

Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with *Lactobacillus casei* GG-derived enzymes

Yelda Sütas, MD,^a Esa Soppi, MD,^a Hannu Korhonen, PhD,^b
Eeva-Liisa Syväoja, PhD,^c Maija Saxelin, PhD,^c Timo Rokka, MSc,^d and
Erika Isolauri, MD^a Tampere, Jokioinen, and Helsinki, Finland

Background: Processing of proteins in the gut and activation of T-cell suppression leads to systemic hyporesponsiveness to ingested protein antigens.

Objective: The study was designed to determine whether lactobacilli, a major part of human intestinal microflora, can contribute to degradation of food antigens in the gut and modify their immunoactivities.

Methods: Lymphocyte transformation tests were carried out in healthy adults to determine the mitogen-induced lymphocyte proliferative responses to bovine caseins hydrolyzed with pepsin and trypsin and to bovine caseins additionally hydrolyzed with enzymes derived from *Lactobacillus casei* strain GG (ATCC 53103).

Results: In experiments done with caseins hydrolyzed with pepsin and trypsin, β - and α_{s1} -caseins significantly suppressed the proliferation of lymphocytes at 0.1 and 10 $\mu\text{g/ml}$, respectively, when compared with corresponding control cultures without these hydrolysates. In contrast, κ -casein significantly stimulated the proliferation of lymphocytes at 10 $\mu\text{g/ml}$. In experiments done with caseins additionally hydrolyzed with *L. casei* GG-derived enzymes, there was one consistent effect on lymphocyte proliferation: suppression by α_{s1} -, β -, and κ -caseins at 0.1, 10, and 1000 $\mu\text{g/ml}$, respectively.

Conclusions: Hydrolysis of caseins with *L. casei* GG-derived enzymes generates molecules with suppressive effects on lymphocyte proliferation. In addition, intestinal bacteria can be beneficial in the downregulation of hypersensitivity reactions to ingested proteins in patients with food allergy. (*J Allergy Clin Immunol* 1996;98:216-24.)

Key words: Cow's milk, caseins, infant food, *Lactobacillus casei* physiology, food hypersensitivity, lymphocyte transformation, immunotherapy

Cow's milk allergy (CMA) is defined as an immune-mediated adverse reaction to cow's milk proteins.¹ The treatment of choice is the complete avoidance of cow's milk antigens. In infants with CMA, it is necessary to use a substitute formula when human milk is unavailable.² Hydrolyzed formulas, based on cow's milk-derived whey or ca-

Abbreviations used

CMA:	Cow's milk allergy
¹²⁵ IUdR:	Iodine 125-labeled 5-iodo-2'-deoxyuridine
PBMCs:	Peripheral blood mononuclear cells
PHA:	Phytohemagglutinin
TFA:	Trifluoroacetic acid
UHT:	Ultra-high temperature

From ^aUniversity of Tampere, Medical School, Tampere; ^bAgricultural Research Centre of Finland, Food Research Institute, Jokioinen; ^cValio Ltd, Research & Development Centre, Helsinki; and ^dUniversity of Helsinki, Department of Food Technology, Dairy Sciences, Helsinki.

Supported by the Academy of Finland.

Received for publication Feb. 6, 1995; revised Oct. 4, 1995; accepted for publication Oct. 13, 1995.

Reprint requests: Erika Isolauri, MD, Medical School, University of Tampere, Department of Pediatrics, P.O. Box 607, 33101 Tampere, Finland.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/1/69886

sein, are used to provide adequate nutrition with a reduced antigenic load.³ The preliminary heat treatment of cow's milk mainly affects the conformation of proteins and facilitates their hydrolysis.⁴ Subsequent enzymatic hydrolysis with pepsin, trypsin, pancreatic extracts, and extracts from the intestinal mucosa causes progressive destruction of sequential epitopes and refines the formulas into

the least antigenic and allergenic form.⁴ Despite the extensive degree of hydrolysis, adverse clinical reactions after the ingestion of hydrolyzed formulas have been reported in a subgroup of infants with CMA.⁵ The hypersensitivity reactions may be partly due to retention of original cow's milk antigens or to release of biologically active peptides during the processing of hydrolyzed formulas.⁵⁻⁷ The ability to dampen these hypersensitivity reactions to cow's milk antigens would be a breakthrough in the treatment and prevention of CMA in infants.

The form in which the antigen is presented to the immune system is crucial for the outcome of the immune response against the antigen.^{8,9} As established in animal models, the development of systemic hyporesponsiveness to a fed antigen (i.e., oral tolerance) depends on its processing in the gut.^{10,11} The release of tolerogenic peptides from the native protein may be related to its enzymatic degradation by the gut microflora.¹² So far, the gut microflora is known to contribute to gut mucosal barrier functions¹³ and to regulate local immune responses against ingested antigens.^{14,15} An isolated human intestinal microfloral strain, *Lactobacillus casei* strain GG (*L. casei* GG, ATCC 53103) has recently been shown to promote local IgA responses against dietary antigens encountered by the enteric route and may therefore aid in immune elimination.¹⁵ It is not yet known whether particular strains of intestinal bacteria could directly modify the immunogenicity of the food antigens and consequently down-regulate hypersensitivity reactions.

The objective of this study was to investigate the immunomodulatory activity of cow's milk proteins hydrolyzed with pepsin and trypsin and cow's milk proteins additionally hydrolyzed with *L. casei* GG-derived enzymes as evaluated by lymphocyte transformation tests in vitro.

