Prophylaxis of experimentally induced ovomucoid allergy in neonatal pigs using Lactococcus lactis

P. Rupa, J. Schmied, B.N. Wilkie

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada N1G 2W1

Abstract

Probiotic Lactococcus lactis (LL) is immunomodulatory and may prevent allergy by biasing from type-2 to a type-1 immune response. We hypothesized that newborn pigs pre-treated orally with LL are protected against allergy to ovomucoid (Ovm). Pigs were assigned to two treatment groups. Piglets were pretreated orally on days of age 1–7, 10, 12, 14, 21, 28 and 35 with LL (n = 30) or medium (control, n = 32) and sensitized to Ovm by intraperitoneal injection together with cholera toxin on days 14, 21 and 35. Pigs were orally challenged with egg white (day 46) and assigned scores for allergic signs. Outcomes were measured as direct skin tests, serum antibody to Ovm [IgG (H + L); IgE; IgG1 and IgG2] and cytokine production by mitogen-stimulated blood mononuclear cells (BMC). Clinical signs and skin test positivity were less frequent in the LL group (p ≤ 0.0001). Serum antibody associated with IgG (H and L), IgE, IgG1 or IgG2 was significantly increased on day 46 (post-sensitization) compared to day 14 (pre-sensitization) (p ≤ 0.0001). The LL-treated pigs had more IgE and IgG2-related antibody activity and lower IgG1/IgG2 and IgE/IgG2 ratios indicating a type-1 bias in immune response (p ≤ 0.05). Concentration of type-2 cytokines interleukin IL-4 and IL-10 were significantly lower in supernatants of stimulated BMC of LL-treated pigs (p ≤ 0.0001). Interferon-γ, TGF-β and IL-13 were not detected in control or treated animals. Thus, oral treatment of neonatal pigs with LL significantly reduced subsequent frequency of allergy to Ovm associated with reduced type-2 immune response correlates hence supporting the “hygiene hypothesis” and potential use of LL as a neonatal immunoregulator.

1. Introduction

Immunological dysregulation reflecting altered gene-environmental interactions underlies the current epidemic of allergy and autoimmunity (Romagnani, 2004) such that the innate and adaptive immune system (IS) interacting within environments conducive to allergy results in regulatory dysfunction leading to allergic disease. Allergy has increased in prevalence in developed societies, such that 4% of adults and 6–8% of children have food aller-

gies (Sampson, 2004). Epidemiological evidence supports the “hygiene hypothesis” which states that normally protection from allergy is due to microbially induced immunological bias away from the allergy-conducive type-2 immune response (IR) towards the non-conducive type-1 IR phenotype as normal flora colonizes surface epithelia (Garn and Renz, 2007). Therefore, the current epidemic of human allergy may be due to failure of modern environments to provide evolutionarily programmed microbial or parasite-derived signals necessary for balanced ontogeny of immune regulation. This has motivated investigation of methods to direct the IR away from predisposition to allergy. Since type-1 IRs do not support allergy and down-regulate type-2 IRs, it would be beneficial to alter bias in
the developing IS from type-2 towards type-1 IR (Akdís and Akdís, 2009).

Allergen-non-specific treatments may control allergic predisposition and clinical allergy generally, regardless of the inciting allergen (Akdís and Akdís, 2009). Such treatments may include exposing neonates to safe microbial stimuli in the form of bacterial cells, live probiotics or other microbial derivatives. Treatment of neonatal pigs by intramuscular injection of heat-killed *Escherichia coli* prior to experimental allergic sensitization, altered IR bias towards type-1 and significantly reduced clinical signs of allergy to Ovm (Rupa et al., 2009a,b). Inclusion of the type-1 cytokine recombinant porcine interferon gamma (rIFN-γ) with the *E. coli* treatment had no additional beneficial effect. This confirms that in the neonatal period a balanced type-1/type-2 IR can be induced by simple treatment with killed whole bacteria. Given these results it was decided to investigate the immunoregulatory and anti-allergic effects of neonatal oral exposure to *Lactococcus lactis*, a potentially practical and physiological approach to favourably altering IR bias of neonates.

The potential of probiotics to prevent allergy has been demonstrated by the administration of *Lactobacillus rhamnosus* GG to neonates, in which *L. rhamnosus* GG caused a switch from a type-2 biased IR in allergic patients towards a balanced type-1/type-2 IR, leading to amelioration of allergy (Furrrie, 2005). *L. rhamnosus* GG and *Bifidobacterium lactis* Bb12 downregulated atopic eczema in children with food allergies (Isolauri et al., 2000).

