

Journal of Pediatric Gastroenterology & Nutrition:

February 1999 - Volume 28 - Issue 2 - pp 191-198

Original Articles

Intestinal Barrier Function and Cow's Milk Sensitization in Guinea Pigs Fed Milk or Fermented Milk

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Received May 13, 1998; revised August 24, 1998; accepted September 21, 1998.



Abstract

Background: The respective effect of milk and fermented milks on intestinal barrier capacity and on sensitization to β -lactoglobulin was studied using a guinea pig model of cow's milk allergy.

Methods: Guinea pigs were fed a control diet or the same diet supplemented with milk, fermented milk (*Streptococcus thermophilus* and *Bifidobacterium breve*), or dehydrated fermented milk. Intestinal barrier capacity to macromolecules was assessed in an Ussing chamber, and sensitization to cow's milk protein was measured by systemic anti- β -lactoglobulin immunoglobulin G1 titers and by intestinal anaphylaxis, the latter assessed by the β -lactoglobulin-induced increase in short-circuit current of jejunal fragments ($\Delta I_{sc\beta-LG}$).

Results: The electrical resistance of jejunum was similar in the four groups ($\sim 80 \Omega/\text{cm}^2$) suggesting the same paracellular permeability. The transport of ^{14}C - β -lactoglobulin from mucosa to serosa was significantly decreased in the animals fed dehydrated fermented milk ($403 \pm 131 \text{ ng/hr}\cdot\text{cm}^2$) compared with that in control animals or animals fed milk ($767 \pm 250 \text{ ng/hr}\cdot\text{cm}^2$ and $749 \pm 475 \text{ ng/hr}\cdot\text{cm}^2$, respectively; $p < 0.05$). Milk fermentation did not modify native β -lactoglobulin concentration but anti- β -lactoglobulin immunoglobulin G1 titers were higher in fermented milk and dehydrated fermented milk ($\log_{10}$ titer = 2.86 and 2.79, respectively) than in guinea pigs fed milk ($\log_{10}$ titer = 2.5; $p < 0.007$). However, β -lactoglobulin-induced intestinal anaphylaxis remained the same in the three groups ($\Delta I_{sc\beta-LG}$, $9.6 \pm 4.1 \mu\text{A}/\text{cm}^2$, $8.5 \pm 4.3 \mu\text{A}/\text{cm}^2$, and $8.5 \pm 3.4 \mu\text{A}/\text{cm}^2$ in milk-fed, fermented milk-fed, and dehydrated fermented milk-fed guinea pigs, respectively).

Conclusions: The intestinal barrier capacity to milk proteins seems to be reinforced by dehydrated fermented milk, but milk and fermented milks are equally efficient in inducing cow's milk allergy in guinea pigs.

The neonatal period is often associated with digestive diseases caused by the exposure of the gastrointestinal tract to considerable amounts of foreign antigens, including food antigens. The intestinal mucosa represents the first line of defense against the penetration of exogenous noxious antigens. Among the factors contributing to the development of the gut mucosal barrier, the intestinal microflora plays an important role. In the newborn, bacterial colonization is established gradually and greatly depends on the nutritional context—that is, breast feeding or formula feeding (1). Bifidobacteria constitute the dominant microflora in breast-fed infants (2), but the weaning period is associated with important changes in the gut flora (3,4). This microflora has been shown to play a major role in oral tolerance to food antigens. Abrogation of oral tolerance to ovalbumin induced by *E. Coli* LT toxin or cholera toxin in mice was rapidly restored in conventional but not in germ-free mice (5).

Some strains of lactic bacteria can survive and temporarily colonize the human gastrointestinal tract after ingestion (6). During infancy, the beneficial effect of lactic bacteria on gastrointestinal symptoms has often been demonstrated in clinical studies. In children with persistent diarrhea, a clinical advantage of feeding yogurt rather than milk has been suggested (7). In children with atopic eczema, the administration of an extensively hydrolyzed whey protein formula enriched with *Lactobacillus casei* GG promotes the reinforcement of the intestinal barrier and a decrease in the release of tumor necrosis factor- α in feces (8). In the same way, *Lactobacillus* GG promotes the rapid recovery of children with rotavirus diarrhea, possibly by increasing the mucosal defense afforded by specific secretory immunoglobulin (Ig) A (9).

Among the different factors affecting the intestinal function at weaning, allergy to cow's milk proteins plays an important role. The beneficial effect of lactic bacteria on gastrointestinal function, mainly in decreasing diarrheal symptoms, may rely on several possible mechanisms: more efficient lactose digestion from fermented milk than from milk (10), possible modification of cow's milk proteins during the fermentation process (11), stimulation of secretory immunity enforcing the intestinal barrier capacity (10,12), and a downregulating effect on immune response to food antigens (13).

To explore these possibilities experimentally, the relative propensity of milk and fermented milks to activate cow's milk protein sensitization was studied in a model of cow's milk allergy in guinea pigs (14). Two strains of lactic bacteria (*Streptococcus thermophilus* and *Bifidobacterium breve*) were used. Fresh and dehydrated fermented milks were prepared and fed during 2 weeks to guinea pigs, and intestinal barrier capacity and sensitization to cow's milk proteins, were quantified.