METHODS

Hydrolysis of cow's milk proteins

Cow's milk was heated at ultra-high temperature (UHT), 135° C for 2 to 4 seconds. UHT-treated milk was fermented with *L. casei* GG for 20 to 24 hours at 37° C. Bovine total casein was purified from cow's milk.¹⁶ Casein components (β -, α_{s1} -, and κ -caseins) were obtained from commercial suppliers (β - and κ -casein; Sigma Chemical Co., St. Louis, Mo.; α_{s1} -casein; Merck, Darmstadt, Germany).

The hydrolysis with pepsin and trypsin was applied separately to milk, fermented milk, casein, and casein components, β -, α_{s1} -, and κ -caseins. Briefly, these

were hydrolyzed initially with 0.1% pepsin in 0.1 mol/L HCl for 3 hours at 37° C, pH 2.5. After pH adjustment with 250 mg of NaHCO₃ and 2 mol/L NaOH, 0.1% trypsin was added to samples, and these were hydrolyzed for 5 hours at 37° C, pH 8.0. The trypsin activity was stopped by cooling the sample to 5° C, and the unhydrolyzed protein was separated by high-speed centrifugation, 12,000 g for 20 minutes at 5° C. The supernatant was filtered through a 0.22 μ m Millex-GV low-protein-binding sterile filter (Nihon Millipore Kogyo Inc., Yonezawa, Japan) and lyophilized.

The hydrolysis with the enzyme mixture derived from *L. casei* GG was applied separately to casein and casein components (β -, α_{s1} -, and κ -caseins). The enzymes were isolated by using the modified method of Exterkate and de Veer.¹⁷ Briefly, the enzymes were released by sonication of frozen bacterial cells. The supernatant of the centrifuged cells was separated and used for the hydrolysis of casein and casein components. The hydrolysis was carried out for 24 hours at 34° C. Finally, pepsin-trypsin hydrolysis was applied to casein and casein components as described previously.

Fast protein liquid chromatography (FPLC System; Pharmacia, Uppsala, Sweden) was used to separate peptide fractions in pepsin-trypsin-hydrolyzed fermented milk. A reversed-phase column (Pep RP HR 16/10, Pharmacia) was linked to a Monitor UV-M absorbance detector (Shimadzu Corp., Kyoto, Japan) at a wavelength of 214 nm. The samples were deproteinized by centrifugation at 9000 g in 0.1% trifluoroacetic (TFA) in water. The supernatants were fractionated (A to D) by a gradient from 0% to 60% of 0.1% TFA in acetonitrile for 30 minutes at a flow rate of 7 ml/min. Peptide fractions (B and C) were collected at 8-minute intervals and dried. On a separate chromatography run, subfractions (BI-IV, CI-CIV; molecular weight < 2500 d) were obtained at 2-minute intervals (Fig. 1).

Lymphocyte transformation test

A modification of the whole blood micromethod for mitogen-induced lymphocyte transformation was used.^{18,19} The same six to eight healthy individuals volunteered as blood donors for the experiments. Briefly, heparinized venous blood was obtained and diluted 1:7 with RPMI 1640 culture medium (Grand Island Biological Co., Grand Island, N.Y.) containing antibiotics. Concanavalin A (Pharmacia) and phytohemagglutinin (PHA) (PHA M: Difco Laboratories, Detroit, Mich.) were diluted with RPMI 1640 containing antibiotics, with ranges of final concentrations in cultures from 1 to 625 μ g/ml and 5 to 1250 μ g/ml, respectively. In pilot experiments, mitogen-induced proliferation in control cultures with PHA was comparable to that in control cultures with concanavalin A. PHA was chosen as the mitogen for further experiments. Lyophilized hydrolysates of milk, fermented milk, and caseins were diluted in RPMI 1640 to their

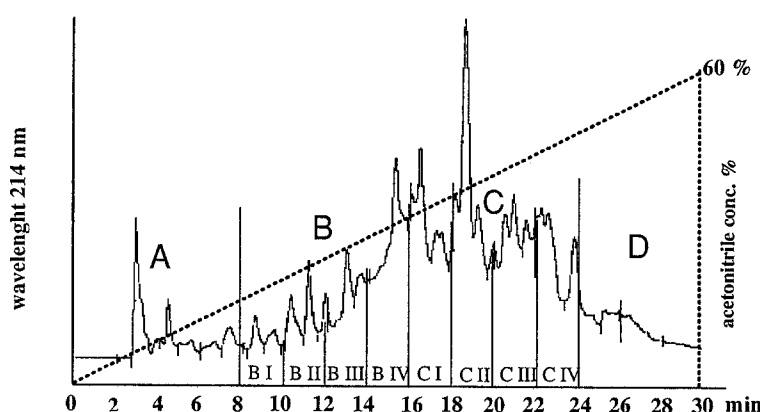


FIG. 1. Peptide profile (A-D) of *L. casei* GG-fermented milk hydrolyzed with pepsin and trypsin. Fractions B to C and subfractions B I to C IV were isolated by fast protein liquid chromatography with a Pep RP HR 16/10 column (Pharmacia) and 0.1% TFA in acetonitrile at a flow rate of 7 ml/min.

final concentrations of 0.1 $\mu\text{g/ml}$ (low), 10 $\mu\text{g/ml}$ (medium), and 1000 $\mu\text{g/ml}$ (high) as proven to be nontoxic on T cells in dye exclusion studies with eosin.