*Lactis* (LL) is a “generally regarded as safe” bacterium commonly found in fermented dairy products and is a candidate food-grade delivery vector of therapeutic proteins commonly found in fermented dairy products and is a candidate treatment for human inflammatory bowel disease and Crohn’s disease (Steidler et al., 2003). *Lactis* expressing intergenic leader (rPLF) with the *E. coli* treatment had no additional beneficial effect. This confirms that in the neonatal period a balanced type-1/type-2 IR can be induced by simple treatment with killed whole bacteria. Given these results it was decided to investigate the immunoregulatory and anti-allergic effects of neonatal oral exposure to *Lactococcus lactis*, a potentially practical and physiological approach to favourably altering IR bias of neonates.

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*Lactis* (LL) is a “generally regarded as safe” bacterium commonly found in fermented dairy products and is a candidate food-grade delivery vector of therapeutic proteins to the intestinal mucosa (Nouaille et al., 2003). *Lactis* can produce and secrete functional proteins in the mammalian intestine without long-term colonization (Hannify et al., 2004; Bermúdez-Humarán et al., 2004), while effectively inducing mucosal immune responses to expressed and secreted proteins (Wells et al., 2004; Huibregtse et al., 2009; Frossard et al., 2007). *Lactis* expressing interleukin (IL-10), for which safety has been confirmed in pigs, is a candidate treatment for human inflammatory bowel disease and Crohn’s disease (Steidler et al., 2003). *L. lactis* and *Acinetobacter lwoffi* F78 are commonly isolated in cow barns, environments epidemiologically associated with low prevalence of allergy in children exposed to them (Debary et al., 2007). When given intranasally to mice, both of these bacteria were able to reduce allergic signs of experimental allergy and induced a type-1 polarization in dendritic cells as indicated by cytokine production by bacterial LPS-activated blood mononuclear cells (BMC) (Debary et al., 2007).

Porcine T-cells and cytokines induce type-1 and type-2 differentiated antibody responses in vitro as influenced by antigen-exposed dendritic cells (Raymond and Wilkie, 2005) and in vivo (Crawley et al., 2003), such that immunoglobulin isotypes reflect type-1 (IgG2) or type-2 (IgG1, IgE) bias in IR (Crawley and Wilkie, 2003). Relative antibody activity associated with IgG1, IgG2 and IgE was used here as a correlate of functional allergic status. Type-1 vs. type-2 IR bias was assessed in treatment groups as expression of allergy to Ovm (skin test [ST] and clinical signs), serum antibody to Ovm associated with IgG (H+L), IgG2, IgG1 and IgE immunoglobulin isotypes and type-1 (IFN-γ), type-2 (IL-4, IL-10 and IL-13) and transforming growth factor (TGF)-β cytokine production by mitogen-stimulated BMC.

2. Methods

2.1. Experimental design and animals

Animal use was approved by the University of Guelph Animal Care Committee under guidelines of the Canadian Council for Animal Care. Two groups of Yorkshire pigs consisting of three litters (*n* = at least 10/litter) per group (*n* = 32; medium control and *n* = 30; LL) were orally treated with 1 ml of 10⁸ cfu/ml bacterial suspension in 1 ml of honey for palatability, using a 3 ml syringe attached to a 5 cm polypropylene tube. Piglets were weaned at 28 days of age. Treatments were on each of 1–7, 10, 12, 14, 21, 28 and 35 days of age. Treatment groups received live LL or M17 medium alone (control) (Fig. 1). On days 14, 21 and 35 pigs were given intraperitoneal (IP) sensitizing injections of 100 μg of oovamucoid (Ovm, prepared here as previously described by Rupa et al., 2008) adjuvanted with 10 μg cholera toxin (CT; List Biologicals, CA, USA) dissolved in PBS. Pigs were fasted overnight on day 45–46 and challenged orally with egg white mixed in yoghurt (3:2, v/v) on day 46. Clinical signs of allergy were monitored and scored as previously described (Rupa et al., 2009a,b) and as described in Section 2.3. Blood was collected from the retro-orbital sinus on days 14 (pre-sensitization) and 45 (post-sensitization) for measurement of serum antibody activity and on day 45 for cytokine quantification from phytohemagglutinin (PHA-P; Sigma, Oakville, ON, CA) stimulated BMCs. Skin reactivity was tested by intradermal injection of 100 μg Ovm in 0.1 ml of phosphate-buffered saline (PBS; 0.01 M, pH 7.2) on days 14, 21 and 35 to monitor sensitization to Ovm. Day 45 skin test data were used to interpret treatment effects. As a negative control, PBS was similarly injected. In each case a single injection was made per pig. Injections were made in marked sites on the medial aspect of the thigh. Skin tests were examined by at least three observers and consensus reached regarding positivity (Rupa et al., 2008).