METHODS

Milk and Fermented Milks

We used commercially available ultra-high temperature whole milk (diet M) to feed one group of guinea pigs. The same batch of milk was used to prepare the fresh fermented milk (diet FM) used in this study. The latter was prepared by addition of equal volumes of milk fermented separately with *Streptococcus thermophilus* or with *Bifidobacterium breve*, and contained approximately 10^8 colony-forming units cfu/ml. Briefly, 5.10^9 cfu *Streptococcus thermophilus* (provided by Diepal-nsa, Steenvorde, France) was inoculated into 1 l of milk, enriched with 1 g/l

yeast extracts and incubated for 4 hours at 44°C. In a parallel procedure, 4.10^9 cfu *Bifidobacterium breve* was inoculated into 1 liter of milk, enriched with 1 g/l yeast extracts and 0.3 g/l cysteine chlorhydrate and incubated for 22 hours at 37°C in anaerobic conditions (Anaerocult A, Merck-Clevenot, France). The dehydrated fermented milk (diet DFM; Lactofidus, prepared by Diepal-nsa) was reconstituted in water as indicated. In the fresh fermented milks, the final concentration of *Streptococcus thermophilus* and *Bifidobacterium breve* were respectively 5.6×10^8 cfu/ml) and 4.17×10^8 cfu/ml. In the dehydrated fermented milk, the lactic bacteria were almost totally dead. The diets had the same lactose concentration (approximately 5 g/100 ml), except the control diet (C) which contained no lactose; pH was 6.4, 5.8, and 4.3 in M, DFM, and FM diets, respectively.

Animals and Experimental Design

Weanling male Dunkin-Hartley guinea pigs, (Charles River, Saint-Aubin les Elboeuf, France) weighing 210 to 250 g, were used. Because they had just been weaned, gut closure was achieved (15). The guinea pigs were divided into four groups. A control group (C, $n = 8$) was fed with a standard chow during the whole feeding protocol. The pellets contained no animal proteins, and we checked that they were β -lactoglobulin (β -LG) free. The other three groups received either milk (M, $n = 12$), fermented milk (FM, $n = 12$), or dehydrated fermented milk (DFM, $n = 8$), according to a protocol that will be described. During the first 5 days, 10^8 fresh bacteria in water were administered orally to FM and DFM guinea pigs. In addition to the standard pelleted chow given ad libitum. From days 6 to 14, the animals received 5 g standard chow in 20 ml milk or fermented milks or water (groups M, FM, DFM, and C, respectively) in the morning and twice that amount in the evening. From days 15 to 21, the animals received 10 g standard chow in 40 ml milk or fermented milks or water in the morning and in the evening.

Two days before death (days 22 and 23), milk proteins were excluded from the diet to keep the intestine free of allergic reactions before testing intestinal anaphylaxis. On day 24, guinea pigs were anesthetized with 1.5 ml/kg pentobarbital, the blood was collected by cardiac puncture and put into the heparinized tubes, and feces and intestinal mucosal scrapings were sampled and stored at -80°C. The intestinal fragments were mounted immediately in Ussing chambers.

β -Lactoglobulin Enzyme-Linked Immunosorbent Assay

β -Lactoglobulin concentration in milk, fermented milks, or in serosal compartments of Ussing chambers after β -LG transport was assessed by a two-site enzyme-linked immunosorbent assay (ELISA) provided by J. M. Wal (CEA, Saclay, France). Briefly, plates were coated with 150 μ l of a capture monoclonal antibody recognizing exclusively native β -LG. After overnight incubation at room temperature, plates were saturated for at least 12 hours with 300 μ l of a blocking solution containing 1% polyvinylpyrrolidone. Fifty μ l of diluted milk, fermented milks, Ussing chamber samples or β -LG standards (20 pg/ml to 20 ng/ml) as well as 50 μ l of a β -LG monoclonal antibody coupled to acetylcholinesterase (117-G4), was added to the well and incubated overnight at 4°C. After washing, 200 μ l Ellman reagent was added and plates were read after 30 minutes at 405 nm. The limit of detection of this test was 0.1 ng/ml.

Anti- β -Lactoglobulin Immunoglobulin G1 Titers in Plasma

Plasma samples were stored at -80°C until used. Anti- β -LG IgG1 titers were quantified by ELISA (16). Briefly, 96-well plates (Dynatech Immulon 2, Polylabo, France) were coated overnight with 100 μ l β -LG 0.1 mg/ml (Sigma, Saint-Quentin Fallavier, France) at 4°C, washed with phosphate-buffered saline-Tween 20, and further incubated with 1% gelatin (Prolabo, Fontenay sous Bois, France) for 1.5 hours at 37°C. Serial dilutions of plasma samples (1:32 to 1:2048) were incubated

for 1.5 hours at room temperature. After washing in PBS-Tween, monoclonal peroxidase-conjugated goat anti-guinea pig IgG1 (Tebu, Le Perray-en-Yvelines, France) was incubated for 1.5 hours at 37°C, and the plates were stained with orthophenylene diamine (Sigma) in citrate buffer 0.05 M (pH 5) containing 0.036% H₂O₂. The plates were read at 495 nm.

Intestinal Function

Part of the proximal intestine was removed, carefully rinsed with cold Ringer's solution to remove the intestinal content, opened along the mesenteric border, and immediately mounted in an Ussing chamber.

