hyper-secretion and airway hyper-responsiveness to a variety of stimuli. It is now generally thought that antigen-specific helper T lymphocyte (Th2) cells and their cytokines orchestrate these pathological features of asthma. On the other hand, Th17 cells and IL-23, a cytokine that preferentially expands Th17 cells, play a significant role in the development of chronic inflammatory diseases, including autoimmune diseases. Recently, several lines of evidence suggest that IL-17A and IL-17F are also involved in airway inflammation, especially in neutrophilic inflammation[14,15]. In addition, it has been shown that IL-17A is expressed in the airways of asthmatic patients[16] and its expression is correlated with the severity of asthma[17]. Moreover, our previous study results on the genetic factors associated with childhood allergic asthma indicated a possibility that the polymorphisms of IL-17A gene may confer risk for paediatric asthma in the Taiwanese population[18]. In a mouse model of asthma, it is also found that IL-23 and Th17 cells enhance not only neutrophilic airway inflammation but also Th2 cell-mediated eosinophilic airway inflammation in a murine asthma model[19,20].

To date, the precise mechanism of *Lactobacillus*-induced immunomodulation has not been fully elucidated, although it was reported that these probiotics can directly but differentially modulate dendritic cells maturation and induce cytokine secretion through toll-like receptors including toll-like receptor 2[21–23]. On the basis of experimental data, the anti-inflammatory effects of probiotics may be, in part, a consequence of the modulation of the cytokine balance: down-regulation of pro-inflammatory (e.g. IL-12 and TNF-α) cytokine production and/or stimulation of anti-inflammatory (e.g. IL-10) cytokine production[24]. However, whether the effect of probiotic bacteria such as *Lactobacillus* can act on IL-17-producing cells is currently unknown.

Materials and methods

Animals and reagents

Female BALB/c mice aged 6–8 weeks were obtained from the Laboratory Animal Center of the College of Medicine, National Cheng Kung University. All experimental animal care and treatment followed the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals, USA. *Dermatophoides pteronyssinus* (Der p) extract (1 g lyophilised whole-body extract in diethyl ether; Allergon, Engelholm, Sweden) was dissolved in pyrogen-free isotonic saline, filtered through a 0·22 μm filter, and stored at −70°C before use. Lipopolysaccharide concentration of the Der p preparations was <0·96 ng/ml (Limulus amebocyte lysate test; E-Toxate; Sigma-Aldrich, St Louis, MO, USA).

Probiotic bacterial preparations

L. gasseri A5, which exhibits probiotic characteristics, was isolated from the intestinal tract of normal, healthy humans at ProMD Biotech Company Limited (Tainan, Taiwan) by using the high-throughput detecting system. Acute oral toxicity studies (FR-AC00235), conducted by the Development Center for Biotechnology (Taipei, Taiwan), in test mice administered the highest dose of 5000 mg/kg *L. gasseri* A5 orally for 14 d, revealed that the treatment had no side-effects on the mice. To prepare the stock solution for *ex vivo* experiments, bacteria from frozen stocks were suspended in de Man–Rogosa–Sharpe liquid medium (Difco Laboratories, Detroit, MI, USA), plated in de Man–Rogosa–Sharpe agar, cultured anaerobically at 37°C for 24 h, then inoculated in fresh de Man–Rogosa–Sharpe broth and grown at 37°C under anaerobic conditions for 48 h in 50-ml tubes. A high-density culture system was used to produce freeze-dried powder of *L. gasseri* A5 at concentrations of more than 10^{11} colony-forming units (CFU)/g supplied by ProMD Biotech Company Limited, and then the powder was stored at a temperature below 4°C. Stability tests demonstrated that cell viability could be maintained at room temperature for up to 3 months. To prepare heat-killed bacterial extracts, cultured bacteria were washed twice with sterile distilled water, heat-killed at 100°C, lyophilised and suspended in PBS.

Treatment protocols

Each experiment included the following groups (*n* 6). Controls were sensitised and the allergen-challenged mice were subcutaneously immunised twice at 1-week intervals with a mixture containing 40 μg of Der p in incomplete Freund's adjuvant (Difco Laboratories) and subsequently challenged intra-tracheally with 50 μl of Der p (0·5 mg/ml) 7 d apart after the last immunisation. Treatment groups received oral administration of high (4×10^6 CFU), middle (2×10^6 CFU) or low dose (1×10^6 CFU) of *L. gasseri* in 700 μl PBS daily via a gavage needle, started from 14 d before the first immunisation with mite extract (day 0) and continued until the day of allergen challenge (day 14). Non-treated sensitised and challenged mice received PBS instead. Der p-sensitised, saline-challenged mice served as control animals. Mice were subjected to measurements of airway responsiveness followed by bronchoalveolar lavage (BAL).