2.2. Bacterial strain and growth

*Lactis* MG1363 (donated by V. Monodero, IATA, Burjassot, Spain) was cultured in M17 broth medium (Difco) supplemented with 0.5% glucose and grown at 30 °C aerobically. Stock cultures of bacteria were diluted 200-fold in GM17 medium and incubated at 30 °C overnight. Within 16 h of culture, a saturation density of 10⁹ colony-forming units per ml was reached. The culture was aliquoted and stored at −80 °C in medium containing 25% glycerol at 10⁹ bacteria in 1 ml of medium.

2.3. Clinical scores

Pigs were challenged orally with egg white on day 46, observed for 2 h and clinical scores were assigned in...
overnight challenge were given orally with honey (Section 2.2). Each treatment group consisted of three litters with 10–12 pigs in each litter. Piglets were sensitized by intraperitoneal (IP) injection of 100 μg of ovomucoid (Ovm) and 10 μg of cholera toxin (CT) in a final volume of 200 μl of phosphate buffered saline (PBS). Controls were pre-treated with PBS. All piglets were fasted on day 45 and orally challenged on day 46 with 40 ml of a mixture of egg white and yoghurt (3:2, v/v) and scored for clinical signs. Blood was collected on days 14 and 45 of age to detect Ovm-specific antibody activity and on day 45 to measure mononuclear cell-derived cytokines.

![Experimental Design](image)

**Fig. 1.** Experimental Design. Six individual litters were used. Oral pre-treatments (double-headed arrows) with live Lactococcus lactis or medium (control) were given orally with honey (Section 2.2). Each treatment group consisted of three litters with 10–12 pigs in each litter. Piglets were sensitized by intraperitoneal injection of 100 μg of ovomucoid (Ovm) and 10 μg of cholera toxin (CT) in a final volume of 200 μl of phosphate buffered saline (PBS). Controls were pre-treated with PBS. All piglets were fasted on day 45 and orally challenged on day 46 with 40 ml of a mixture of egg white and yoghurt (3:2, v/v) and scored for clinical signs. Blood was collected on days 14 and 45 of age to detect Ovm-specific antibody activity and on day 45 to measure mononuclear cell-derived cytokines.

a blinded manner by four experienced observers. Scores were as follows: 0 = no signs; 0.5 = repeated sneezing; 1 = isolation and immobility; focal cutaneous reddening, emesis (single incident); 2 = partially confluent cutaneous reddening, repeated emesis, respiratory difficulty; 3 = confluent cutaneous reddening. Total scores for each animal were obtained by adding scores for individual signs.

### 2.4. Enzyme linked immunosorbent assay (ELISA)

To measure anti-Ovm serum antibody activity, ELISA assays used standard methods, for IgG (H + L), IgG1, IgG2 and IgE-related antibody. Ovm-specific porcine IgG (H and L chain specific) and IgE (H chain specific) antibodies were detected by ELISA as previously described (Rupa et al., 2009a,b). Antibody associated with IgG1 and IgG2 was detected using biotinylated murine monoclonal antibodies (Dr. K. Nielsen, ADRI, Nepean, Canada). In brief, polystyrene, flat-bottomed, Immulon 2HB, 96 well plates (Dynex Technologies Inc, VWR International, Mississauga ON) were coated with 100 μg/ml of Ovm in 0.05 M carbonate-bicarbonate coating buffer (pH 9.6). Plates were washed 3 times (ELX 405 automatic plate washer; Biotek Instruments, Winooski, VT, USA) with 200 μl per well of 0.05% Tween PBS (PBST, pH 7.4). Wells were blocked by adding 3% Tween in PBS and incubating 1 h at room temperature (RT). Washing was repeated and sera diluted at 1:100 in 0.05% PBST was added at 100 μl per well in triplicate. Controls included wells without sera and negative (pooled serum from pre-immunized piglets) and positive (pooled serum from post-immunized piglets) sera. Plates were incubated for 2 h at RT and washed. The following primary antibodies were used: alkaline phosphatase (Alk-phos)-conjugated rabbit anti-pig IgG (H and L chain specific, Sigma), rabbit anti-pig IgE (Rupa et al., 2009a,b), biotinylated mouse anti-IgG1 and IgG2. The anti-pig IgE was detected by Alk-phos- conjugated goat-anti rabbit IgG (H and L specific, Sigma). The anti- IgG1 and IgG2 were detected by streptavidin-Alk-phos (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). Plates were washed and chromogen (polynitrophenol-phosphate [pNPP], Sigma) in diethanolamine buffer, 0.1 M pH 9.8 was added and incubated in the dark at RT. Reaction product and background optical densities (OD) were quantified at 405 nm (96-well plate reader, EL808, Bio-Tek Instruments Inc.) when the OD of the positive control reached 1.0. Results were expressed as percentage of the positive control as follows: sample % OD of positive control = (sample OD / [positive control OD – negative control OD]) × 100. Sera (day 14 and day 45) from one litter were analyzed on the same day. Relative activities by isotype were expressed as simple ratios of respective percent positive control values.