Two sets of experiments were undertaken to investigate the mitogen-induced proliferative responses of peripheral blood mononuclear cells (PBMCs) to: (1) milk, fermented milk, casein, and casein components hydrolyzed with pepsin and trypsin and (2) casein and casein components hydrolyzed with *L. casei* GG-derived enzymes and with pepsin and trypsin. Experiments consisted of four control cultures in triplicates containing: (1) 50 μl of RPMI 1640, (2) 25 μl of RPMI 1640 and 25 μl of 5 $\mu\text{g/ml}$ PHA, (3) 25 μl of RPMI 1640 and 25 μl of 125 $\mu\text{g/ml}$ PHA, (4) 25 μl of RPMI 1640 and 25 μl of 1250 $\mu\text{g/ml}$ PHA and corresponding test cultures with 25 μl of hydrolyzed products at three different concentrations. Finally, 150 μl of diluted blood was added to flat-bottomed wells of a microtiter plate. The total incubation time in a humidified 5% CO_2 atmosphere at 37° C was 96 hours. Sixteen to 18 hours before harvesting, 20 μl of iodine 125-labeled 5-iodo-2'-deoxyuridine ($^{125}\text{IUdR}$) containing 0.25 μCi of iodine 125 with specific activity of 2000 Ci/mol/L (The Radiochemical Centre, Amersham, U.K.) was added to each well together with 5-fluoro-2'-deoxyuridine at a final concentration of 4×10^{-6} mol/L to enhance the uptake of $^{125}\text{IUdR}$.²⁰ To avoid interference of different $^{125}\text{IUdR}$ radioactivity for different experiment dates with our results, all counts were adjusted according to the reference day of $^{125}\text{IUdR}$ as indicated by the manufacturer.

The mitogen-induced proliferation of PBMCs was expressed as counts per minute, background excluded. The mitogen-induced proliferation of PBMCs was observed consistently to result in its maximum in control cultures containing 125 $\mu\text{g/ml}$ PHA and RPMI 1640. The results were then presented for control cultures containing 125 $\mu\text{g/ml}$ PHA and RPMI 1640 and for the three test cultures containing 125 $\mu\text{g/ml}$

PHA and hydrolyzed products at the low, medium, and high concentrations.

Statistical analysis

A nonparametric pairwise test (Wilcoxon signed-rank test) was used to compare the values in counts per minute of each of the test cultures with those of the control cultures. The level of significance was $p < 0.05$. Significant results of pairwise comparisons were presented as suppression or stimulation according to decrease or increase of the values in counts per minute in the test culture.

RESULTS

In vitro mitogen-induced proliferative responses of PBMCs to milk and caseins hydrolyzed with pepsin and trypsin

In the experiments done with milk hydrolyzed with pepsin and trypsin, the mitogen-induced proliferation of PBMCs was suppressed at the concentrations of 0.1, 10, and 1000 $\mu\text{g/ml}$ ($p = 0.015$, $p = 0.007$, and $p = 0.007$, respectively) (Fig. 2). Likewise, in the experiments done with casein hydrolyzed with pepsin and trypsin, proliferation was suppressed at the concentrations of 0.1, 10, and 1000 $\mu\text{g/ml}$ ($p = 0.015$, $p = 0.015$, and $p = 0.007$, respectively) (Fig. 3, A).

In experiments done with β -casein hydrolyzed with pepsin and trypsin, there was suppression of proliferation only in test cultures containing 0.1 $\mu\text{g/ml}$ β -casein ($p = 0.02$) (Fig. 3, B). In experiments done with α_{s1} -casein hydrolyzed with pepsin and trypsin, proliferation was suppressed only at 10 $\mu\text{g/ml}$ ($p = 0.007$) (Fig. 3, C). In experiments done with κ -casein hydrolyzed with pepsin and trypsin, proliferation was stimulated only at 10 $\mu\text{g/ml}$ ($p = 0.03$) (Fig. 3, D).

In vitro mitogen-induced proliferative responses of PBMCs to caseins additionally hydrolyzed with *L. casei* GG-derived enzymes

In the experiments done with casein and casein components (β -, α_{s1} -, or κ -caseins) additionally hydrolyzed with *L. casei* GG-derived enzymes, the mitogen-induced proliferation of PBMCs was suppressed at 0.1, 10, and 1000 $\mu\text{g/ml}$ ($p = 0.03$, $p = 0.03$, and $p = 0.03$) (Fig. 4, A-D). Altogether, the pattern of the proliferative responses was identical to that of suppression and consistent at all concentrations in this second set of experiments.

A separate set of experiments was also undertaken for *L. casei* GG-fermented milk hydrolyzed with pepsin and trypsin and for its peptide fractions (B and C) and subfractions (BI-BIV and CI-CIV). In experiments done with the *L. casei* GG-fermented milk hydrolyzed with pepsin and trypsin and in those done with its peptide fractions, the mitogen-induced proliferation of PBMCs in the test cultures at 0.1 and 1000 $\mu\text{g/ml}$ was significantly different from that of the control cultures (Table I). In experiments done with the subfractions, BII did not cause any significant changes in proliferation; whereas BI, BIII, BIV, and CI-CIV did. Proliferation was suppressed in experiments done with BIII, BIV, CII, CIII, and CIV at the concentration of 0.1 $\mu\text{g/ml}$; in those done with BI, BIII, BIV, CI, and CII at 10 $\mu\text{g/ml}$; and in those done with BI, BIII, BIV, CI, CII, and CIV at 1000 $\mu\text{g/ml}$ (Table I for BI and CI). In all three sets of experiments, none of the hydrolyzed products induced proliferation of PBMCs in the absence of mitogenic stimulation (data not shown).

