

Dietary Whey Protein Hydrolysate Suppresses Development of Atopic Dermatitis-like Skin Lesions Induced by Mite Antigen in NC/Nga Mice

Nobuko Shimizu¹, Kazuo Dairiki¹, Saori Ogawa¹ and Tetsuo Kaneko¹

ABSTRACT

Background: Oral administration of enzymatic hydrolysate of cow's milk whey protein (WPH) has been reported to produce an anti-inflammatory effect. Since inflammation plays a role in dermatitis of allergic disease, we examined the influence of WPH on the development of atopic dermatitis (AD)-like skin lesions, induced in NC/Nga mice by the mite antigen *Dermatophagoides pteronyssinus* (Dp).

Methods: AD-like skin lesions were induced on the pinnae and backs of NC/Nga mice by daily application of Dp for 4 weeks. Mice were fed cow's milk casein (control), WPH or casein protein hydrolysate (CPH) diets for 2 weeks prior to Dp application. Clinical skin conditions were evaluated periodically by a clinical severity score, total serum IgE and soluble E-selectin levels were measured by enzyme linked immunosorbent assay (ELISA).

Results: WPH-fed mice showed significantly less AD-like skin lesions than those fed casein diets at 2 and 4 weeks after Dp application. In contrast, CPH-fed mice had manifestations in a similar manner as casein-fed mice did, and did not show an inhibitory effect. Serum soluble E-selectin levels, known as a marker of disease activity in AD patients, were significantly lower in the WPH diet group.

Conclusions: Our results suggest that in addition to its hypoallergenicity an anti-inflammatory function, dietary WPH might be useful for reducing the severity of AD-like skin lesions.

KEY WORDS

atopic dermatitis, *Dermatophagoides pteronyssinus*, E-selectin, mice, whey protein

INTRODUCTION

Whey protein comprises approximately 20% of the protein found in milk.¹ Certain whey proteins, such as lactoferrin, have been demonstrated to have anti-inflammation properties.² Lactoferrin has actions to regulate levels of tumor necrosis factor (TNF) and interleukin 6 (IL-6), thus decreasing inflammation. Furthermore, whey protein hydrolysate (WPH) has been reported to possess antioxidant³ and anti-inflammation effects.⁴ However, WPH's effects on atopic diseases have not been studied.

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by pruritis, erythema, edema, excoriation, and dryness. Various serum pro-

teins, such as soluble adhesion molecules and chemokines, have been reported to correlate with the inflammation in AD.⁵ Recently, NC/Nga mice have been used as a model for investigating the mechanism of moderate AD, with several reports of AD-like skin lesions induced by mite antigen.⁶⁻⁹

In this study, we examined the suppressive effects of WPH diets on development of AD-like lesions in NC/Nga mice. In addition, we searched for a serum marker that is correlated with the skin lesions in the mice, as well as in humans with AD.

METHODS

ANALYSIS OF WHEY PROTEIN HYDROLYSATE

Whey protein (Meiji Dairies Corporation) was enzy-

¹Food Science Institute, Division of Research and Development, Meiji Dairies Corporation.

Correspondence: Nobuko Shimizu, Food Science Institute, Division of Research and Development, Meiji Dairies Corporation, 540 Naruda, Kanagawa 250-0862, Japan.

Email: NOBUKO_SHIMIZU@MEIJI-MILK.COM

Received 31 August 2005. Accepted for publication 6 December 2005.

©2006 Japanese Society of Allergology

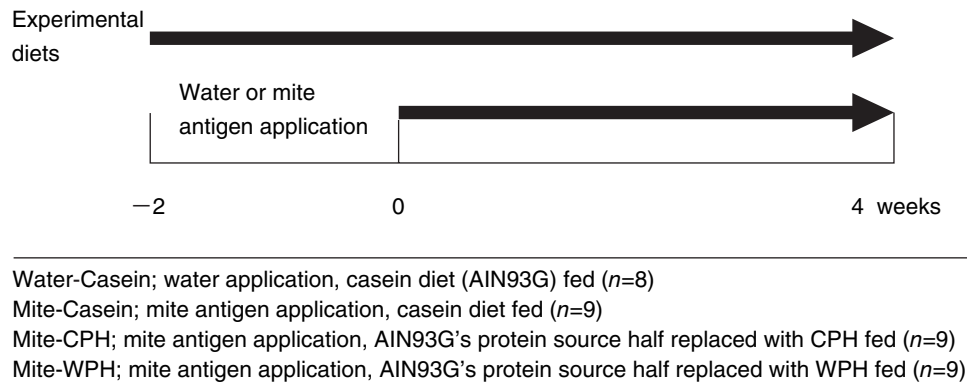


Fig. 1 Experimental design for the induction of atopic dermatitis like lesions in NC/Nga mice.