### 2.5. Cytokine analysis

Blood was collected on day 45 to measure cytokine concentrations in supernatant of PHA-P-stimulated BMCs. Blood (3 ml) was diluted (1:1, v/v) with PBS (3 ml) and under-layered with an equal volume (6 ml) of Histopaque (Sigma); BMCs were isolated by centrifugation at 400 × g for 30 min based on the manufacturer's guidelines (Sigma) and suspended in complete Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum, L-glutamine (2 mM) and penicillin (100 U/ml)/streptomycin (100 μg/ml). Cells were cultured in 24 well plates in triplicate at 2.5 × 10^5 cells/ml as unstimulated (control) or
stimulated with 10 µg/well of PHA-P. Each well contained 1 ml of medium. Culture supernatants were collected after 96 h incubation at 37 °C and stored in aliquots at −20 °C until analyzed. Cytokine concentration was measured in supernatants using quantitative sandwich immunoassay porcine cytokine ELISA kits as outlined by the manufacturers (Invitrogen, [IL-4, IL-10, IFN-γ and TGF-β]; Kingfisher, St. Paul, MN, [IL-13]). Briefly, plates were coated with mouse monoclonal anti-porcine IL-4, IL-13, IL-10, IFN-γ and TGF-β overnight at 4 °C. Test samples from stimulated vs. unstimulated BMC and standards of known cytokine concentration were added to duplicate wells and plates were incubated at room temperature for 2 h. After washing, 50 µl of biotinylated anti-cytokine antibody was added to each well and incubated for 1 h. After washing, 100 µl of streptavidin-peroxidase was added. Following a final wash colour was developed with substrate solution (tetramethylbenzidine; Sigma) for 30 min at RT in the dark, stop solution (1.8 N sulphuric acid) was added, and OD was measured at 450 nm using a microplate reader. Optical density was directly proportional to the concentration of cytokine. Linear standard curves were plotted for concentrations between 0 and 1000 pg/ml with KC Junior software (Bio-Tek Instruments Inc., USA) and used to determine cytokine concentration in test samples. Data were expressed as sample means for test replicates.

2.6. Statistical analysis

For skin test and clinical score data Fisher’s exact test (GraphPad Instat, GraphPad, San Diego, CA, USA) was used to compare frequency by groups. Significance of difference between means for antibody activity and cytokine production were assessed using the unpaired Student’s t-test for normally distributed data. Welch’s correction was applied for non-normally distributed data as determined by the F-test. Data are reported as means plus/minus (±) the 95% confidence interval and p ≤ 0.05 was taken to indicate significance. All calculations were performed with GraphPad Prism software 4.0 (GraphPad).

3. Results

3.1. Reduction in frequency of positive direct skin tests

Positive skin reaction to Ovm on day 45 was observed in 30 of 32 pigs in the control and 15 of 30 pigs in the LL-treated group (p < 0.0001) (Table 1). There was no reaction at the PBS injection sites.

3.2. Reduction in clinical signs after oral challenge

Allergic signs were more frequent in the control group (13 of 32) than in the LL group (1 of 30) (p ≤ 0.0001) on day 46 (Fig. 2, Table 1). In addition, 3 of 30 pigs in the LL group expressed immediate onset of moderate cutaneous erythema on day 35 in response to the IP systemic injection of Ovm and CT (Fig. 1).