Intestinal Anaphylaxis

Eight jejunal segments mounted in an Ussing chamber (exposed area 0.5 cm²), were bathed on both sides with 10 ml Ringer's solution containing (in millimolar): 140 Na⁺, 5.2 K⁺, 120 Cl⁻, 25 HCO₃⁻, 1.2 Ca²⁺, 2.4 HPO₄²⁻, 1.2 Mg²⁺, and 2 glutamine. Segments were then thermostated, oxygenated, and maintained at pH 7.4 and in atmosphere containing 5% CO₂ and 95% O₂. The mucosal and serosal bathing solutions were connected by agar bridges to calomel electrodes for measurement of transmural potential difference (PD) and to Ag-AgCl electrodes for current application. The tissues were kept under short-circuit conditions by an automatic clamping device (World Precision Instruments, New Haven, CT, U.S.A.) that canceled out fluid resistance. The short-circuit current was constantly recorded, and the tissue was pulsed at 1.0 mV every 30 seconds. The increase in short-circuit current (I_{sc}) deflection was used to calculate the electrical resistance and ionic conductance (G) according to Ohm's law. The local intestinal anaphylaxis was measured as a maximum increase in I_{sc} in response to 100 µg/ml β-LG added to the basal compartment.

Intestinal Transport of a Bystander Protein and a Sensitizing Protein

The transport of a bystander protein horseradish peroxidase (HRP type VI; molecular weight [MW], 40,000) and of the sensitizing protein β-LG (isoforms A+B, MW, 36,000; both from Sigma) was assessed in jejunal fragments mounted in a small Ussing chamber (exposed area, 0.15 cm²), bathed on both side with 1.5 ml Ringer's solution, and maintained under short-circuit conditions. Electrical parameters, including PD, I_{sc}, G, were recorded at regular intervals during a 2-hour period of experimentation. After equilibration, macromolecular markers were added to the apical compartments. Two fragments of tissue were used for HRP transport and two others for β-LG transport. Horseradish peroxidase was added at the final concentration of 0.4 mg/ml, with a tracer dose of ³H-HRP (37 kBq/ml) (17). β-Lactoglobulin was added at a final concentration of 0.4 mg/ml, including 31 kBq ¹⁴C-β-LG, prepared according to Jentoft and Dearborn (18). The transport was monitored by sampling 800 µl of the serosal compartment at 30-minute interval for 130 minutes. Radioactivity was counted on 500-µl samples by liquid scintillation photometry. The rate of intact HRP transport was determined by its enzymatic assay on 200 µl aliquots, according to Maehly and Chance (19) and the rate of intact β-LG transport was assessed by ELISA as described earlier. ³H-Equivalent HRP fluxes were calculated using ³H counts that constituted the total amount of HRP transported in either intact or degraded form. Horseradish peroxidase fluxes were expressed in nanograms per hour per square centimeter. ¹⁴C-Equivalent β-LG fluxes, which constituted the total amount of β-LG transported in either intact or degraded form, were calculated using ¹⁴C counts. Flux values are reported at steady state (i.e., as the mean values during the second hour after apical addition of tracers).

Absorptive and Secretory Intestinal Functions

To analyze further a possible effect of feeding fermented milks to guinea pigs on the overall intestinal function, we tested in the four experimental groups the Na-glucose cotransport and the chloride secretion stimulated with different agonists. Histamine and carbachol were used as Ca^{2+} -dependent secretagogues, and forskolin as a cyclic adenosine monophosphate-dependent secretagogue. Glucose absorption was measured by the increase in I_{sc} after mucosal addition of 10 mM glucose, and chloride secretion was measured as the increase in I_{sc} after serosal addition of 10^{-4} M histamine or carbachol or 10^{-5} M forskolin.

Statistical Analysis

Results are expressed as means $\pm$ standard deviation, with n = number of animals. Data were analyzed using commercial statistical analysis software (SAS institute, Cary, NC, U.S.A.). Analysis of variance was performed using the general linear model (GLM) procedure, which is an analysis of variance procedure best fitted for unbalanced situations with unequal number of observations for the different groups. Differences between mean values were studied using the least-square means statement, to analyze differences between the means separately.

RESULTS

Concentration of β -Lactoglobulin in Milk and Fermented Milks

The native β -LG concentration measured by ELISA was 0.11, 0.14, and 0.13 mg/ml in UHT whole milk, fermented milk, and dehydrated fermented milk, respectively. Although the milk used was not processed (not extensively or partially hydrolyzed), the amount of native β -LG was much less than the concentration observed in crude milk (3.3 mg/ml). This result does not mean that no more antigenic β -LG was present in the milk and fermented milks: The antigenicity probably remained high in milk and fermented milks, but the β -LG was probably unfolded and the native form had disappeared, probably during the heating procedure.

Systemic IgG1 Response to β -Lactoglobulin and Intestinal Anaphylaxis

The control guinea pigs fed standard chow showed no systemic IgG1 response against β -LG ([Fig. 1A](#)). In contrast, IgG1 antibodies to β -LG, which are reagenic antibodies in the guinea pig, were detected in the plasma of animals fed milk or fermented milks. Moreover, the IgG1 titers were higher in guinea pigs fed fermented milk ($\log_{10}$ titer = 2.85 ± 0.26) than in those receiving milk ($\log_{10}$ = 2.5 ± 0.35 ; $p < 0.03$) or dehydrated fermented milk ($\log_{10}$ titer, 2.73 ± 0.5 ; $p = 0.16$).

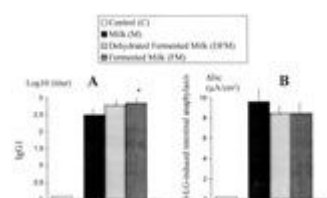


Fig. 1

Intestinal anaphylaxis was quantified by the increase in I_{sc} induced by serosal addition of β -LG. In control animals which did not receive milk proteins, β -LG did not induce a change in I_{sc} . In contrast, in guinea pigs fed milk or fermented milk, I_{sc} increased after serosal addition of β -LG, indicating that the animals were sensitized to β -LG and secreted chloride ions in response to β -LG.