matically hydrolyzed and its molecular weight distribution determined by gel-permeation highperformance liquid chromatography (GP-HPLC) in a TOSOH G2000SWXL separating column (7.8 mm ID $\times$ 30 cm; TOSOH, Tokyo, Japan) with 0.1% TFA, 45% acetonitrile as the mobile phase, a flow rate of 0.5 ml/min, room temperature and UV detection at 215 nm. Casein protein hydrolysate (CPH) was also characterized in the same manner.

MICE

Four-week-old NC/Nga female mice (Charles River Japan, Kanagawa, Japan) were housed at $21 \pm 2^\circ\text{C}$ in $55 \pm 15\%$ humidity with a 12-hour light-dark cycle (light on 7 AM–7 PM) in SPF condition. After preliminary breeding for 1 week, the mice were divided into four groups, Water-casein, Mite-casein, Mite-CPH and Mite-WPH as shown in Figure 1. All experiments were performed in accordance with the animal care ethics guidelines of Meiji Dairies Corporation.

INDUCTION OF ATOPIC DERMATITIS

AD-like skin lesions were induced using a modification of the procedure described by Unno *et al.*⁶ The mite *Dermatophagoides pteronyssinus* (Dp; LSL, Tokyo, Japan) was extracted and adjusted to 4.5 mg/ml protein concentrations (BSA conversion). Mice were fed experimental diet for 2 weeks and water or mite antigen applied daily for 4 weeks to the sheared back skin and pinnae with a brush. Prior to each application, 4% SDS was applied to disrupt the skin barrier (Fig. 1).

SKIN STATUS

Skin status was observed weekly. The degree of erythema/hemorrhage, edema, excoriation/erosion, and dryness were each scored from 0 points (none) to 3 points (severe). The sum of these scores was defined as the skin score.¹⁰

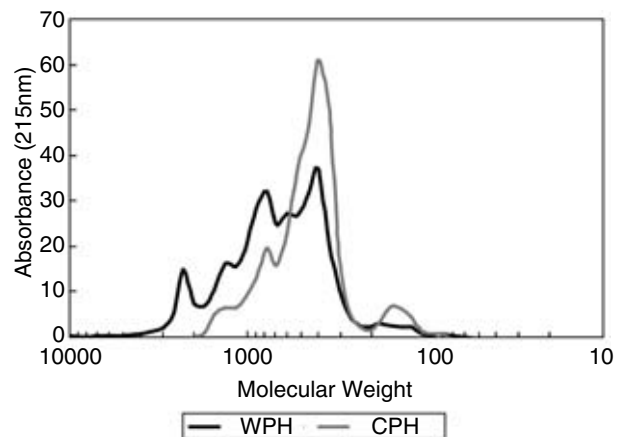


Fig. 2 Chromatogram of whey protein hydrolysate (WPH) and casein protein hydrolysate (CPH) was monitored by UV detector.

SERUM IgE LEVELS MEASUREMENT

Serum samples were collected on days 0, 14, 21 and 28 after starting the antigen application and stored at -40°C for analysis. Total IgE levels in the serum were assessed by sandwich enzyme linked immunosorbent assay (ELISA). Antibodies used were: capture antibody; clone R35-72, and biotin-conjugated antibody; clone R35-118 (BD Pharmingen, San Diego, CA, USA). Standard IgE used was clone C38-2 (BD Pharmingen).

SERUM SOLUBLE E-SELECTIN MEASUREMENT

Soluble E-selectin in serum samples was measured using a mouse serum soluble E-selectin assay kit (R&D; Minneapolis, MN, USA).

STATISTICAL ANALYSIS

Skin score data was analyzed by cumulative chi square test: 1 way type. Serum IgE and sE-selectin