3.3. Immunoglobulin isotype-related bias reflects treatment effects

All Immunoglobulin isotype-related antibody activity for IgG1, IgG2, and IgE measured on day 45 (post-sensitization) was significantly increased from day 14 (pre-sensitization) (p ≤ 0.0001). Pre-treatment of neonatal pigs with LL, followed by IP sensitization with Ovm and CT, induced greater IgG2 (in pigs a type-2 isotype) antibody activity than in controls (p = 0.0119) (Fig. 3). There was more IgE-related antibody activity in pigs pre-treated with LL than in controls (p ≤ 0.001). Immunoglobulin IgG1 (in pigs a type-2 isotype) or IgG (H + L) antibody activity did not differ between the LL group and controls.

3.4. Immunoglobulin-isotype bias of antibody reflects reduced allergic phenotype

To compare antibody associated with IgG1 and IgG2, IgG1/IgG2 antibody ratios were calculated for day 45 (Fig. 4). It was hypothesized that control pigs would produce higher IgG1/IgG2 ratios (type-2 bias), and LL-treated pigs, lower IgG1/IgG2 ratios (type-1 bias). As hypothesized, the IgG1/IgG2 ratio of anti-Ovm antibody was lower in the LL-treated pigs than in controls (p = 0.0004). In LL-treated pigs, the IgE/IgG2 ratio was lower (p = 0.0245, type-1 bias) and the IgE/IgG was higher (p = 0.02), than in the control group (Figs. 3 and 4).

3.5. Cytokine expression by stimulated BMC correlates with treatment effects and response to challenge

The cytokines IL-13, IFN-γ and TGF-β were not detected in any of the samples measured in spite of the internal

Table 1
Clinical signs and skin test positivity.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Percentage of pigs expressing clinical signs of allergy (day 46)</th>
<th>Percentage of pigs expressing skin test positivity (day 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 32)</td>
<td>40</td>
<td>93.75</td>
</tr>
<tr>
<td>L. lactis (n = 30)</td>
<td>3</td>
<td>50</td>
</tr>
</tbody>
</table>

Percentage of pigs showing clinical signs post-ovomucoid challenge and skin test positivity on day 45 in the two different treatment groups. Treatment groups differed significantly in frequency of pigs expressing allergic signs and positive skin tests (p ≤ 0.05; Fisher’s exact test, GraphPad Instat).

Fig. 2. Clinical scores for individual pigs. Individual clinical scores for pigs that expressed clinical signs post-ovomucoid challenge on day 46 in the two groups. Treatment groups differed significantly (p ≤ 0.05; Fisher’s exact test, GraphPad Instat) in frequency of pigs expressing allergic signs.

Allergic signs were more frequent in the control group (13 of 32) than in the LL group (1 of 30) (p ≤ 0.0001) on day 46 (Fig. 2, Table 1). In addition, 3 of 30 pigs in the LL group expressed immediate onset of moderate cutaneous erythema on day 35 in response to the IP systemic injection of Ovm and CT (Fig. 1).

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The cytokines IL-13, IFN-γ and TGF-β were not detected in any of the samples measured in spite of the internal
positive standard controls being positive in the measurable range of 0–1000 pg/ml for all cytokines. In LL-treated pigs there was less IL-4 ($8.15 \pm 2.21$) and IL-10 ($6.58 \pm 0.06$) than in controls (IL-4, $27.98 \pm 43.37$; IL-10, $35.21 \pm 23.69$) (Fig. 5) ($p < 0.05$). This cytokine profile reflects reduction in type-2 response and may indicate a type-1 response bias. There were no detectable cytokines in the unstimulated control samples.

4. Discussion

Oral pre-treatment of neonatal pigs with probiotic bacteria LL significantly reduced frequency of experimental allergy to orally administered Ovm. Several correlates were investigated including skin test positivity, antibody and antibody related type-1 and type-2 Ig isotype bias and BMC cytokine production.

