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Milk basic protein supplementation exerts an anti-inflammatory effect in a food-allergic enteropathy model mouse

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ABSTRACT

To examine novel functions of milk basic protein (MBP) in T-cell-related inflammatory diseases, such as autoimmune diseases and allergies, we evaluated the effects of MBP on the causative responses of ovalbumin (OVA)-specific T cells in a food-allergic enteropathy model, OVA23–3 mice, which express an OVA-specific T-cell receptor gene. The OVA-specific $CD4^+$ T cells of the mesenteric lymph nodes (MLN) from OVA23–3 mice were cultured with $CD11c^+$ dendritic cells of MLN from BALB/cA mice in the absence or presence of MBP following stimulation with OVA; then the levels of CD69 expression and the levels of cytokine production by $CD4^+$ T cells were measured to evaluate activation. The effects of MBP supplementation of OVA 23–3 mice were assessed by feeding a diet containing OVA (OVA diet) with or without MBP for 28 d. Intestinal inflammation, together with activation and cytokine production of $CD4^+$ T cells by MLN, as well as femoral bone mineral density, were measured. In in vitro culture, MBP inhibited excess activation and IL-4 production by $CD4^+$ T cells. The supplementation of MBP to the OVA diet attenuated OVA-specific IgE production in OVA-diet-fed OVA23–3 mice and slightly resolved developing enteropathy caused by excess IL-4 production by $CD4^+$ T cells. Feeding OVA diet to OVA23–3 mice exhibited bone loss accompanied with enteropathy, whereas MBP supplementation prevented bone loss and increased osteoprotegerin, an osteoclastogenesis inhibitory factor, in the mice. The inhibition of T-cell-activation in both MLN and bone marrow by MBP supplementation may help prevent increased IgE levels caused by excessive IL-4 production and bone loss accompanied by enteropathy. Our findings show that MBP may help attenuate both T-cell-related inflammation and bone loss.

Key words: milk basic protein, food-allergic enteropathy, anti-inflammation, bone

INTRODUCTION

An increase in inflammatory diseases such as autoimmune diseases and allergies caused by T-cell activation (Lindelöf et al., 2009; Chen and Flies, 2013; Luo et al., 2015; Kuwabara et al., 2017) is becoming an important social problem. Inflammation is an essential reaction for host defense, but in food-allergic inflammation, excessive immune responses to food proteins cause severe symptoms in different tissues of the body, as well as in the intestinal tract. The prevalence of food allergies is apparently increasing (Simons et al., 2015; Grabenhenrich et al., 2016); thus, preventing food allergies is important. Food allergies are believed to be caused by abnormal Th2 immune responses to food allergens, and activated $CD4^+$ T cells play a central role in several symptoms involving allergen-specific IgE production. Ovalbumin (**OVA**) is a leading egg allergen, and OVA-specific T-cell receptor transgenic mice (**OVA23–3**) show food allergic intestinal inflammation and weight loss when solely fed an OVA-based diet (Nakajima-Adachi et al., 2006). Severe intestinal inflammation in the OVA-fed mice was caused by the induction of excessive IL-4-producing $CD4^+$ T cells in the mesenteric lymph nodes (**MLN**; Nakajima-Adachi et al., 2014). Recent clinical studies have shown that intestinal inflammation, such as inflammatory bowel disease and celiac disease caused by the ingestion of wheat gluten (Bernstein and Leslie, 2003; Bernstein, 2006; Alaedini and Greem, 2005; Gordon, 2006) is associated with an increased risk of osteoporosis. Receptor activator of NF-κB ligand (**RANKL**), a factor required for the differentiation, maturation, and activation of osteoclasts, and osteoprotegerin (**OPG**), a decoy receptor of RANKL, are known to be key factors in bone remodeling, and an imbalance in serum concentration of RANKL and OPG has been reported in inflammatory bowel disease and celiac patients (Silvennoinen et

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al., 1995; Franchimont et al., 2004; Taranta et al., 2004; Moschen et al., 2005; Fiore et al., 2006), suggesting a relationship between intestinal inflammation and bone loss. Given this potential relationship, it is important to determine whether bone loss occurs in OVA-fed OVA23–3 mice that develop enteropathy caused by excessive IL-4-producing $CD4^+$ T cells.

