Down-Regulation of Anti-CD3 Antibody-Induced IL-4 Production by Bovine Caseins Hydrolysed with Lactobacillus GG-Derived Enzymes

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A prerequisite for systemic hyporesponsiveness to dietary antigens is their processing in the gut. This study investigated whether bovine caseins degraded by enzymes of an intestinal bacterial strain, Lactobacillus GG (ATCC 53103), could regulate the cytokine production by anti-CD3 antibody-induced peripheral blood mononuclear cells of 14 atopic patients, aged 5–29 (mean, 16) months. Purified casein up-regulated the interleukin-4 and interferon-γ production, \( P = 0.008 \) and \( P = 0.008 \), respectively. Conversely, Lactobacillus GG-degraded casein down-regulated the interleukin-4 production, \( P = 0.003 \), with no effect on interferon-γ. These results indicate that intestinal bacteria may modify immunomodulatory properties of native food proteins and introduce a promising tool to provide protection from potentially harmful dietary antigens at a young age.

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INTRODUCTION

Atopic dermatitis (AD) is associated with IgE production to common environmental allergens. Interleukin-4 (IL-4) induces IgE production, whereas interferon-γ (IFN-γ) suppresses it [1, 2]. AD patients manifest high production of IL-4 and little or no production of IFN-γ in their peripheral blood [3], indicating that an imbalance of the cytokine production profile is relevant to the pathogenesis of AD. To restore this imbalance would comprise a new objective in the treatment of AD.

Systemic hyporesponsiveness to ingested protein antigens, i.e., oral tolerance, depends on their processing in the gut [4]. Intestinal bacteria may contribute to the generation of tolerogens from native protein antigens [5]. Bovine caseins degraded by enzymes of an isolated human intestinal bacterial strain, Lactobacillus GG (ATCC 53103), suppress the proliferation of phytohaemagglutinin (PHA)-induced peripheral blood mononuclear cells (PBMC) [6], suggesting their ability to modulate T-cell responses. We hypothesize here that such an effect of Lactobacillus GG-degraded antigens may help restore the cytokine imbalance in AD. We therefore investigated whether Lactobacillus GG-degraded casein can modulate the IL-4- and IFN-γ-producing immune responses in AD patients.

MATERIALS AND METHODS

Patients. Altogether, 14 patients aged 5–29 (mean, 16) months who fulfilled the Hanifin criteria for atopic dermatitis [7], and eight age-matched non-atopic healthy children were studied. None were receiving systemic corticosteroids at the time of the study.

Reagents. OKT3 (anti-CD3-antibody) containing ascites fluid was a kind gift from Dr M. Kaartinen, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland. Bovine total casein had been purified from bovine milk [8]. Purified casein was hydrolysed with Lactobacillus GG-derived enzymes as described previously [6]. Purified casein or Lactobacillus GG-degraded casein were lyophilized and stored at room temperature (24°C). Before experiments, they were diluted in RPMI 1640 and filter sterilization (0.1 μm, Millipore corporation, Bedford, MA, USA) was applied.

Cytokine assays. Complete culture medium consisted of RPMI 1640 with 10% fetal calf serum, 10 mM Hepes buffer, 2 mM L-glutamin (Gibco Life Technologies, Paisley, UK), 50 U/ml benzylpenicillin (Sigma, St Louis, MO, USA), 10 mg/ml gentamycin (Roussel Laboratories Ltd, Uxbridge, Middlesex, UK). Peripheral venous blood (5 ml) was obtained. PBMC containing >90% lymphoid cells was isolated by Ficoll–Paque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation and suspended at 1 × 10^6 cells/ml in complete culture medium. Culture wells were precoated with anti-CD3 antibody containing ascites fluid at a pretested optimal dilution. The test culture additionally contained...
dilution of casein or Lactobacillus GG-degraded casein at a final concentration of 1 mg/ml. These experiments were repeated for purified bovine αs1-casein or Lactobacillus GG-degraded αs1-casein. After 24 h of incubation in a humidified 5% CO₂ atmosphere at 37°C, supernatants were collected and stored at −70°C for cytokine assays. IL-4 and IFN-γ in culture supernatants were determined by commercially available ELISA kits according to manufacturers’ instructions (IL-4: CLB, Compact Human Interleukin-4 ELISA kit, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands; IFN-γ: E-IFNG, Endogen Inc., Cambridge, MA, USA). The results from different runs were equalized employing the comparison of standard curves and were expressed as pg/ml. The sensitivity of the assays for IL-4 was 2.3 pg/ml and for IFN-γ, 5 pg/ml. Wilcoxon signed-rank test was used in statistical comparisons of the test cultures to the control cultures. The level of significance was P < 0.05.