Measurement of airway hyper-responsiveness and bronchoalveolar lavage examination

The airway resistance of mice was measured by a single-chamber, whole-body plethysmograph (Buxco Electronics, Inc., Troy, NY, USA) as described in the manual provided. Airway resistance was expressed as Penh (enhanced pause).

Increasing doses of methacholine were administered by nebulisation for 3 min, and Penh was measured over the subsequent 3 min. Mice were killed with an overdose of sodium pentobarbitone. The trachea was exposed, cannulated, and BAL was performed with two 1-ml aliquots of saline. A total of 1·8–1·9 ml of BAL fluid was consistently recovered by this technique. The total cells in cytocentrifuge preparations of BAL fluid were stained with Liu's stain for differential cell counts.

Lung histochemistry

The lungs of mice were first inflated with 0·5 ml of formalin (Sigma-Aldrich) via trachea, then excised and fixed in 4% buffered formalin overnight at 4°C. Tissues were embedded in paraffin, cut into 5-μm sections and stained with haematoxylin and eosin. Inflammatory infiltrates and lung architecture were assessed using light microscopy.

Total IgE and *Dermatophoides pteronyssinus*-specific IgG1 and IgG2a/2b antibodies

Serum samples were collected from the retro-orbital venous plexus 3 d after the intra-tracheally allergen challenge. The total IgE, Der p-specific IgG1 and IgG2a/2b serum antibody titres were determined by ELISA, with the required reagents and monoclonal antibodies purchased from BD PharMingen, San Jose, CA, USA.

Splenocytes culture

The spleens were removed from three mice for each experiment, and the pooled splenocytes (10^6 cells) were incubated with transforming growth factor (TGF)-β (2 ng/ml) plus IL-6 (20 ng/ml) at 37°C for 72 h in 1 ml of Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS), 10 μM-2-mercaptoethanol, 10 mM-HEPES, penicillin and streptomycin. Various doses of Der p extract (1 μg/ml or 10 μg/ml) and heat-killed *L. gasseri* (10^7 bacteria, multiplicity of infection = 10 or 10^6 bacteria, multiplicity of infection = 1) were added to the culture according to experimental condition. Cell cultures were set up in duplicate or triplicate in six-well flat-bottom polystyrene microtitre plates. A culture without the addition of TGF-β plus IL-6 or heat-killed bacteria and Der p was included as a control. Culture supernatants were harvested and stored at −80°C until assayed for cytokines, as described next.

Assay for cytokines

The amounts of thymus and activation-regulated chemokine (TARC), IL-10, IL-12, IL-17A, IL-23 and TNF-α in the BAL fluid supernatants and IL-17F in culture supernatants were determined by ELISA kits (R&D Systems, Minneapolis, MN, USA). The minimum detectable doses were 2, 7, 4, 1·5, 5 and 2 pg/ml, respectively.

Isolation of alveolar macrophages from lung

The lung tissues of mice were digested with collagenase and hyaluronidase and minced. After lysis of erythrocytes, the dissociated cells were underlaid with 5 ml of lymphocyte separation solution (Mediatech, Herndon, VA, USA) and centrifuged at 2200 rpm for 20 min. The mononuclear cells in the middle layer were collected for flow cytometric analysis. The neutrophils were collected from the bottom of the tube. For lung macrophage isolation, the aforementioned cells were incubated in a six-well plate for 2 h and the adherent cells were harvested as lung macrophages.

Flow cytometric analysis

The BAL fluids cells from mice were incubated with 1 μg/10^6 cells of Fc receptor-blocking antibody (Ab) (clone 24G2; American Type Culture Collection, Manassas, VA, USA), and then stained with phycoerythrin (PE)-Cy3-conjugated anti-mouse CD3, allophycocyanin-cy5-conjugated anti-mouse CD11b, PE-conjugated anti-mouse DX5 (pan-NK cells), allophycocyanin-conjugated anti-mouse c-Kit and PE-conjugated anti-mouse F4/80 Abs. After cell-surface staining, cells were washed, fixed and permeabilised with Fix-Perm solution for intracellular staining with fluorescein isothiocyanate-conjugated anti-mouse IL-17 Ab (clone eBio17B7, eBioscience, San Diego, CA, USA). All fluorophore-conjugated Abs and the corresponding isotypes were purchased from eBioscience. The stained cells were used for flow cytometric analysis (BD LSR II).