The β -LG-induced intestinal anaphylaxis was similar in the three groups ($\Delta I_{sc}\beta$ -LG: $9.6 \pm 4.2 \mu\text{A}/\text{cm}^2$, $8.5 \pm 4.3 \mu\text{A}/\text{cm}^2$, and $8.5 \pm 3.4 \mu\text{A}/\text{cm}^2$ in animals in the M, FM, and DFM groups, respectively; [Fig. 1B](#)). When β -LG was placed on the mucosal side of the tissue, the β -LG response, $\Delta I_{sc}\beta$ -LG, was smaller than that observed when the protein was placed on the serosal side. This response remained identical in the three groups ($2.7 \pm 1.5 \mu\text{A}/\text{cm}^2$, $5.8 \pm 2.9 \mu\text{A}/\text{cm}^2$, and $4.4 \pm 3.7 \mu\text{A}/\text{cm}^2$ in the M, FM, and DFM groups, respectively; $n = 4$ -7 guinea pigs).

Intestinal Function

Basal Electrical Parameters

The basal electrical parameters, I_{sc} , PD, and G, in animals receiving the different diets are shown in [Table 1](#). The ionic conductance, an index of the paracellular permeability (i.e., of tight junction integrity) was similar in all groups, indicating that milk or fermented milk feeding did not alter the paracellular permeability of the intestinal epithelium. However, there was a significant increase in PD associated with a small increase in I_{sc} in the DFM group compared with those parameters in the other groups. This results suggests the stimulation of the electrogenic activity of the tissue.

Parameter	Control (C)	Milk (M)	Fermented with (FM)	Dehydrated Fermented with (DFM)
Basal conductance (G) ($\mu\text{S}/\text{cm}^2$)	14.6 ± 3.2	14.9 ± 3.2	13.3 ± 3.1	8.3 ± 3.8
Potential difference (PD) (mV)	3.1 ± 0.9	3.1 ± 0.6	3.8 ± 0.7	$4.2 \pm 1.2^*$
Short-circuit current (I _{sc}) ($\mu\text{A}/\text{cm}^2$)	46.9 ± 13.8	45.4 ± 13.1	47.2 ± 8.8	56.8 ± 19.4

Results are expressed as mean $\pm$ SD ($n = 5$ -12 guinea pigs).

* Significantly different from C and M groups ($p < 0.05$).

Table 1

Intestinal Permeability to the Bystander Protein ^3H -Horseradish Peroxidase

Total HRP fluxes, represented by ^3H -HRP fluxes, are shown in [Figure 2](#) (left). Fluxes in ^3H -HRP observed in group C ($1091 \pm 353 \text{ ng}/\text{hr}\cdot\text{cm}^2$) were similar to those observed in M ($921 \pm 497 \text{ ng}/\text{hr}\cdot\text{cm}^2$; $p = 0.45$), DFM ($800 \pm 274 \text{ ng}/\text{hr}\cdot\text{cm}^2$; $p = 0.24$), an FM guinea pigs ($1429 \pm 591 \text{ ng}/\text{hr}\cdot\text{cm}^2$; $p = 0.17$). However, among animals receiving fermented milks, those fed with the fresh FM (living bacteria) exhibited significantly higher ^3H -HRP fluxes than those fed the dehydrated fermented milk (dead bacteria; $p = 0.01$). Intact HRP fluxes ([Fig. 2](#), right) were significantly decreased in DFM group guinea pigs ($28.6 \pm 20 \text{ ng}/\text{hr}\cdot\text{cm}^2$) compared with fluxes in C and M group animals ($62.7 \pm 39.4 \text{ ng}/\text{hr}\cdot\text{cm}^2$, $p = 0.06$; and $61.1 \pm 39.4 \text{ ng}/\text{hr}\cdot\text{cm}^2$, $p = 0.05$). The percentage of protein degraded during the intestinal transport (approximately 94%) was not modified by the diet.

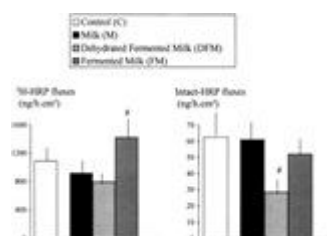


Fig. 2

Intestinal Permeability to the Sensitizing Protein ^{14}C - β -LG

^{14}C - β -Lactoglobulin fluxes, representing the total transport of the protein, are shown in [Fig. 3](#) (left). Total transport of β -LG was similar in C and M group animals ($767 \pm 251 \text{ ng}/\text{hr}\cdot\text{cm}^2$ and $749 \pm 476 \text{ ng}/\text{hr}\cdot\text{cm}^2$, respectively). The transport rates of intact β -LG were also comparable in the C and M groups ($16.4 \pm 8.0 \text{ ng}/\text{hr}\cdot\text{cm}^2$ vs. $12.1 \pm 4.0 \text{ ng}/\text{hr}\cdot\text{cm}^2$, respectively). However, ^{14}C - β -Lactoglobulin fluxes were significantly lower in the DFM group guinea pigs (403 ± 131

ng/hr·cm²) than in M group ($p = 0.05$) or C group ($p = 0.06$) guinea pigs. The intracellular degradation of β -LG during the epithelial transport was notable ($\sim 98\%$), because intact β -LG fluxes were 16.4 ± 8.0 ng/hr·cm² in C group guinea pigs. Intact β -LG fluxes were decreased in DFM (6.8 ± 3.05 ng/hr·cm²; $p = 0.017$) and FM group animals (10.4 ± 9.3 ng/hr·cm²; $p = 0.09$), compared with those in C group guinea pigs (Fig. 3, right). The percentage of protein degraded during the transport was not modified by the diet.