DISCUSSION

The results of this study indicate that the manipulation of cow's milk caseins in vitro with *L. casei* GG-derived enzymes modifies their immunoactivity as evaluated by lymphocyte transformation tests.

The first objective of this study was to assess the immunomodulatory activity of cow's milk hydrolyzed with pepsin and trypsin. Pepsin and trypsin hydrolysis was chosen as a model because it mimics the basic enzymatic degradation in the gut. The lymphocyte transformation test was used to demonstrate the peripheral events in vivo during exposure to cow's milk antigens. Although comparable with regard to sterilizing efficiency, UHT treatment has been used instead of high temperature, short time treatment to provide complete inactivation of microorganisms and their products,

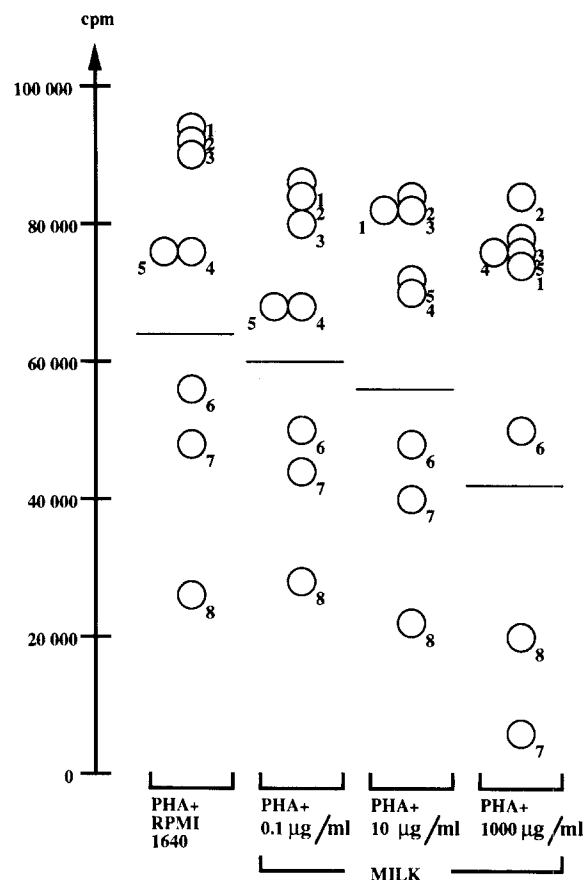


FIG. 2. Mitogen-induced proliferative responses of PBMCs in vitro to milk hydrolyzed with pepsin and trypsin. The results are expressed as mean counts per minute for cultures without hydrolyzed milk (PHA + RPMI 1640) and with hydrolyzed milk (PHA + hydrolyzed milk) at three concentrations (0.1, 10, and 1000 $\mu\text{g/ml}$). Horizontal lines represent geometric means of counts per minute of the eight experiments, enumerated the same for each individual.

regardless of the palatability of milk. Caseins, the predominant proteins in cow's milk, are frequent stimulators of lymphocytes in patients with CMA.²¹ Heat treatment and enzymatic degradation of cow's milk proteins would destroy the conformational and sequential structure of epitopes and consequently might reduce their immunogenicity.^{4,6} We observed that in the absence of the mitogenic induction, milk and caseins hydrolyzed with pepsin and trypsin did not stimulate the proliferation of PBMCs in vitro. Nevertheless, our results indicated that when induced by mitogen, milk and caseins hydrolyzed with pepsin and trypsin modulated the proliferation of PBMCs in vitro. In this first set of experiments, a suppressive effect was obtained with milk and casein hydrolyzed with pepsin and trypsin. A suppressive trend was observed in experiments