RESULTS

In atopic patients, both IL-4 and IFN-γ production were increased in cultures containing purified casein when compared to control cultures, P = 0.008 and P = 0.008, respectively (Fig. 1a,b). No such effect of bovine casein was observed when degraded by enzymes of Lactobacillus GG. Conversely, the IL-4 production in cultures containing Lactobacillus GG-degraded casein was significantly less than in control cultures, P = 0.003 (Fig. 1a), and the IFN-γ production in these cultures was comparable to control cultures, P = 0.10 (Fig. 1b).

In healthy children, the production of IL-4 and IFN-γ in cultures containing purified casein were comparable to control cultures, P = 0.10 and P = 0.10 (Fig. 1c,d). In parallel to the findings in atopic patients, healthy children had significantly less IL-4 production in cultures containing Lactobacillus GG-degraded casein than in control cultures, P = 0.01 (Fig. 1c) and the IFN-γ production in these cultures remained comparable to control cultures, P = 0.50 (Fig. 1d). Parallel results were obtained with αs1-casein and Lactobacillus GG-degraded αs1-casein (Fig. 2).

DISCUSSION

Specific immunotherapy can reduce IL-4 production by allergen-specific CD4⁺ T cells [9]. Indeed, our results confirm that cytokine profiles in atopic individuals are mutable and indicate that intestinal bacteria can help down-regulate the IL-4 production capacity in atopics.

We have previously shown a suppressive effect of Lactobacillus GG-degraded bovine caseins on the lymphocyte proliferation in healthy individuals [6]. Before our experiments,
the methodology had been ascertained by fast protein liquid chromatography that the hydrolysates were deproteinized and contained no residual enzymatic activity. In the present study, we extended our investigations to atopic patients. Atopic patients manifested differential patterns of IL-4 and IFN-γ production in cultures containing purified caseins and in cultures containing degraded caseins. Degraded caseins down-regulated the IL-4 production in both atopics and nonatopics, with no effect on IFN-γ. The IL-4-associated downregulatory effect was consistent in a separate series of experiments performed with purified T cells or with phorbol esters plus calcium ionophore as mitogen in cultures (data not shown), reinforcing the possibility of a direct interaction between Lactobacillus GG-degraded caseins and T cells. Clinical studies with Lactobacillus GG indicate that fortification of maternal diet with Lactobacillus GG can reduce the severity scores of skin symptoms in breast-fed infants with atopic dermatitis (H. Majamaa et al., unpublished observations). Moreover, when cow milk allergic infants were fed with an extensively hydrolysed whey formula fortified with Lactobacillus GG, reduction in the severity of atopic dermatitis was significantly greater than patients with no Lactobacillus GG in the diet. Altogether, intestinal bacteria can degrade dietary allergens and modify their original immunomodulatory properties. IgE antibodies to foods are common in infants around 3–8 months of age, at the age when new foods are introduced into the diet [10]. In nonatopic infants, allergen-specific IgE titres in sera are low and followed by the suppression of IgE and delayed-type hypersensitivity responses and the preservation of IgA, IgG production and IFN-γ-producing T-cell responses, i.e. immune deviation [10, 11]. However, in atopic infants who manifest allergic disease later in life, the IgE titres are high and persistent concomitantly with high IL-4 and low IFN-γ production by their PBMC [10, 3]. These observations indicate that primary allergen sensitization plays a key role in the development of allergic disease. Thus, effective regulation of immune responses at such an initial phase of sensitization may secure the ‘immune deviation’ and control the frequency of allergic disease. Our findings with Lactobacillus GG degraded bovine caseins. This study was supported by the Academy of Finland and the Foundation of Nutrition Research.

**REFERENCES**


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