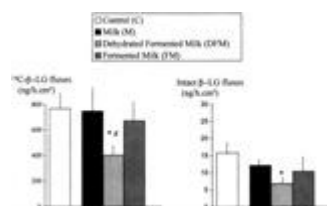


Fig 3

Intestinal Absorption and Secretion

The parameters used to evaluate the overall absorptive and secretory intestinal function in the four groups of guinea pigs are shown in Table 2. The Na-glucose co-transport was not modified by the different diets. However, the chloride secretion induced by the Ca²⁺-dependent secretagogues, histamine and carbachol, but not by the cyclic adenosine monophosphate-dependent secretagogue forskolin, was significantly decreased in the FM group, compared with that in the other groups. This may indicate that there is a desensitization of the enterocyte receptors to histamine and carbachol, possibly because of an increased stimulation in vivo.

	Control (C)	Milk (M)	Fermented milk (FM)	Dehydrated Fermented milk (DFM)
2hr glucose	12.2 ± 6.6	9.2 ± 6.1	9.4 ± 6.2	10.6 ± 6.2
2hr histamine	7.5 ± 6.1	10.0 ± 6.6	7.7 ± 6.2*	9.7 ± 6.1
2hr carbachol	10.2 ± 6.1	9.4 ± 6.0	7.5 ± 6.3*	9.6 ± 7.1
2hr forskolin	6.1 ± 6.6	7.4 ± 6.5	6.3 ± 6.6	7.8 ± 6.2

Experiments on the different compounds used are presented in an Online chapter for the increase in short-circuit current due to microamps per square centimeter, after removal of glucose or removal of glucose or removal of histamine, carbachol, and forskolin. Results are expressed as mean ± SD in n = 12 guinea pigs.

* Significantly different from M ($p < 0.05$) and DFM ($p < 0.05$) groups.

† Significantly different from C ($p < 0.05$) group.

Table 2

DISCUSSION

The present study, feeding guinea pigs with dehydrated fermented milk (but not fresh fermented milk) reinforced intestinal barrier capacity for food proteins. However, sensitization to milk proteins developed equally in animals receiving milk, dehydrated fermented milk, or fresh fermented milk, suggesting that macromolecular permeability was not the unique factor playing a role in the sensitization process in guinea pigs.

The use of probiotics or biotherapeutic agents is generally considered healthy (20) and is currently used in the treatment of various gastrointestinal diseases including persistent diarrhea and inflammatory bowel disease. A clinical advantage of feeding yogurt rather than milk in children with persistent diarrhea (7) has been noted. Moreover, a series of studies from Finland have found a positive effect of *Lactobacillus casei* GG in promoting systemic and intestinal immune responses to rotavirus in infants with rotavirus gastroenteritis (8). It also has been found to increase the gut IgA immune response in patients with Crohn's disease (21) and to promote endogenous barrier mechanisms and to alleviate intestinal inflammation in children with atopic dermatitis and food allergy (22).

The present study was intended to determine, by using a well-described model of allergy-prone guinea pigs, whether various milk-based diets, including or not including living or dead lactic acid bacteria, could interfere with the development of cow's milk protein allergy (14). In this model, oral sensitization is achieved by feeding milk proteins for 2 to 3 weeks and is described as an immediate-type, IgG1-dependent hypersensitivity. The local intestinal response can be

assessed at the intestinal level in an Ussing chamber by recording an Isc after β -LG has been added to the serosal compartment. This reaction is probably caused by mast cell degranulation and the activation of chloride secretion.

Various hypotheses can be raised on the possible interference of lactic acid bacteria in the sensitization process: they could interfere with the degradation of milk antigens; alter antigen absorption and processing at the intestinal level; stimulate the immune system, particularly the secretory IgA pathway; or modify the relationship between the intestinal epithelium and the local immune system.

It is not easy to determine the impact of fermentation on the nature of milk proteins. In the present study, the native β -LG concentration was less (~ 0.13 mg/ml) in milk or fermented milks than that in crude milk (present data, 3.3 mg/ml), and microbial acidification had no effect on the concentration of native β -LG. The immunoenzymatic assay used in this study detected native β -LG only and not the unfolded or denatured protein (probably present in the UHT milk after the heating procedure). Therefore, the results of this ELISA cannot be considered an index of the milk's antigenicity, because denatured proteins have the same or even higher potential of sensitization.

Another possibility for lactic acid bacteria to interfere with the sensitization process is to modify the antigen absorption and processing at the intestinal epithelial level (i.e., to change the intestinal permeability). Because it is generally accepted that an increased permeability to macromolecules could favor the development of food antigen sensitization, it could be suggested that the stabilizing capacity of probiotics on intestinal permeability could interfere with sensitization. The enterocyte has been shown to absorb food-type antigens by transcytosis along two functional pathways: a direct pathway, along which the protein remains intact and a major degradative pathway, allowing more than 90% of the protein to be degraded within the cell before its release into the mucosa (17). This degradation allows the formation of amino acids but also of peptides (23) with potential capacity of activating the local immune system.