Statistical analysis

Mediator contents in culture supernatants and antibody production values were analysed by one-way ANOVA, followed by a *post hoc* comparison between groups. The cellular accumulation and mediator contents in BALF and serum Ab titre were analysed by using the Mann–Whitney U test. Results are expressed as means with their standard errors. A P value < 0·05 was considered significant.

Results

Lactobacillus gasseri suppresses Th2 and Th17 cytokine levels in bronchoalveolar lavage fluids

After the mite-allergen challenge, the levels of TARC, TNF-α, IL-23 and IL-17A were significantly increased in BAL fluid in mite-sensitised mice as compared to saline-challenged mice (Fig. 3). The levels of IL-12 and IL-10 were not significantly changed, compared with saline controls. Oral administration of high dose of *L. gasseri* in Der p-sensitised and -challenged mice significantly attenuated the increase in TARC, TNF-α and IL-17A but did not affect IL-23 levels. In contrast, the levels of IL-12 and IL-10 were significantly elevated when compared with non-treated, sensitised and challenged mice. Although middle- and low-dose *L. gasseri*-treated mice also had significantly lower levels of TARC and higher level of IL-12 (middle dose) in BAL fluids, the levels of other cytokines were not significantly altered (Fig. 3).

The change of IgE, IgG1 and IgG2a antibodies in the sera and the cytokines production from splenocytes

To determine whether the attenuated allergen-induced Th2 and Th17 cytokine response was an entirely local effect or a result of systemic immunological changes due to *L. gasseri* ingestion, we measured the changes in serum antibody concentration and cytokine production from culture supernatants of mite-allergen-stimulated splenocytes. While mite-allergen-sensitised mice produced a large amount of total IgE and Der p-specific IgG1 antibodies, compared to naïve mice, oral administration of *L. gasseri* did not significantly change these two antibodies, compared to Der p-sensitised mice, except that there was a significant increase in concentration of Der p-specific IgG2a antibody in the serum of high-dose *L. gasseri*-treated mice (Fig. 4(a)). There was also no significant change of Th1 (interferon-γ) and Th2 (IL-4) cytokines production in the culture supernatant of mite-allergen-stimulated splenocytes from *L. gasseri*-treated mice as compared to non-treated sensitised mice. In contrast, The IL-10 cytokine production was significantly increased in both high- and middle-dose-treated mice than in non-treated sensitised mice ($P<0.05$, Fig. 4(b)).

Oral administration of *Lactobacillus gasseri* suppresses IL-17 production in murine splenocytes