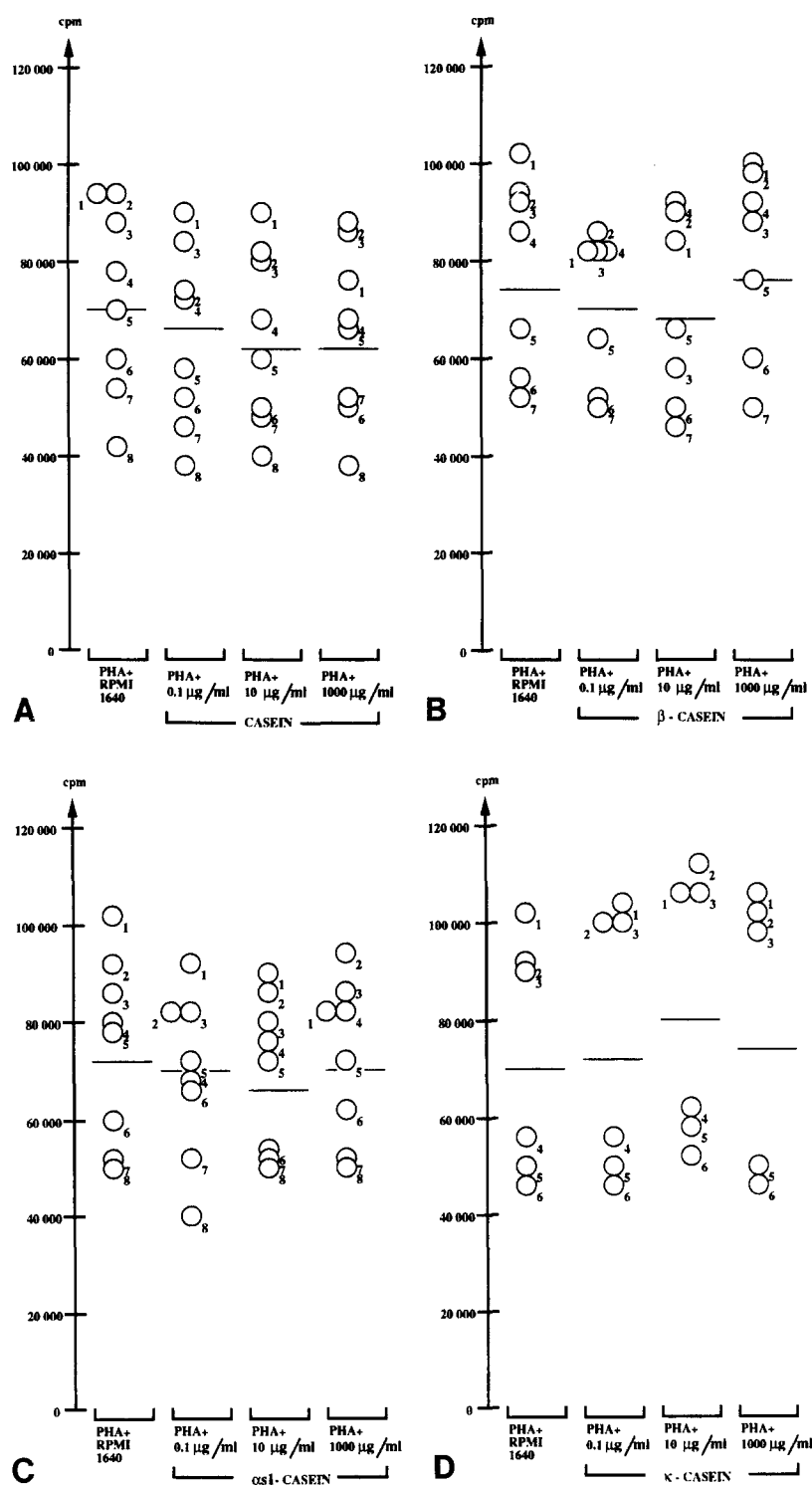


FIG. 3. Mitogen-induced proliferative responses of PBMCs in vitro to casein (**A**) and casein components, β - (**B**), α_{s1} (**C**), and κ -casein (**D**) hydrolyzed with pepsin and trypsin. Results are expressed as mean counts per minute for cultures without hydrolyzed product (PHA + RPMI 1640) and with hydrolyzed product at three concentrations (0.1, 10, and 1000 $\mu\text{g/ml}$). *Horizontal lines* represent the geometric mean of counts per minute of the six experiments, enumerated the same for each individual.