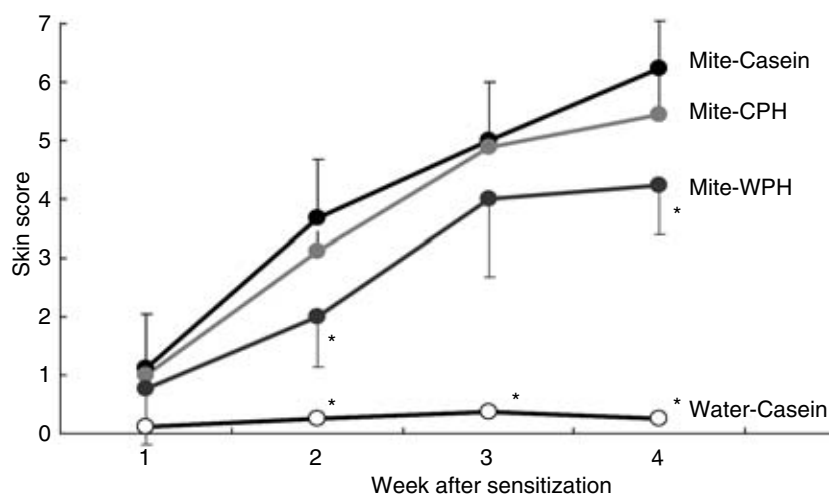


Fig. 3 Skin score observed weekly after water/mite antigen application in NC/Nga mice. Water-Casein: open circles; Mite-Casein: closed circles; Mite-CPH: light grey circles; Mite-WPH: dark grey circles. Each point indicates the mean $\pm$ SD. (* $P < 0.05$)

levels were analyzed by Student's *t* test. A *p* value of less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

HPLC CHART OF WPH

Molecular weight distributions of WPH and CPH are shown in Figure 2. The mean molecular weights of WPH and CPH were nearly 800 and 400 Daltons, respectively.

SUPPRESSIVE EFFECTS OF WPH DIET ON SKIN LESION

Mite antigen-applied mice appeared to develop AD-like skin lesion. Skin scores of casein-fed and CPH-fed mice increased 2 weeks after the beginning of the mite extract application (Fig. 3). In contrast, WPH-fed mice showed significantly lower skin scores than casein-fed mice after both 2 and 4 weeks of application. Mice receiving water applications did not show AD-like skin findings.

Typical photographs of each group after 4 weeks of application are shown in Figure 4 (A)–(D). The mean itemized skin scores are shown in Figure 3 (E). Skin scores for groups (A)–(D) were 1 (Water-Casein), 7 (Mite-Casein), 9 (Mite-CPH) and 4 (Mite-WPH), respectively. Erythema/hemorrhage and excoriation/erosion scores were significantly lower in WPH-fed mice than in casein-fed mice. Scores for each item were similar between the casein-fed and CPH-fed mice. Scratching behavior of mice was recorded for 1 minute. There was no significant difference among the groups (data not shown).

TOTAL IgE AND DP-SPECIFIC IGE LEVELS

Total IgE levels increased in all 3 groups that had received mite antigen. However, there was no significant difference among the groups (Fig. 5). Total IgE levels of mice receiving water applications were less than 1 μ g/ml.

SOLUBLE E-SELECTIN LEVELS

WPH-fed mice showed significantly lower sE-selectin levels than CPH-fed mice (Fig. 6). A significant correlation between the skin score and sE-selectin was observed. On the other hand, serum RANTES and sICAM-1 levels, which have also been reported as serum markers in AD patients¹¹, did not correlate with skin scores (data not shown).

DISCUSSION

In this study, we investigated the suppressive effect of WPH against AD-like skin lesions induced by mite antigen in NC/Nga mice. Skin scores of WPH-fed mice were significantly lower than those of CPH-fed mice at both 2 and 4 weeks after beginning antigen application. Hematoxylin and eosin stained histological sections from ears showed that pathologic tissue of WPH-fed mice tend to have less infiltration of inflammatory cells such as neutrophils than that of CPH-fed mice (data not shown). On the other side, scratching behavior did not correlate with skin score. Perhaps longer periods of observation are needed.⁹

Since a significant difference in the severity of the skin lesions was observed among Mite-Casein, Mite-CPH and Mite-WPH groups, a difference in total serum IgE was expected. However, total IgE levels were similarly elevated in all antigen groups, irre-