To know the regulatory effects of *L. gasseri* on Th17 immune response in mite-allergen-sensitised and -challenged mice, we first examined its effect on IL-17 production in cultured splenocytes. The addition of TGF-β plus IL-6 drastically enhanced IL-17 production in Der p-sensitised mice as compared to naive mice (990·6 (SEM 128·9) *v.* 467·7 (SEM 72·3) pg/ml, $P<0.05$). Addition of Der p-allergen (10 μg/ml) into the culture condition significantly increased IL-17 production from splenocytes of Der p-sensitised mice (1594·7 (SEM 164·5) *v.* 990·6 (SEM 128·9) pg/ml, $P<0.05$), but not
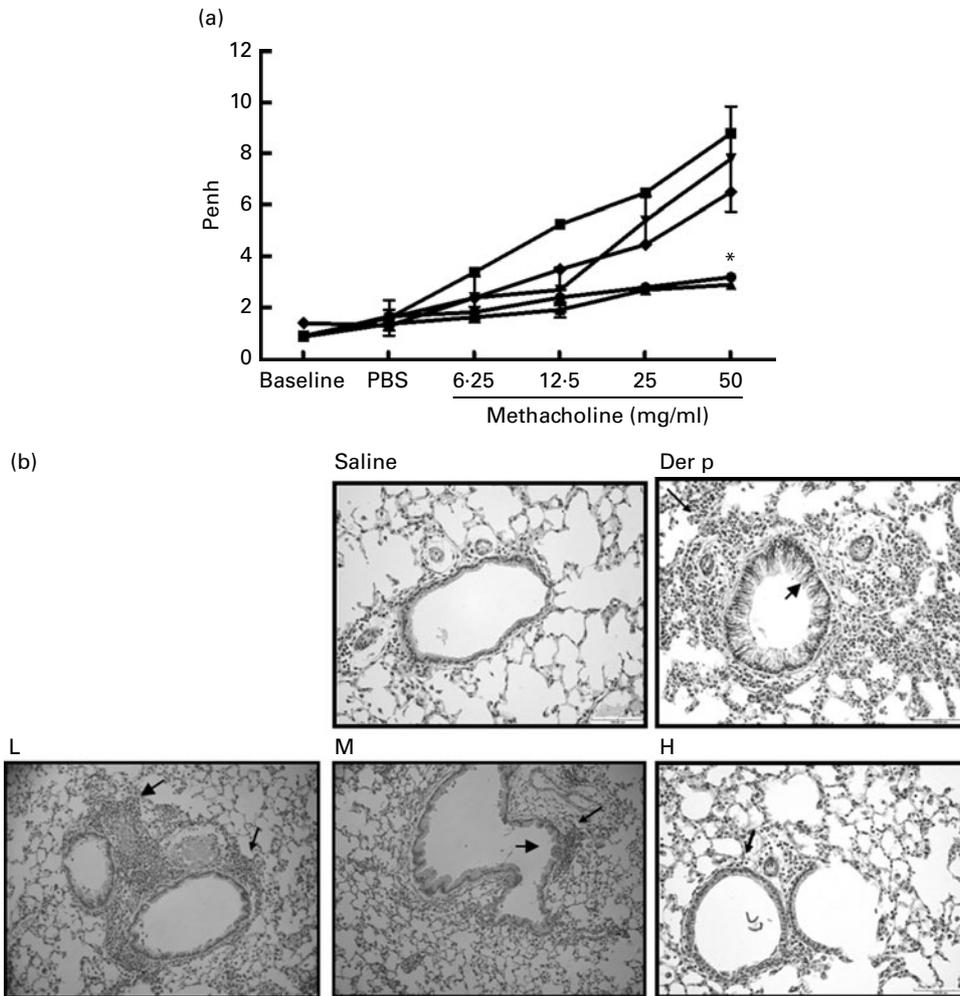
Fig. 1. (a) The airway hyper-responsiveness (AHR) in *Dermatophoides pteronyssinus* (Der p, —■—)-sensitised mice treated with different doses of *Lactobacillus gasseri*. Measurements of AHR were using whole-body plethysmography in unrestrained mice after airway exposure to increased concentrations of methacholine. Values are means with their standard errors represented by vertical bars (*n* 6). * Mean values were significantly different from those of the Der p group ($P<0.05$). —▲—, Saline. (b) Histopathologic examination of representative lungs taken from mice treated with different doses (low (L, —▼—), 1×10^6 colony-forming units (CFU); medium (M, —◆—), 2×10^6 CFU; high (H, —●—), 4×10^6 CFU) of *L. gasseri*. Mice were killed 3 d after Der p challenge. Lung sections were prepared and stained with haematoxylin and eosin (400 × magnification). Arrows indicate inflammatory cells infiltrated in the parenchyma and arrowheads represent damaged epithelial cells in the bronchi.