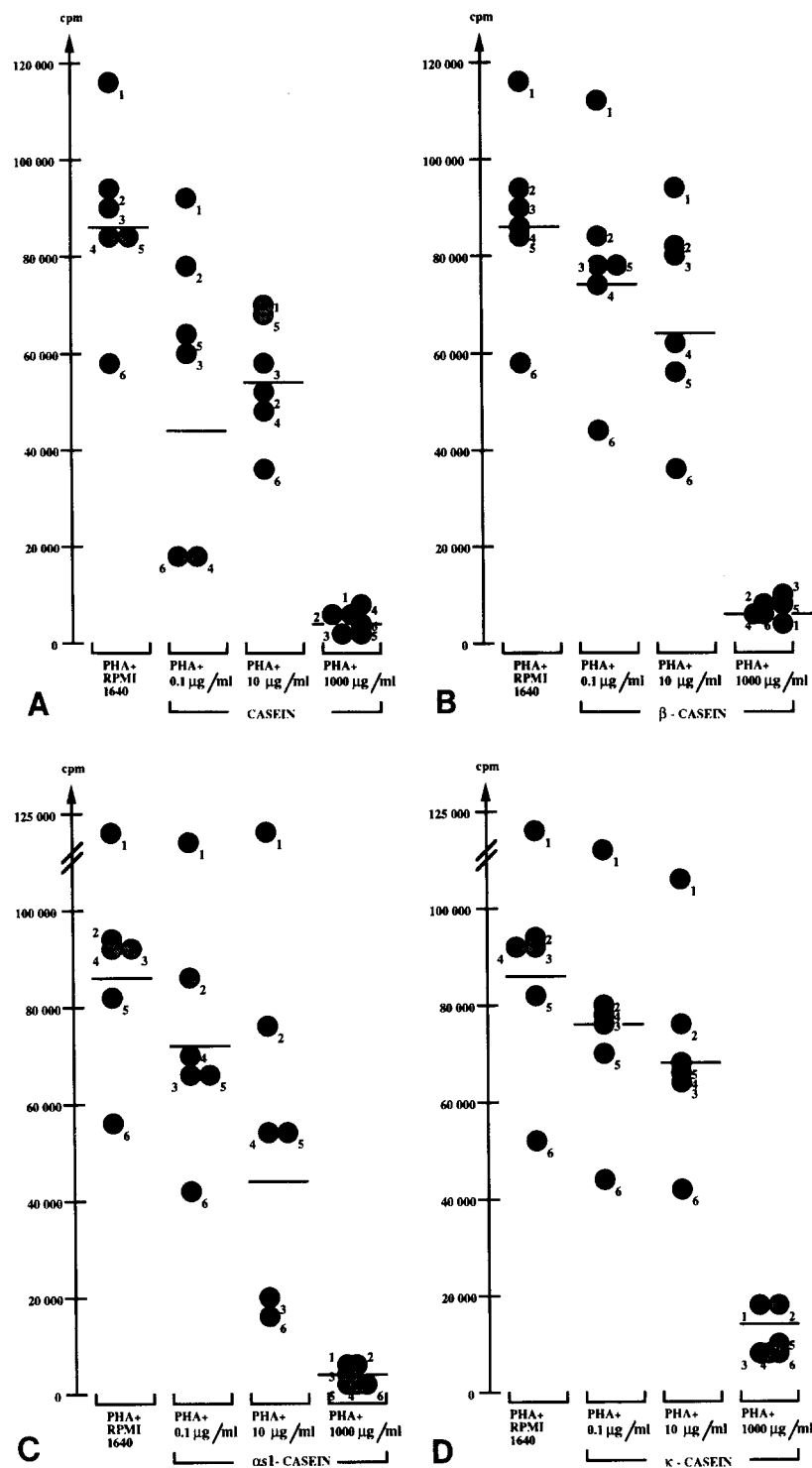


FIG. 4. Mitogen-induced proliferative responses of PBMCs in vitro to casein (**A**) and casein components, β (**B**), α_1 (**C**), and κ -casein (**D**) additionally hydrolyzed with *L. casei* GG-derived enzymes. Results are expressed as the mean counts per minute for cultures without hydrolyzed product (PHA + RPMI 1640) and with hydrolyzed product at three concentrations (0.1, 10, and 1000 μ g/ml). Horizontal lines represent the geometric mean of counts per minute of the six experiments, enumerated the same for each individual.

TABLE I. Mitogen-induced proliferative responses in experiments done with *L. casei* GG-fermented milk, hydrolyzed pepsin and trypsin and in experiments done with its peptide fractions B, C and subfractions BI, CI

	Mitogen + RPMI 1640	Mitogen + 0.1 µg/ml hydrolysate	Mitogen + 10 µg/ml hydrolysate	Mitogen + 1000 µg/ml hydrolysate
Fermented milk (<i>n</i> = 7)	68,000 44,000–105,000	60,000* 40,000–94,000	63,000 39,000–104,000	58,000* 35,000–99,000
Fraction B (<i>n</i> = 7)	72,000 54,000–97,000	69,000 50,000–96,000	67,000 45,000–98,000	51,000* 32,000–82,000
Fraction C (<i>n</i> = 5)	75,000 54,000–102,000	74,000 51,000–108,000	68,000 49,000–94,000	58,000 30,000–114,000
Subfraction BI (<i>n</i> = 6)	83,000 63,000–111,000	75,000 55,000–104,000	66,000* 46,000–95,000	55,000* 37,000–80,000
Subfraction CI (<i>n</i> = 6)	80,000 62,000–103,000	80,000 60,000–108,000	67,000* 49,000–91,000	64,000* 49,000–84,000

Data presented are geometric mean(s) of measurements counts per minute of number of individual experiments (*n*) with 95% confidence intervals for the mitogen-induced cultures of peripheral blood without hydrolysate (mitogen + RPMI 1640 culture medium) and with hydrolysate at concentrations of 0.1 µg/ml (mitogen + 0.1 µg/ml hydrolysate), 10 µg/ml (mitogen + 10 µg/ml), and 1000 µg/ml (mitogen + 1000 µg/ml hydrolysate).

*Comparison with the control culture is statistically significant (*p* < 0.05) as determined by Wilcoxon signed-rank test.

with the hydrolyzed α_{s1} -casein, and a stimulatory trend was observed in experiments with the hydrolyzed κ -casein. As suggested in earlier studies, κ -caseins have the potency to suppress humoral immune responses to cow's milk antigens, whereas α_{s1} -caseins stimulate it.^{22,23} In our experiments the reverse was observed for PHA-induced proliferation, which is more likely to reflect T-lymphocyte responses.²⁴ Hence, our results with milk and caseins hydrolyzed with pepsin and trypsin first confirm that essential parts of caseins are resistant to heat treatment and enzymatic hydrolysis,⁴ and second, suggest that they retain their immunogenic characteristics at a certain level, as described for healthy individuals.

The second objective of this study stems from the data indicating that gut-processed food antigens result in an antigen-specific systemic hyporesponsiveness, whereas peripheral exposure to food antigens results in hyperresponsiveness.^{11,12} On the basis of the conception that intestinal microflora might be associated with the development of oral tolerance, earlier studies^{15,25,26} aimed to delineate the immune mechanisms with which particular strains (i.e., lactobacilli) could interfere. These studies established that feeding lactobacilli enhanced mucosal antibody responses to food antigens^{15,25} and feeding lactic acid bacteria-fermented milk enhanced the secretion of interferon- γ , a cytokine secreted mainly by activated T-helper lymphocytes.²⁶ In this study we investigated the effect of *L. casei* GG-fermented milk, hydrolyzed with pepsin and trypsin, on lymphocyte proliferative responses. The hydrolyzed *L. casei* GG-fermented milk modulated

the lymphocyte proliferation in vitro at some concentrations. By contrast, when its peptide fractions were isolated, a suppressive effect on the proliferation was detected with some of them (BIII, BIV, CII) at all three concentrations. A residual enzymatic activity is unlikely as the cause of this suppression because of deproteinization of products before fast protein liquid chromatography and a pH of 0.1% TFA in water and acetonitrile that was too low to retain the activity of pepsin and trypsin. These results suggest that *L. casei* GG-fermented milk might contain immunomodulatory peptides and that hydrolysis of fermented milk with pepsin and trypsin would not liberate these peptides.