In the guinea pig, the permeability to macromolecules decreases rapidly 48 hours after birth (15), but a residual macromolecular uptake remains even after weaning. Previous results show that sensitization to cow's milk proteins increases intestinal permeability to the bystander protein HRP, especially in the presence of the sensitizing antigen β -LG (24) and can also decrease the absorption of sensitizing antigens (25). In the current study, we found that sensitization did not modify the basal intestinal permeability to ^3H -HRP or ^{14}C - β -LG, when compared with that in control guinea pigs. This discrepancy could be because in this study the unstripped small intestine was mounted in an Ussing chamber, whereas in our previous study, mucosa stripped of their muscular layers were used (26). It is possible, that during the stripping procedure a mechanical stress stimulated the degranulation of mast cells, leading to the increase of basal permeability. A tendency toward an increase in ^3H -HRP transport was noted in animals fed fresh fermented milk. This phenomenon was not observed with ^{14}C - β -LG transport. This observation remains unexplained but could be caused by a basal "physiological" inflammation induced by living bacteria. By contrast, a significant decrease in intact HRP and β -LG absorption was observed in animals fed with the dehydrated fermented milk (but not with the fresh fermented milk) suggesting a reinforcement of the intestinal barrier capacity to intact proteins. This was probably not because of a reinforcement of the transjunctional permeability, because the ionic conductance, an index of this permeability, was not modified in the four groups of guinea pigs. Therefore, it can be speculated that the decrease in intact protein permeability is caused by some modification of the intracellular processing of the proteins within the enterocytes or by an increased IgA secretory response that impairs the transport of intact proteins. Such enforcement of intestinal permeability has already been shown in rats fed a cow's milk enriched with *Lactobacillus casei* GG (27). In fact, epithelial surface secretory IgA has

been shown to prevent the uptake of macromolecular antigens at mucosal surfaces [\(28,29\)](#).

It is remarkable to note that although some decrease was noted in the intestinal permeability to β -LG in animals fed the fermented milk, the level of sensitization was the same as that shown in intestinal anaphylactic responses or was even greater (IgG1 titers) than that observed in milk-fed guinea pigs. This indicates that minute amounts of proteins were sufficient to induce sensitization and that even when the permeability was reduced (DFM group), full sensitization was observed

Taken together, the present results indicate that the intestinal barrier capacity for food proteins could be reinforced by dehydrated, but not fresh, fermented milk, but that milk and fermented milks were equally efficient in inducing cow's milk allergy in guinea pigs.

Acknowledgement: The authors thank Isabelle Goderel for technical assistance.

This study was conducted in collaboration with Diepal-nsa, Steenvorde, France, who provided partial funding.

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10.1016/j.earhumdev.2006.12.002

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10.1016/j.foodres.2009.12.003

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Gill, HS

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10.1016/S1521-6918(03)00074-X

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Proceedings of the Nutrition Society

How dietary antigens access the mucosal immune system

Heyman, M

Proceedings of the Nutrition Society, 60(4): 419-426.

Keywords:

Cow's milk allergy; Fermented milk; Intestinal function; Mucosal immunity; Probiotics

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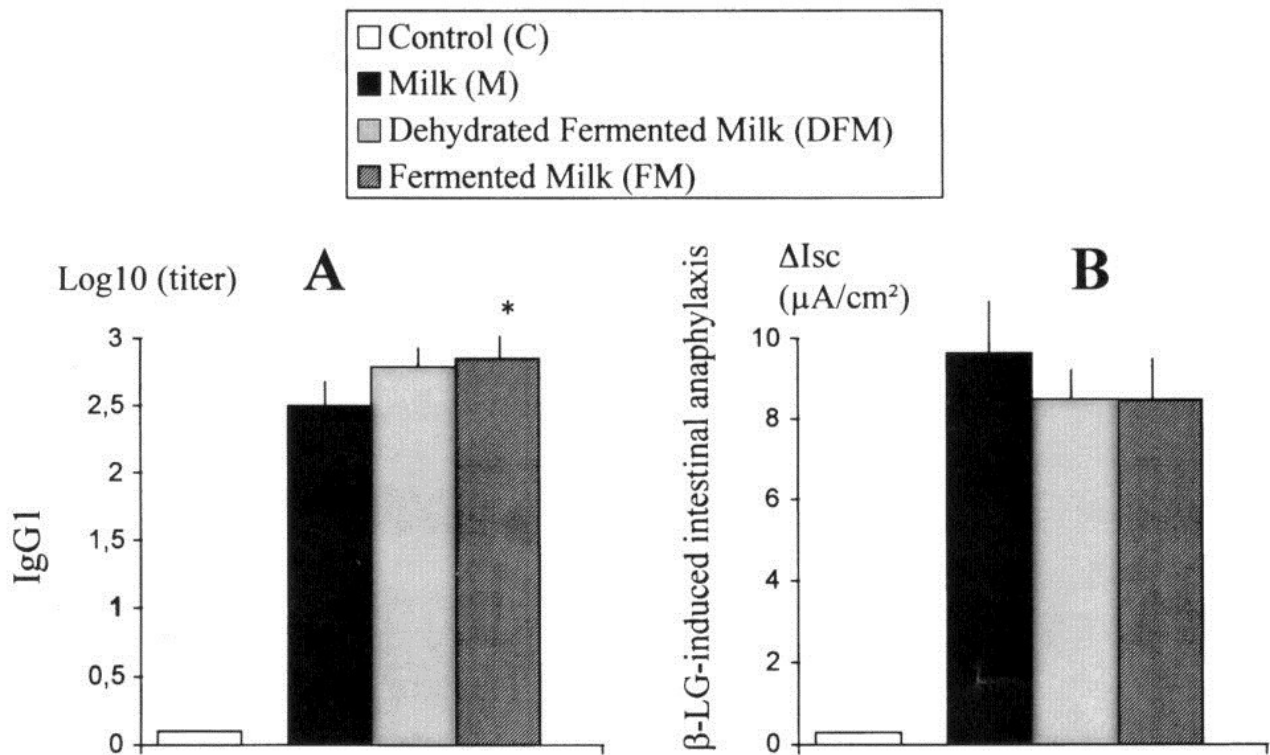


FIG. 1. Sensitization to milk proteins measured by systemic immunoglobulin (Ig) G1 antibody titers and intestinal anaphylaxis to β -lactoglobulin (β -LG). (A) Systemic β -LG-specific IgG1 antibodies measured by enzyme-linked immunosorbent assay (ELISA) and expressed as log10 of 1 titer. *Significantly different from milk-fed animals. (B) Intestinal anaphylaxis measured by the increase in short-circuit current (Isc) induced by the serosal addition of β -LG to the small intestinal fragments mounted in Ussing chambers. Results are expressed as mean $\pm$ SD ($n = 7$ -12 guinea pigs).