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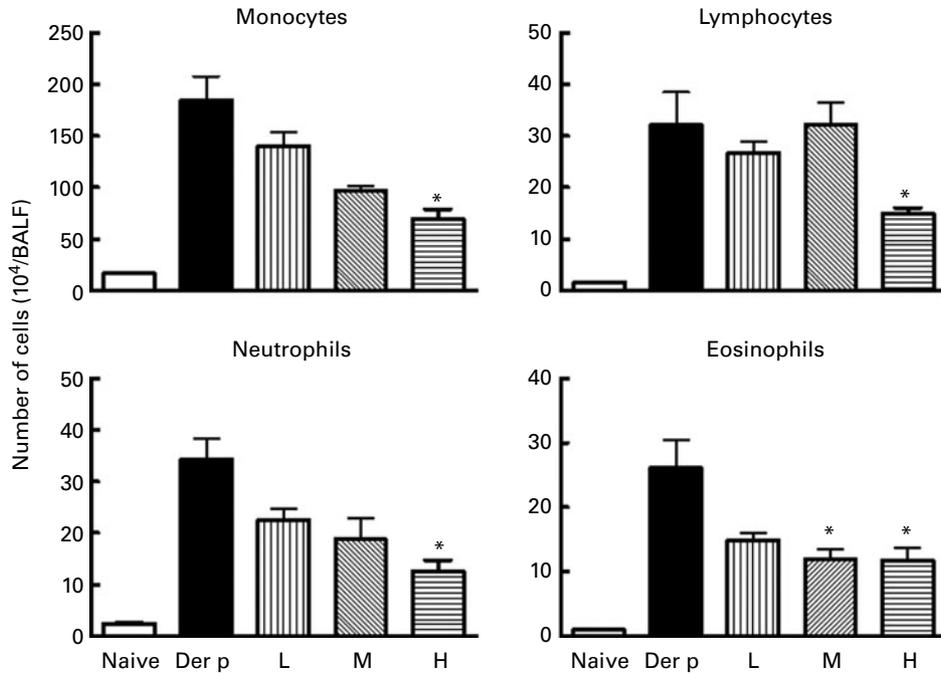
Fig. 2. Differential cell counts of bronchoalveolar lavage fluid (BALF) from different doses (low (L), 1×10^7 colony-forming units (CFU); medium (M), 1×10^8 CFU; high (H), 1×10^9 CFU) of *Lactobacillus gasseri*-treated and non-treated *Dermatophoides pteronyssinus* (Der p)-sensitised and -challenged mice. The cells in BALF were collected 3 d after intra-tracheal challenge of 50 μl (1 mg/ml) Der p. Values are means with their standard errors represented by vertical bars (*n* 6). * Mean values were significantly different from those of the Der p group ($P < 0.05$).
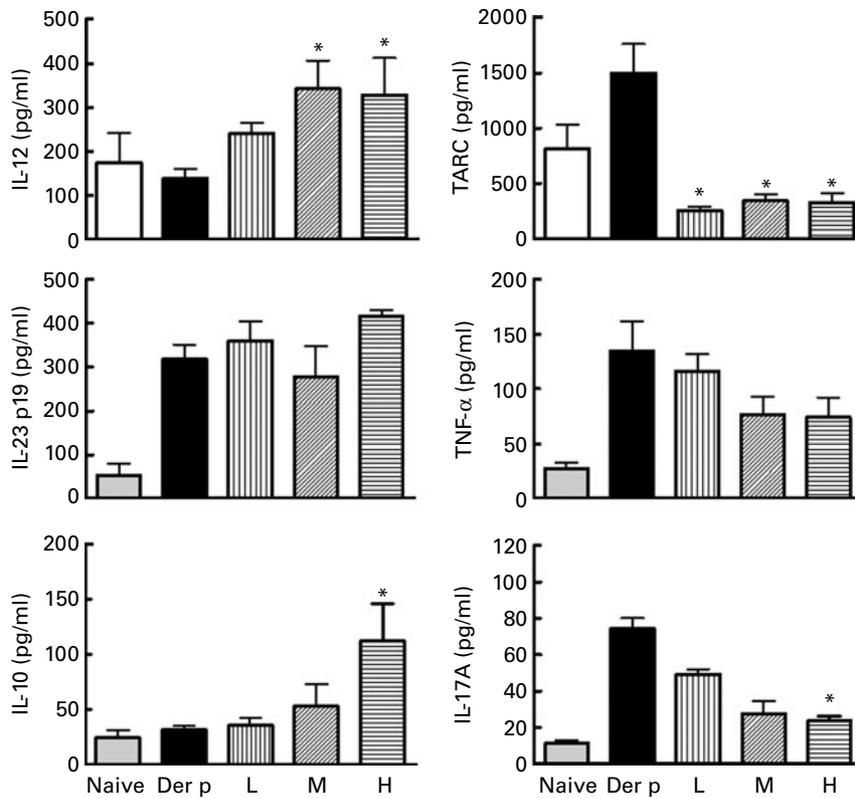
Fig. 3. Cytokines production in bronchoalveolar lavage fluid (BALF) of naive and different doses of *Lactobacillus gasseri*-treated and non-treated *Dermatophoides pteronyssinus* (Der p)-sensitised and -challenged mice. BALF was collected 3 d after Der p challenge and cytokine levels were measured using the respective ELISA kits. Values are means with their standard errors represented by vertical bars (*n* 6). * Mean values were significantly different from those of the Der p group ($P < 0.05$). TARC, thymus and activation-regulated chemokine; L, low; M, medium; H, high.