On the basis of these findings, we further investigated the direct effect of bacterial degradation on the lymphocyte proliferative responses to cow's milk proteins. We chose caseins that were shown in the first set of experiments to have dual effects—suppression or stimulation—on proliferation. In the second set of experiments, when additionally hydrolyzed with *L. casei* GG-derived enzymes, these caseins exerted a single effect—suppression of proliferation. Measures were taken to stop enzymatic activity during preparation of these hydrolysates,¹⁷ and culture conditions were unfavorable for bacterial enzyme activity.^{18,19} Therefore the suppression appears to be a consequence of immunologic mechanisms. These results indicate that *L. casei* GG can directly modify the immunogenicity of cow's milk caseins. The effect of intestinal bacteria on processing of gut antigens has previously been demonstrated for different food

proteins.²⁷ Our results, as confined to bovine caseins, support the conception¹² that in the gut, enzymatic degradation by intestinal bacteria can release tolerogenic peptides from the native protein.

Clinical implications drawn from these results first focus on the treatment of food allergy. Elimination diets provide remission of clinical symptoms and promote the development of hyporesponsiveness to food antigen.^{28, 29} Elimination diets could be effective in reducing the proliferative responses of PBMCs to food antigens in vitro²⁸ and in enhancing mucosal antibody responses.^{28, 29} As demonstrated in animal models, systemic hyporesponsiveness to a food antigen is achieved by active immunoregulation,³⁰ and the kinetics of the response depend on the structure of the antigen.³¹ In this study, cow's milk caseins, additionally hydrolyzed by *L. casei* GG-derived enzymes, were found to be suppressors of the proliferation. In infants who are severely allergic to cow's milk, hypersensitivity reactions to other food antigens and to extensively hydrolyzed cow's milk formulas are not uncommon.^{1, 3, 5} The reason is that the antigenic epitopes can react with antigen-specific antibodies and cause IgE-mediated reactions.³² At this point, immunosuppressive peptides could be used to downregulate hyperresponsiveness of the host in general and consequently promote the tolerogenicity of cow's milk substitute formulas.

Of equal interest are the implications of our results for the prevention of food-induced hypersensitivity reactions. Various environmental factors have been documented as risk factors predisposing children to the development of atopy and food allergy.³³ In health, as a result of continuous allergen exposure, the suppression of immediate and delayed-type hypersensitivity mechanisms with simultaneous preservation of IgG production and interferon- γ -producing T-cell reactivity (i.e., immune deviation) evolves after a phase of T-cell-mediated regulation.³⁴ Empirical elimination diets or prolonged breast-feeding could prevent the sensitization of children at risk and thereby delay the onset of symptoms.³⁵ Such preventive interventions should also be vital to secure the immune deviation early in life. In this context, peptides with suppressive effects on lymphocyte proliferation could contribute to the efficacy of preventive interventions.

This study has established that degradation of food antigens with *L. casei* GG-derived enzymes modifies their immunomodulatory activity and consequently leads to suppression of the proliferation

of PBMCs in health. It is still a long way from suppressing PHA responses in vitro to suppressing immune responses in vivo. Our investigations of these products are in progress to delineate their cellular mechanisms, as well as to assess their peripheral effects in vitro in patients with food allergy.

REFERENCES

1. Sampson HA. Food allergy. *Curr Opin Immunol* 1990;2:542-7.
2. Kleinman RE, Bahna S, Powell GF, Sampson HA. Use of infant formulas in infants with cow milk allergy. A review and recommendations. *Pediatr Allergy Immunol* 1991;4:146-55.
3. Committee on Nutrition. Hypoallergic infant formulas. *Pediatrics* 1989;83:1068-9.
4. Jost R, Fritsche R, Pahud JJ. Reduction of milk protein allergenicity through processing. In: Somogyi JC, Müller HR, Ockhuizen T, editors. *Bibliotheca nutricia et diata: food allergy and intolerance. Nutritional aspects and developments*. 1st ed. Basel: Karger, 1991;48:127-37.
5. Sampson HA, Bernhisel-Broadbent J, Yang E, Scanlan SM. Safety of casein hydrolysate formula in children with cow milk allergy. *J Pediatr* 1991;118:520-5.
6. Wahn U, Wahl R, Rugo E. Comparison of the residual allergenic activity of six different hydrolyzed protein formulas. *J Pediatr* 1992;121:S80-4.
7. Britton JR, Kastin AJ. Biologically active polypeptides in milk. *Am J Med Sci* 1991;301:124-32.
8. Mowat AM. The regulation of immune responses to dietary protein antigens. *Immunol Today* 1987;8:93-8.
9. Walker WA. Allergen absorption in the intestine: implication of food allergy in infants. *J Allergy Clin Immunol* 1986;78:1003-9.
10. Challacombe SJ, Tomasi T. Systemic tolerance and secretory immunity after oral immunization. *J Exp Med* 1980;152:1459-72.
11. Strobel S, Mowat AMcI, Ferguson A. Prevention of oral tolerance induction to ovalbumin and enhanced antigen presentation during a graft-versus-host reaction in mice. *Immunology* 1985;56:57-64.
12. Peng HJ, Turner MW, Strobel S. The generation of a tolerogen after the ingestion of ovalbumin is time-dependent and unrelated to serum levels of immunoreactive antigen. *Clin Exp Immunol* 1990;81:510-5.
13. Heyman M, Crain-Denoyelle AM, Corthier G, Morgat JL, Desjeux JF. Postnatal development of protein absorption in conventional and germ-free mice. *Am J Physiol* 1986;14:G326-331.
14. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting-cell response in human diarrhea by a *Lactobacillus* strain. *Pediatr Res* 1992;32:141-4.
15. Isolauri E, Majamaa H, Arvola T, Rantala I, Virtanen E, Arvilommi H. *Lactobacillus casei* strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. *Gastroenterology* 1993;105:1643-50.
16. Syväoja EL. Quantitative determination of the main casein components and purification of α_{s2} - and κ -casein from bovine milk. *Milchwissenschaft* 1992;47:563-66.
17. Exterkate F, de Veer G. Partial isolation and degradation