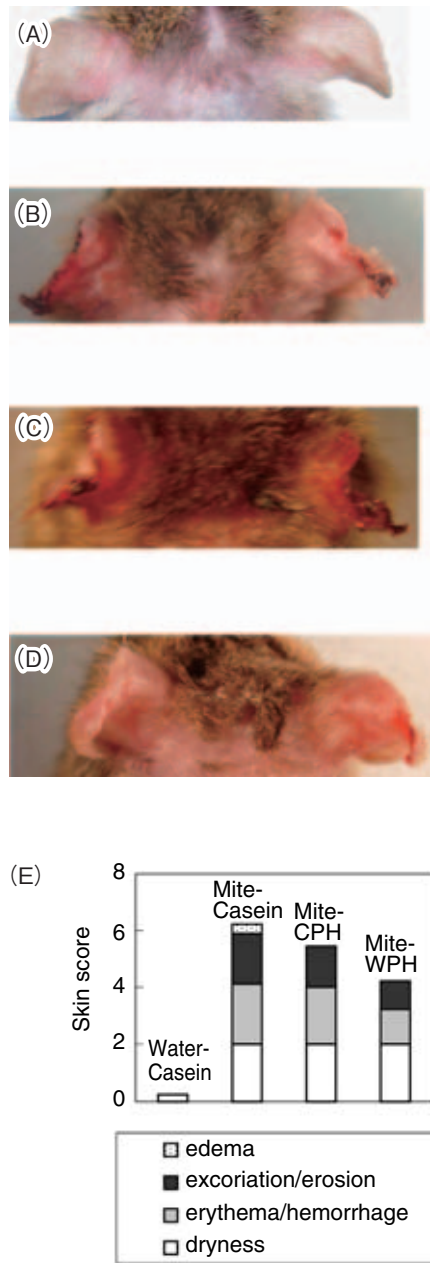


Fig. 4 Skin features of NC/Nga mice after 4 weeks mite extract-Dp application. A-D: Typical photograph of each group, E: Mean itemized skin score.

spective of diet. The mechanism for suppressive effect of WPH on AD-like skin findings is therefore thought to be independent of the induction of IgE production.

We investigated serum markers in our mouse model, which have been reported to correlate with the skin lesions in human AD. Serum sE-selectin levels in WPH-fed mice were found to be significantly lower than those of CPH-fed mice.

The cell expression of E-selectin is regulated by cy-

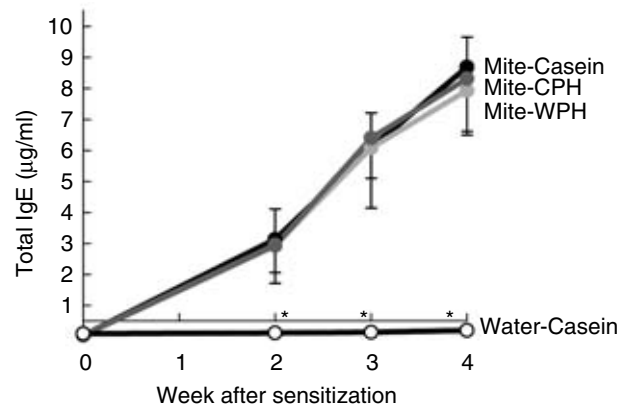


Fig. 5 Serum total IgE levels in NC/Nga mice. Water-Casein: open circles; Mite-Casein: closed circles; Mite-CPH: light grey circles; Mite-WPH: dark grey circles. Each point indicates the mean $\pm$ SD. (* P < 0.05)

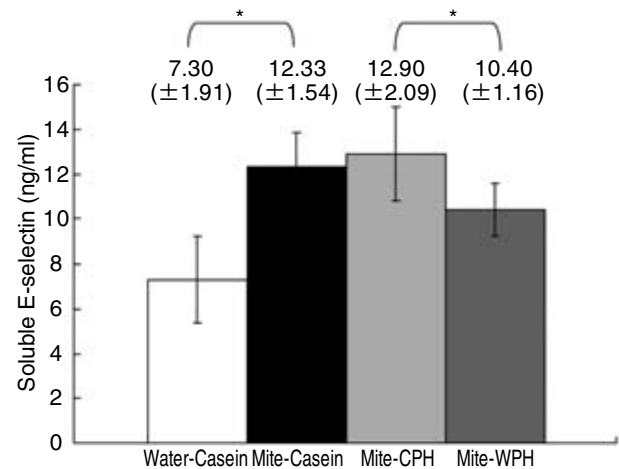


Fig. 6 Serum soluble E-selectin levels in NC/Nga mice 4 weeks after water/mite antigen application. Water-Casein: white; Mite-Casein: black; Mite-CPH: light grey; Mite-WPH: dark grey. Each point indicates the mean $\pm$ SD. (* P < 0.05)

tokines such as $\text{TNF-}\alpha$. In this study, there was no significant difference among the three groups that had received mite antigen in cell proliferation or cytokine (such as $\text{TNF-}\alpha$) production from cervical lymph nodes (data not shown). The suppressive effect of WPH on AD-like skin lesions therefore did not involve T-cells, but was likely due to macrophages at the inflammation site.