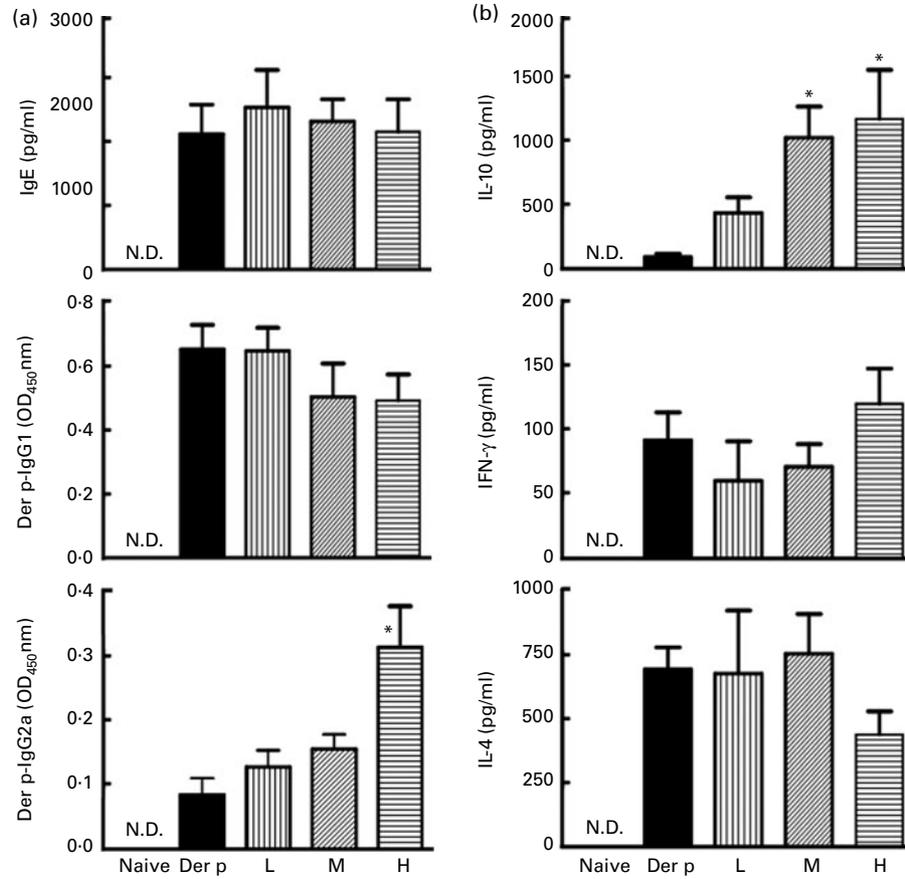
Fig. 4. (a) Total IgE, *Dermatophoides pteronyssinus* (Der p)-IgG1 and Der p-IgG2a in sera from naive mice and Der p-sensitised mice treated with different doses of *Lactobacillus gasseri*. Sera from all groups were collected 3 d after allergen challenge and IgE and IgG concentrations were detected by ELISA. Values are means with their standard errors represented by vertical bars (*n* 6). *Mean values were significantly different from those of the Der p group (*P*<0·05). (b) Inter-feron-γ (IFN), IL-4 and IL-10 productions from Der p-stimulated splenocytes of mice treated with different doses of *L. gasseri*. Mice splenocytes were collected 3 d after Der p challenge and incubated with 10 μg/ml Der p for 48 h. Culture supernatants were assayed by ELISA. Values are means with their standard errors represented by vertical bars (*n* 6). *Mean values were significantly different from those of the Der p group (*P*<0·05). L, low; M, medium; H, high; N.D., not detectable.