Skin test positivity was induced in 93.75% of controls and 50% of LL-treated pigs. These data suggest that reduction in frequency of positive skin tests reflects reduced ability to sensitize for and to manifest allergic signs. Paradoxically, the least reactive group, LL, had higher average IgE-related antibody to Ovm than controls. While IgE-related anti-Ovm antibody and skin test positivity may be prerequisite for manifestation of allergic signs it is not sufficient to ensure this. In our experience of over 100 pigs treated with the sensitization protocol, no skin test negative pig has expressed allergic signs after oral challenge (unpublished data). It might have been expected that reduced clinical signs and skin test positivity would
Antibody associated with type-1 (IgG, assumed at antibody activity was measured by enzyme-linked immunosorbent assay prior to sensitization (day 14), and post-sensitization (day 45) and serum-for the LL-treated group in comparison to the controls. Blood was collected the LL-treated group similarly confirms that bias to IgG2 addition, the LL-treated group had a lower IgE/IgG2 ratio induced in all pigs regardless of treatment group (Fig. 3). This suggests that bias to IgG2-associated, type-1 antibody correlates with protection against allergic signs (Fig. 4). In the LL-treated group compared with controls. However, had IL-12 been measured this may have indicated increase in the LL group as reported for LL-treated mice, concomitant with observed reduction in type-2 cytokines (Debarry et al., 2007). There is a growing body of evidence that suggests interactions between type-1 and type-2 immune elements are not solely antagonistic, but may in fact modulate the IR in a much more complex manner involving other regulatory mediators (Gor et al., 2003). Also, cytokines were measured here in mitogen-stimulated BMC since exposure to antigen failed to result in detectable cytokines (unreported results). Antigens are clonally specific in their interactions with T-cells and are compared to controls. Given that porcine IgG2 is a type-1 isotype these results may indicate treatment-induced type-1 IR bias, with related anti-allergic effects, consistent with previous reports of L. lactis treatment of mice increasing expression of type-1 IgG-related antibody (Debarry et al., 2007). Alternatively, IgG2 may itself mediate protection, as for instance by blocking IgE (Strait et al., 2006), although this remains to be tested for porcine IgG isotypes only two of which have been studied functionally (Crawley and Wilkie, 2003). This may explain the lack of allergic signs in LL-treated pigs in the face of high IgE-related antibody to Ovm.

The type-1 cytokine IFN-γ, type-2 cytokine IL-13 and the regulatory cytokine TGF-β were unexpectedly not detectable in pigs of either group in spite of high sensitivity against test standards in the assay (Section 2.4). While the IFN-γ and IL-13 assays are porcine specific, the TGF-β assay was anti-human and may not have been able to detect porcine TGF-β although claimed by the supplier (Invitrogen) to be cross-reactive with the pig cytokine. Similarly, an anti-human TGF-β (R&D Systems, MN, USA) that detected the bovine cytokine was not reactive with porcine TGF-β (unpublished results). For these assays the single time point used, 96 h, may have compromised our ability to detect, however, this time was chosen based on unpublished results in which cells from similar aged pigs housed in the same facility produced IFN-γ. Consistent with the proposed type-1 bias in the LL-treated group, the concentration of the type-2 cytokine IL-4 was lower in controls (Fig. 5). Similarly, in the LL-treated group IL-10 was less than in controls (Fig. 5). Both IL-4 and IL-10 appear to reflect type-2 cytokine behaviour and are lowest in the LL treatment group. However, had IL-12 been measured this may have indicated increase in the LL group as reported for LL-treated mice, concomitant with observed reduction in type-2 cytokines (Debarry et al., 2007). There is a growing body of evidence that suggests interactions between type-1 and type-2 immune elements are not solely antagonistic, but may in fact modulate the IR in a much more complex manner involving other regulatory mediators (Gor et al., 2003). Also, cytokines were measured here in mitogen-stimulated BMC since exposure to antigen failed to result in detectable cytokines (unreported results). Antigens are clonally specific in their interactions with T-cells and are
therefore less sensitive inducers of cytokines and other indicators of immune regulation.

While the mechanisms involved in allergy reduction are not known, we report that treatment of piglets with LL reduced Ovm-induced allergy in pigs corresponding with reduced frequency of skin test positivity, decreased BMC-derived IL-4 and IL-10 and increased expression of allergen-specific antibody associated with the type-1 isotype IgG₂. It has recently been reported that LL peptidoglycan (Pgn) matrices given intranasally strongly stimulate type-1 IRs in neonatal mice by a TLR2-dependent mechanism mediating both antigen-specific and innate defence against otherwise lethal infection with Yersinia pestis (Ramirez et al., 2010). The induction of type-1 IR by LL is reportedly mediated by Pgn and LPS as ligands for NOD2 and TLR4 (Debarry et al., 2007; Ramirez et al., 2010). Although pigs housed in conventional conditions are constantly exposed to various PAMPs, it is known that pigs housed indoors, with or without antibiotic treatment, have a less beneficial gut microflora than those housed outdoors (Mulder et al., 2010). This suggests oral treatment with LL in the neonatal period prior to sensitization is an allergen non-specific method for prophylaxis of food allergy that may simulate beneficial environmental effects under the “hygiene hypothesis” by inducing type-1 IR bias (Garn and Renz, 2007).

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References