Milk has long been accepted as a beneficial food because it contains various health nutrients essential for the human body. In addition, it has been found that milk contains immunomodulatory effective components such as lipids and proteins. Milk lipids such as polar lipids (phospholipids and sphingolipids), CLA, and n-3 long-chain PUFA are known to prevent diabetes and cardiovascular diseases to suppress chronic inflammation (Lordan and Zabetakis, 2017). Milk proteins, especially whey proteins, are also reported to suppress the inflammation, and prevent colitis and arthritis (Wong and Watson, 1995; Low et al., 2003). Milk whey proteins, particularly in those with basic properties, have been identified as anti-inflammatory bioactive compounds (Otani, 2006; Mohanty et al., 2016). Therefore, we have focused on proteins with alkaline isoelectric points, isolated the fraction containing them from defatted milk using a cation exchange chromatography, and named this protein fraction as milk basic protein (**MBP**). Milk basic protein is composed of some proteins; the principal protein components of MBP are lactoferrin (**LF**), lactoperoxidase (**LPO**), angiogenin (**ANG**), and cystatin C (**CysC**) as previously reported (Matsuoka et al., 2002; Aoe et al., 2005; Kruger et al., 2007; Morita et al., 2008, 2011, 2012; Ishida et al., 2017). In addition, other milk proteins such as high mobility group (**HMG**)-like protein and kininogen fragment 1/2 are slightly included (Yamamura et al., 1999, 2000, 2006). It has also reported that the content of casein in MBP was only a trace amount [GRAS Notice 000196 (2006); Goodman et al., 2007]. In each protein component such as LF, LPO, ANG, and CysC purified from MBP, their own biological activities have not been fully examined. However, because no heat is added in the MBP preparation process, it is suggested that their conformation is preserved as it exists in fresh milk, that is, LF preparations from milk contained ANG (Tanigawa et al., 2001; Morita et al., 2012), and conversely, ANG preparation contained LF (Bharadwaj et al., 2009), suggesting that LF and ANG might form a complex structure. Therefore, it is possible that the components of MBP (at least LF and ANG) exert their functions as a complex when administrated.

Dietary supplementation with MBP prevented bone loss in ovariectomized animal models (Kato et al., 2000; Toba et al., 2000; Morita et al., 2012) and postmenopausal women (Aoe et al., 2001, 2005; Aoyagi et al., 2010). In addition the previous studies imply that MBP improves bone remodeling by promoting osteoblast-mediated bone formation and suppressing osteoclast-mediated bone resorption (Yamamura et al., 1999, 2000; Matsuoka et al., 2002; Morita et al., 2008, 2011), although little is known about the immunomodulatory effects of MBP. We thought that it might be possible for MBP to function as a basic whey protein with anti-inflammatory bioactive compounds as described above.

In this study, we examined the anti-inflammatory functions of MBP in T-cell-related inflammatory diseases by evaluating the suppressive effects of MBP on the causative responses of OVA-specific $CD4^+$ T cells in OVA23–3 mice. The supplementation of MBP inhibited excessive IL-4 production by $CD4^+$ T cells, thereby possibility resolving the developing enteropathy, and also prevented bone loss in these mice. Therefore, MBP is a useful food for preventing both enteropathy and bone loss caused by T-cell-related inflammation.

MATERIALS AND METHODS

Mice

Six-week-old male BALB/cA mice were purchased from CLEA Japan Inc. (Tokyo, Japan), and OVA23–3 mice with a BALB/cA mouse genetic background were kindly provided by S. Habu (Tokai University School of Medicine). Mice were fed a commercial CE-2 diet (CLEA Japan Inc.) until they were used in the experiment. All mice were housed individually in stainless-steel cages in a temperature- and humidity-controlled room (23°C and 40 ± 5% relative humidity) with a 12 h light/dark cycle. The mice were treated in accordance with the animal experimentation regulations of the Milk Science Research Institute of Megmilk Snow Brand Co., Ltd., which are based on the guidelines proposed by the Science Council of Japan.

Preparation of MBP

The preparation of MBP from bovine milk was described by Toba et al. (2000). In brief, fresh bovine milk was defatted by centrifugation and loaded onto a column packed with a cation-exchange resin. Acidic milk protein including casein and lactose were removed in the flow through fractions, whereas the basic protein was bound to the cation-exchange resin. The column was thoroughly washed with deionized water, and then the adsorbed protein was eluted with 1 *M* sodium chloride. The eluted fraction was desalted using cellulose membrane (cutoff 14 kDa) and then lyophilized as MBP. The protein content of the MBP sample was determined to be 98% (wt/wt) by Kjeldahl method