- of caseins by cell wall proteinase(s) of *Streptococcus cremoris*. Appl Environ Microbiol 1985;49:328-32.
18. Eskola J, Soppi E, Viljanen M, Ruuskanen O. A new method for lymphocyte stimulation using whole blood. Immunol Comm 1975;4:297-303.
 19. Viljanen MK, Eskola J. PPD induced lymphocyte transformation in vitro using whole blood. Clin Immunol Immunopathol 1977;8:28-33.
 20. Asantila T, Toivanen P. Potentiation of flourodeoxy-uridine of ¹²⁵I-deoxyuridine uptake by human and chicken lymphocytes in the quantitation of mitogenic response. J Immunol Methods 1974;6:73-82.
 21. van Sickle GJ, Powell GK, McDonald PJ, Goldblum R. Milk and soy protein induced enterocolitis: evidence for lymphocyte sensitization to specific food proteins. Gastroenterology 1985;88:1915-21.
 22. Otani H, Monnai M, Hosono A. Bovine κ -casein as inhibitor of the proliferation of mouse splenocytes induced by lipopolysaccharide stimulation. Milchwissenschaft 1992;47:512-4.
 23. Kim SM, Enomoto A, Hachimura S, Yamauchi K, Kaminogawa S. Serum antibody response elicited by a casein diet is directed to only limited determinants of α_{s1} -casein. Int Arch Allergy Immunol 1993;101:260-5.
 24. Press OW, Rosse C, Clagett J. Phytohemagglutinin-induced differentiation and blastogenesis of precursor T cells from mouse bone marrow. J Exp Med 1977;146:735-40.
 25. Perdígón G, de Macías MEN, Alvarez S, Oliver G, de Ruiz Holgado AP. Systemic augmentation of immune response in mice by feeding fermented milks with *Lactobacillus casei* and *Lactobacillus acidophilus*. Immunology 1988;63:17-23.
 26. De Simone C, Salvadori B, Negri R, Ferrazzi M, Baldinelli L, Vesely R. The adjuvant effect of yogurt on production of gamma-interferon by ConA-stimulated human peripheral blood lymphocytes. Nutr Rep Int 1986;33:419-33.
 27. Moreau MC, Corthier G. Effect of the gastrointestinal microflora on induction and maintenance of oral tolerance to ovalbumin in C3H/HeJ mice. Infect Immun 1988;56:2766-8.
 28. Isolauri E, Suomalainen H, Kaila M, Jalonen T, Soppi E, Virtanen E, Arvilommi H. Local immune responses in patients with cow milk allergy: follow-up of patients retaining allergy or becoming tolerant. J Pediatr 1992;120:9-15.
 29. Agata H, Kondo N, Fukutomi O, Shinoda S, Orii T. Effect of elimination diets on food specific IgE antibodies and lymphocyte proliferative responses to food antigens in atopic dermatitis patients exhibiting sensitivity to food allergens. J Allergy Clin Immunol 1993;91:668-79.
 30. Mowat A. Depletion of suppressor T cells by 2'-deoxyguanosine abrogates tolerance in mice fed ovalbumin and permits the induction of intestinal delayed-type hypersensitivity. Immunology 1986;58:179-84.
 31. Wold AE, Dahlgren UIH, Hanson LA, Mattsby-Baltzer I, Midvedt T. Difference between bacterial and food antigens in mucosal immunogenicity. Infect Immun 1989;57:2666-73.
 32. Sampson HA, James JM, Bernhisel-Broadbent J. Safety of an amino acid-derived infant formula in children allergic to cow milk. Pediatrics 1992;90:463-5.
 33. Bos JD, Kapsenberg ML, Sillevius Smitt JH. Pathogenesis of atopic eczema. Lancet 1994;343:1338-41.
 34. McMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. J Exp Med 1993;178:889-99.
 35. Zeiger RS, Heller S, Mellon M, O'Connor R, Hamburger RN. Effectiveness of dietary manipulation on the prevention of food allergy in infants. J Allergy Clin Immunol 1986;78:224-38.