from naive mice. When heat-killed *L. gasseri* was added to the culture condition, there was a significant reduction of IL-17 production in both Der p-stimulated and Der p-unstimulated splenocytes (Fig. 5(a)). The suppressive effect of *L. gasseri* on IL-17 production was also found in the *L. gasseri*-treated, mite-sensitised and -challenged mice. Splenocytes collected from the high- and middle-dose *L. gasseri*-treated mice had significantly lower production of IL-17 when stimulated with Der p as well as combined stimulations with heat-killed *L. gasseri* (Fig. 5(b)).

Lactobacillus gasseri decreased IL-17-producing alveolar macrophages in allergic lung inflammation

Previously, it has been suggested that macrophages rather than Th17 cells are the main producers of IL-17 in allergic inflammation related to asthma[25]. Therefore, it is imperative to know whether the ability of *L. gasseri* to decrease IL-17 cytokine production in BAL fluid in mite-sensitised and -challenged mice was related to the change of IL-17-producing alveolar macrophages (AM). Fig. 6(a) shows the levels of cytokine production of stimulated and non-stimulated AM collected from mite-sensitised and -challenged mice.

Der p-allergen-stimulated AM produced increasing amounts of IL-17 and IL-23p19 and decreased levels of IL-10 than non-stimulated AM did. In contrast, heat-killed *L. gasseri* (multiplicity of infection 10) significantly increased production of IL-12 and decreased amounts of IL-17 and IL-23p19 as compared to Der p-stimulated AM. Moreover, we found a significantly increased percentage of IL-17-producing AM, which showed CD11b$^+$/IL-17A$^+$ in cytometry analysis (Fig. 6(b)), in BAL fluid in mite-sensitised and -challenged mice as compared to naive mice (Fig. 6(c)). In contrast, oral administration of high dose of *L. gasseri* significantly decreased the percentage of IL-17-producing AM in BAL fluid as compared to non-treated sensitised and challenged mice (Fig. 6(c)).

Discussion

In the present study, we hypothesised that *L. gasseri* is able to attenuate allergen-induced airway inflammation as well as decrease Th17 pro-inflammatory response in the murine model of asthma, which may explain the beneficial response of probiotics supplement in the prevention of allergic diseases and asthma. Accumulating data suggest that IL-17 or Th17 plays an important role in the development of various allergic

(a)

(b)

Fig. 5. (a) Effects of *Lactobacillus gasseri* on IL-17A production in murine splenocytes. Spleens were removed from naive (□) and *Dermatophoides pteronyssinus* (Der p, ■)-sensitised and -challenged mice (*n* 6), and the pooled splenocytes (1 × 10⁶ cells) were incubated with transforming growth factor (TGF)-β (2 ng/ml) plus IL-6 (20 ng/ml). Different doses of Der p and heat-killed *L. gasseri* were added, respectively, or combined into culture conditions. Culture supernatants (*n* 3) were harvested, pooled and assayed for IL-17A concentrations using an ELISA kit. * Mean values were significantly different from those of the conditional medium without Der p or *L. gasseri* (*P* < 0·05). † Mean values were significantly different from those of the conditional medium without Der p or *L. gasseri* (P < 0·05). ‡ Mean values were significantly different from those of the conditional medium with Der p alone (*P* < 0·05). (b) Effects of *L. gasseri* on IL-17A production in murine splenocytes removed from naive and different doses (low (L), ▦; medium (m), ▨; high (h), ▤) of *L. gasseri*-treated or non-treated Der p-sensitised and -challenged mice (*n* 6). * Mean values were significantly different from those of the Der p group (*P* < 0·05). MOI, multiplicity of infection.

diseases that have classically been considered to be Th1- or Th2-mediated disorders[26]. Although it was speculated that IL-17 may have a negative effect on the established allergic asthma[27], IL-17/IL-17R signalling has been proved to be a critical requirement for the development of allergic asthma[28]. The concentration of IL-17 was significantly increased in BAL fluids, sputum and blood from patients with asthma[29,30]. In a mouse model of allergic airway inflammation, systemic blockade of IL-17 inhibits the allergen-induced accumulation of neutrophils in the airway[31]. These findings suggest that IL-17 plays an important role in the pathophysiological process of allergic asthma. Despite the important role of IL-17 in allergic asthma, the development and function of CD4⁺Th17 cells and its relationship with

Th2 cytokine response in allergic asthma have not yet been fully identified, hindering the further elucidation of the mechanisms underlying the onset of allergic asthma[32].

Our data demonstrated that oral administration of *L. gasseri* is able to alleviate allergen-induced airway inflammation in mite-sensitised and -challenged mice. The *L. gasseri*-treated mice resulted in significant attenuation of inflammatory cell influx to the lung and airway responsiveness to methacholine (Fig. 1). The decreases in cellular influx and airway hyper-responsiveness were associated with reduced levels of inflammatory, Th2 chemokine (TNF-α, TARC) and Th17 cytokine (IL-17A), as well as increased production of Th1 (IL-12) and inducible regulatory T cell response (IL-10) in BAL fluids (Figs. 2 and 3). These findings are similar to recent studies showing that LAB decrease the Th2 type CD4⁺T cell responses to sensitised allergen in a mouse model of asthma[33–35]. However, the immunomodulation mechanism or the immunological target cells of LAB for the beneficial effect on allergic disease model animals is apparently not confined to the simple notion of the restoration of the Th1/Th2 balance. Particularly, our study of oral administration of *L. gasseri* did not result in a decrease of serum total IgE and IL-4 levels or an increase of interferon-γ production from Der p-stimulated splenocytes collected from *L. gasseri*-treated mice (Fig. 4).