Parameter	Control (C)	Milk (M)	Fermented milk (FM)	Dehydrated fermented milk (DFM)
Ionic conductance (G) (mS/cm ²)	14.6 $\pm$ 3.3	14.9 $\pm$ 3.2	13.3 $\pm$ 3.1	8.5 $\pm$ 3.4
Potential difference (PD) (mV)	3.3 $\pm$ 0.9	3.1 $\pm$ 0.6	3.6 $\pm$ 0.7	4.2 $\pm$ 1.2 ^a
Short-circuit current (Isc) (μA/cm ²)	46.9 $\pm$ 13.9	45.4 $\pm$ 11.3	47.2 $\pm$ 8.8	56.0 $\pm$ 19.4

Results are expressed as means $\pm$ SD ($n = 7$ -12 guinea pigs).

^a Significantly different from C and M groups ($p < 0.04$).

TABLE 1. Basal electrical parameters in the small intestine of guinea pigs receiving a normal diet or a diet enriched with milk, fresh fermented milk, or dehydrated fermented milk

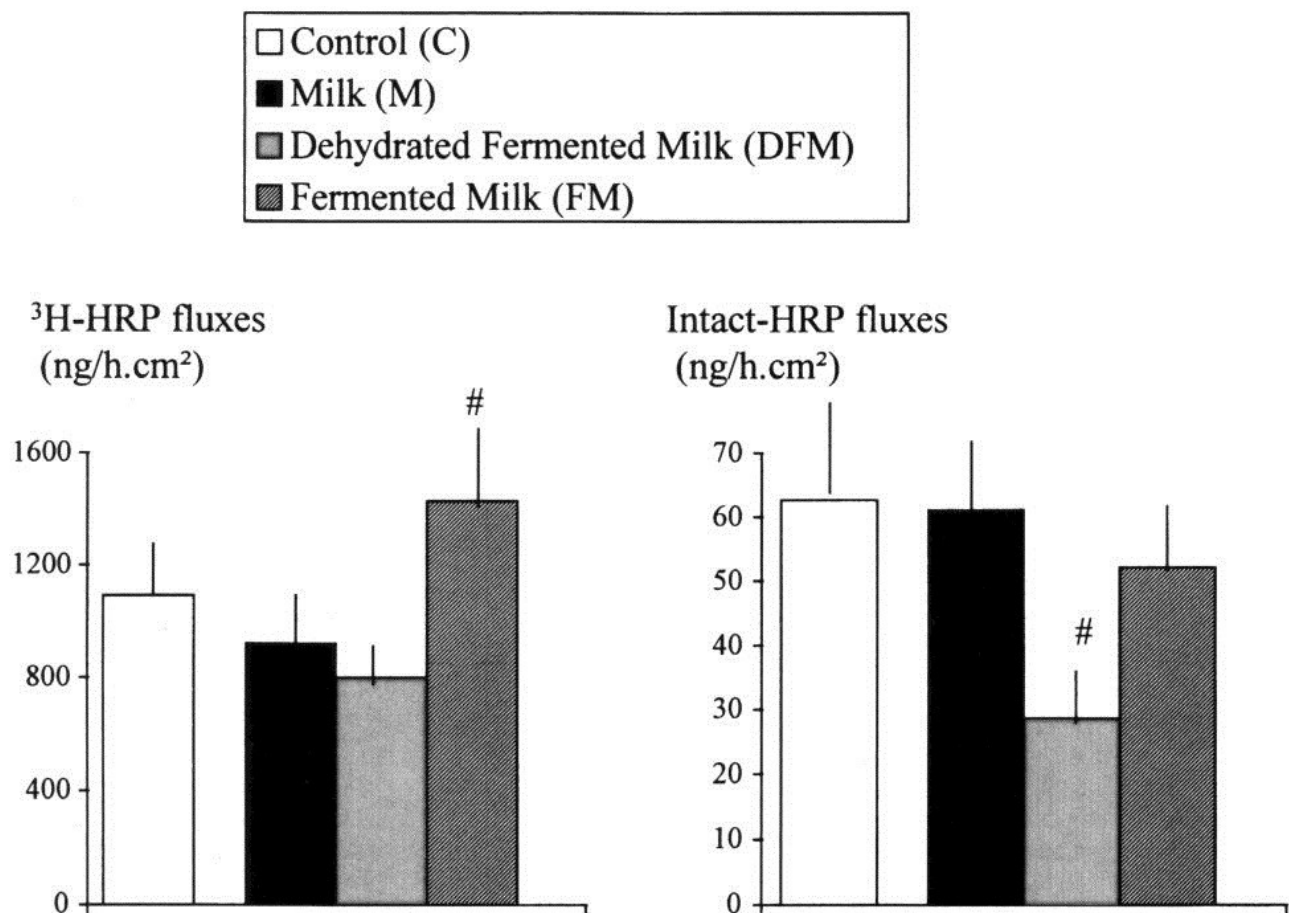


FIG. 2. Horseradish peroxidase (HRP) transport across guinea pig small intestinal fragments mounted in an Ussing chamber. The ^3H -HRP fluxes represent the total flux of HRP, and intact HRP fluxes represent the amount of protein transported intact across the intestinal layer. Results are expressed as means $\pm$ SD ($n = 7-10$ guinea pigs in each group). # Significantly different from milk ($p < 0.03$) and dehydrated fermented milk groups ($p < 0.01$).