The different effects of WPH and CPH in this study are of interest. In addition, mite antigen induced AD-like skin lesions was suppressed by the whey protein diet (AIN93G's protein source half replaced with whey protein) fed mice, compared with those receiving the all-casein diet (data not shown). Not only is

the amino acid proportion but also the protein components are different between casein and whey protein. In addition, the peptides resulting from protein digestion are different. Whey proteins such as lactoferrin possess anti-inflammation effects,² and WPH has also been reported to possess anti-inflammation effects.⁴ In contrast, casein peptides enhance TNF- α and IL-6 production by bone marrow macrophages in mice.¹² These biological differences between whey and casein proteins might explain the difference in the severity of skin lesions observed in WPH- and CPH-fed mice exposed to mite antigens. Further experiments are necessary to clarify the mechanisms.

Milk proteins are used in infant formula. WPH and CPH have been used as the major protein source in formulas for infants with allergic disease to reduce antigen reactions.¹³⁻¹⁵ In this study, we showed that oral administration of WPH could reduce the severity or delay development of AD-like skin lesions in NC/Nga mice, compared with CPH. That is, WPH-based infant formula is likely to have not only low antigen reactivity, but also to have a favorable clinical effect over CPH-based infant formula for infants with atopic dermatitis.

ACKNOWLEDGEMENTS

We are grateful to Dr. Hiroshi Kano for excellent guidance and helpful discussion.

REFERENCES

1. Marshall K. Therapeutic applications of whey protein. *Altern. Med. Rev.* 2004;**9**:136-156.
2. Machnicki M, Zimecki M, Zagulski T. Lactoferrin regulates the release of tumour necrosis factor alpha and interleukin 6 *in vivo*. *Int. J. Exp. Pathol.* 1993;**74**:433-439.
3. Hernandez-Ledesma B, Davalos A, Bartolome B, Amigo L. Preparation of antioxidant enzymatic hydrolysates from alpha-lactalbumin and beta-lactoglobulin. Identification of active peptides by HPLC-MS/MS. *J. Agric. Food Chem.* 2005;**53**:588-593.
4. Kume H. [Applying Nutrigenomics to Food Science -10- Anti-inflammation Effect of Whey Proteins and Their Trypsin-hydrolyzed Peptides on Hepatic Injury Induced by Concanavalin A -DNA Microarray Analysis-]. *ILSI* 2005;**83**:9-17 (in Japanese).
5. Morita E, Hiragun T, Mihara S *et al.* Determination of thymus and activation-regulated chemokine (TARC) - contents in scales of atopic dermatitis. *J. Dermatol. Sci.* 2004;**34**:237-240.
6. Unno T, Suto H, Yoshiike T, Ogawa H, Ra C. [Induction of atopic dermatitis-like skin lesion in NC/Nga mice—the influence of the skin barrier destroying solution to the induction of dermatitis]. *Alerugi* 2001;**50**:1152-1162 (in Japanese).
7. Gao XK, Nakamura N, Fuseda K *et al.* Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. *Biol. Pharm. Bull.* 2004;**27**:1376-1381.
8. Heishi M, Imai Y, Katayama H *et al.* Gene expression analysis of atopic dermatitis-like skin lesions induced in NC/Nga mice by mite antigen stimulation under specific pathogen-free conditions. *Int. Arch. Allergy Immunol.* 2003;**132**:355-363.
9. Matsuoka H, Maki N, Yoshida S *et al.* A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* 2003;**58**:139-145.
10. Matsuda H, Watanabe N, Geba GP *et al.* Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int. Immunol.* 1997;**9**:461-466.
11. Jahnz-Rozyk K, Targowski T, Paluchowska E, Owczarek W, Kucharczyk A. Serum thymus and activation-regulated chemokine, macrophage-derived chemokine and eotaxin as markers of severity of atopic dermatitis. *Allergy* 2005;**60**:685-688.
12. Chaowu Xiao LJ, Xin Z. Bovine casein peptides co-stimulate naive macrophages with lipopolysaccharide for proinflammatory cytokine production and nitric oxide release. *J. Sci. Food Agric.* 2000;**81**:300-304.
13. Sampson HA, Bernhisel-Broadbent J, Yang E, Scanlon SM. Safety of casein hydrolysate formula in children with cow milk allergy. *J. Pediatr.* 1991;**118**:520-525.
14. Cantani A, Micera M. Immunogenicity of hydrolysate formulas in children (Part 2):41 case reports. *J. Investig. Allergol. Clin. Immunol.* 2001;**11**:21-26.
15. Cantani A, Micera M. Allergenicity of a whey hypoallergenic formula in genetically at risk babies: four case reports. *Eur. Rev. Med. Pharmacol. Sci.* 2005;**9**:179-182.