Previous studies[36,37] have shown strong immunomodulatory properties of lactobacilli, which include anti-inflammatory and anti-tumour activity, modulation of autoimmune diseases and prevention of infections. LAB was also demonstrated in the treatment of inflammatory disorders like pouchitis[38], ulcerative colitis[39] and experimental arthritis in mice[40], which are hallmarks of Th17-mediated inflammatory diseases. Our data have clearly demonstrated that live *L. gasseri* suppressed IL-17 production both in TGF-β and in IL-6-stimulated splenocytes collected from Der p-sensitised mice (Fig. 5) and decreased IL-17-producing AM in the lungs of treated mice (Fig. 6). Th17 cells are generated from naive CD4⁺T cells, and Th17 cell differentiation is driven by stimuli such as TGF-β plus IL-6[26]. Indeed, allergen-specific stimulation alone was not enough to induce splenocytes from allergen-sensitised mice to produce IL-17 cytokine (data not shown). In contrast, TGF-β plus IL-6 combined with Der p-allergen induced a remarkable enhancement of IL-17 production in Der p-sensitised murine splenocytes (Fig. 5), representing the ongoing systemic Th17 immune response that resulted in allergen-induced airway inflammation in sensitised and challenged mice.

In general, Th17 cells are abundant at steady state in gut-associated tissues, particularly in the small-intestinal lamina propria. In the absence of a luminal commensal microbiota, the numbers of Th17 cells are significantly reduced[41]. Recently, it has been found that IL-17, a cytokine required for the initiation of allergic inflammation in a mouse model mimicking allergic asthma, was mainly produced by CD11b⁺AM, rather than Th17, natural killer (NK) cells or others[25]. Our present study also confirmed that there was a significant increase of production of IL-17 as well as cell number of CD11b⁺/IL-17⁺AM (Fig. 6), but not CD4⁺Th17 cells (data not shown), in the BAL fluids of sensitised and challenged mice.

Fig. 6. (a) Cytokine production in *Dermatophoides pteronyssinus* (Der p) or *Lactobacillus gasseri* (L.g.)-stimulated alveolar macrophages (AM). The AM were isolated and pooled from Der p-sensitised and -challenged mice (*n* 6), and analysed as described in the Materials and methods section. Mononuclear cells from bronchoalveolar lavage fluid were stained for the analysis of CD3, CD11b, F4/80 and IL-17. The CD3⁻ cells were gated. Among them, the CD11b⁺IL-17⁺cells were further gated for the analysis of F4/80. The result of (b) flow cytometric analysis and the (c) percentage of IL-17A-producing macrophages in AM of different doses of L.g.-treated or non-treated mice were shown. * Mean values were significantly different from those of the Der p group ($P < 0.05$). MOI, multiplicity of infection.

The decrease of IL-17-producing AM in *L. gasseri*-treated mice was also accompanied with the increase of IL-10 production in the culture supernatant of stimulated AM, which is suggested to be a negative regulator for IL-17-producing AM[25]. Nevertheless, there was also a possibility for *L. gasseri* to interact with antigen-presenting cells in the intestine, then regulating the development of natural regulatory CD4⁺Foxp3⁺T cells that may limit the expansion of Th17 cells[42,43].

Finally, it should be noted that regulation of IL-17 or Th17 immune response by LAB, particularly in allergic inflammation, is complicated by the involvement of many different immune cells and mediators. One study showed that ATP produced by commensal bacteria can drive the differentiation of Th17 cells[44]. In the large intestine, commensal-dependent expression of IL-25 by intestinal epithelial cells limits the expansion of Th17 cells[45]. Another investigation found that mice deficient in toll-like receptor 9, a receptor that can interact with commensal DNA, have reduced numbers of IL-17-producing and interferon-γ-producing effector T cells[46]. Many mechanisms of the inhibitory effect of probiotics for IL-17 cytokine response still remain to be discovered.

In conclusion, we have found that oral administration of *L. gasseri* in Der p-allergen-sensitised and -challenged mice resulted in significant attenuation of major characteristics of an asthmatic response, including airway inflammatory cell influx, local cytokine responses and airway hyper-reactivity. Our results also indicate that the immune-regulatory effects of oral treatments with *L. gasseri* are confined not only to the restoration of Th1/Th2 immune balance, but also to the inhibitory effects of systemic IL-17 response and local IL-17-producing AM. However, further studies are required to explore the mechanisms of anti-allergic effects of specific strains of LAB and to identify specific components of bacteria–host interaction that confer potential beneficial immune response, which may aid in the design or therapeutic use of clinically effective agents in the prevention of allergic asthma.

Acknowledgements

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