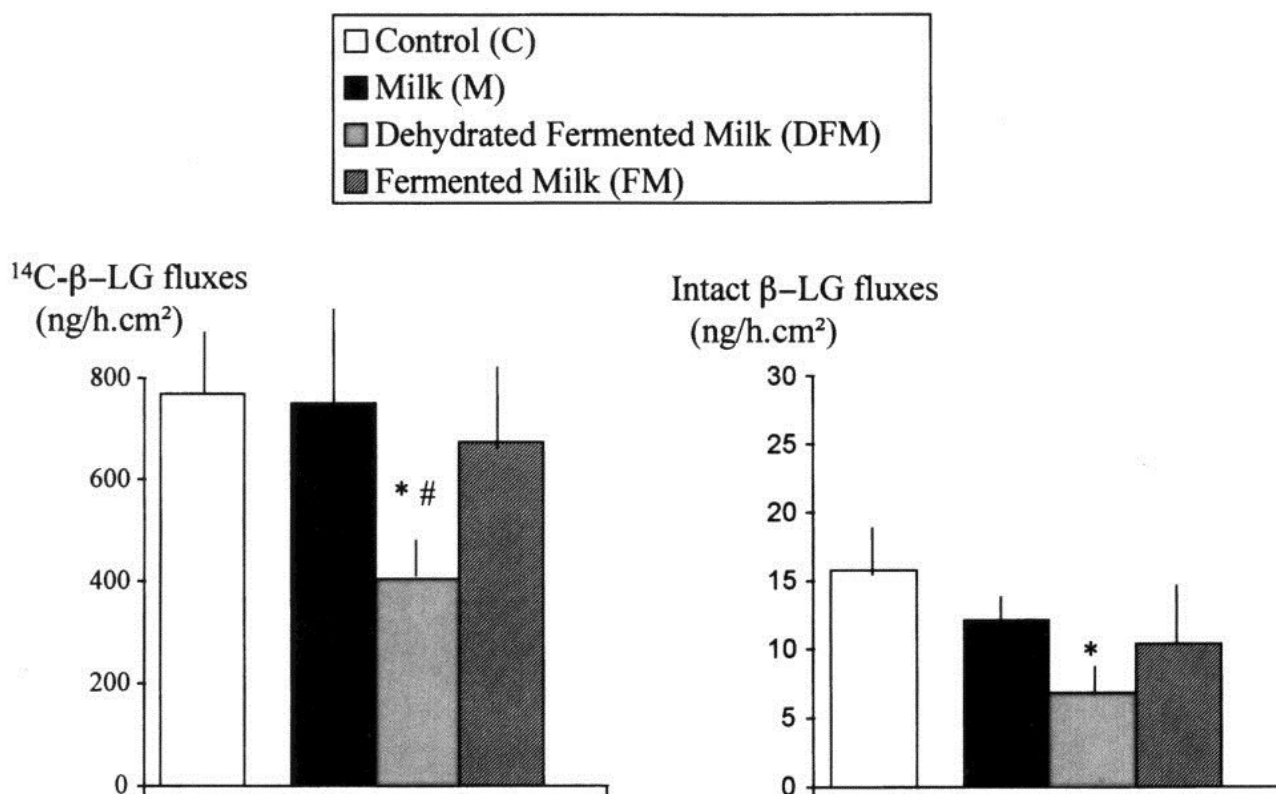


FIG 3. β -Lactoglobulin (β -LG) transport across small intestinal fragments of guinea pigs mounted in an Ussing chamber. ^{14}C - β -Lactoglobulin fluxes represent the total flux of β -LG, and intact β -LG fluxes represent the amount of protein transported in intact form across the intestinal layer. Results are expressed as means $\pm$ SD ($n = 7$ -10 guinea pigs). *,# Significantly different from control and milk groups, respectively.

	Control (C)	Milk (M)	Fermented milk (FM)	Dehydrated fermented milk (DFM)
$\Delta\text{Isc glucose}$	12.2 ± 6.6	9.4 ± 4.1	9.4 ± 4.2	10.8 ± 6.2
$\Delta\text{Isc histamine}$	7.3 ± 5.1	10.0 ± 4.9	3.7 ± 5.6^a	9.7 ± 6.3
$\Delta\text{Isc carbachol}$	10.2 ± 8.1	9.4 ± 8.0	3.5 ± 6.3^b	6.9 ± 7.1
$\Delta\text{Isc forskolin}$	4.1 ± 4.4	2.4 ± 1.5	4.5 ± 8.6	2.8 ± 3.2

Responses to the different compounds used are measured in an Ussing chamber by the increase in short-circuit current (Isc in microamps per square centimeter), after mucosal addition of glucose or serosal addition of histamine, carbachol, and forskolin. Results are expressed as means $\pm$ SD ($n = 7$ -12 guinea pigs).

^a Significantly different from M ($p < 0.02$) and DFM ($p < 0.01$) groups.

^b Significantly different from C ($p < 0.05$) group.

TABLE 2. Intestinal function (absorption and secretion) of guinea-pigs fed four milk diets