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Evaluation of the antigenicity of hydrolyzed cow's milk protein formulas using the mouse basophil activation test

Hiroshi Iwamoto[1,*], Takeshi Matsubara[1], Yuki Nakazato, Kazuyoshi Namba, Yasuhiro Takeda

Nutritional Science Institute, Morinaga Milk Industry Co., Ltd., 5-1-83, Higashihara, Zama, Kanagawa 252-8583, Japan

HIGHLIGHTS

- Mouse basophil activation test (BAT) was applied to the evaluation of antigenicity.
- Mouse BAT is highly useful for the evaluation of antigenicity.
- Mouse BAT may be used as a substitute for the in vivo systemic anaphylaxis test.

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ABSTRACT

Hypoallergenic infant formulas are widely used for infants with cow's milk allergy. The aim of this study was to assess the utility of the mouse basophil activation test (BAT) in the evaluation of residual antigenicity in these formulas. Whole blood samples derived from β-lactoglobulin- or casein-immunized mice were incubated with one of the following formulas: conventional, partially hydrolyzed, or extensively hydrolyzed. Basophilic activation was analyzed by flow cytometry using an IgE-dependent activation marker CD200R1 and an IgG-dependent activation marker CD200R3. Systemic anaphylaxis was induced by i.v. injection of milk formula and results were compared. Conventional formula induced pronounced changes in CD200R1 and CD200R3 expression on basophils, whereas extensively hydrolyzed formulas did not elicit any changes in these markers. Similarly, challenge with conventional formula induced anaphylaxis, whereas extensively hydrolyzed formulas did not induce anaphylaxis. Although the partially hydrolyzed formula also induced basophilic activation and systemic anaphylaxis, the magnitude of these effects was smaller than that observed with the conventional formula. Compared to CD200R1, the observed trend in CD200R3 expression resembled the results obtained from systemic anaphylaxis test more closely. These findings show that mouse BAT, in particular using CD200R3, is highly useful for the evaluation of antigenicity of milk formulas.

1. Introduction

After hen's egg, cow's milk is the second most common food allergen in infants and young children (Fiocchi et al., 2010). Since the principle of cow's milk allergy management is to avoid consumption of cow's milk protein, appropriate breast-milk substitute with reduced antigenicity is essential for bottle-fed infants. Hydrolyzed cow's milk protein formulas were introduced in the 1940s for this purpose. Extensively hydrolyzed formulas, with maximally reduced antigenicity, are successfully used as breast-milk substitutes in many infants with cow's milk allergy (Fiocchi et al., 2010). In more recent years, partially hydrolyzed formulas have been developed for preventive use in high-risk infants. Although partially hydrolyzed formulas are not "hypoallergenic", their antigenicity is greatly reduced compared to that of conventional infant formulas (Docena et al., 2002; Niggemann et al., 1999).

From the development phase to clinical application, it is important to evaluate the antigenicity of hydrolyzed formulas for their safe usage. Although clinical tests such as double-blind,

Abbreviaions: BAT, basophil activation test; CN, casein; β-LG, β-lactoglobulin; APC, allophycocyanin; PreCP/Cy55, peridinin–chlorophyll protein/cyanine 5.5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; CMF, conventional milk formula; PHF, partially hydrolyzed formula; EHF, extensively hydrolyzed formula.

* Corresponding author. Fax: +81 46 252 3077.
E-mail address: h_iwamot@morinagamilk.co.jp (H. Iwamoto).

[1] These authors contributed equally to this work.

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Table 1
Milk formulas evaluated in this study.

	Product name	Classification	Detected milk antigen[a] [ppm]	
			CN	β-LG
CMF	Hagukumi®	Conventional infant formula	4.7×10^4	1.9×10^4
PHF	E-akachan®	Partially hydrolyzed formula	1.7	1.1×10^2
EHF1	New MA-1®	Extensively hydrolyzed formula	<0.5	<0.5
EHF2	MA-mi®	Extensively hydrolyzed formula	<0.5	<0.5

[a] Data are expressed as part per million of antigenic equivalent protein in powdered formula products.

placebo-controlled food challenge are the most reliable, several non-clinical procedures are also useful in the assessment of the safety of hydrolyzed formulas. Evaluation of antibody binding activity, e.g., by ELISA on animal antibodies (Docena et al., 2002; Plebani et al., 1997) or CAP inhibition in serum from patients with cow's milk allergy (Bellioni-Businco et al., 1999; Ehn et al., 2004) are relatively simple in vitro quantitative techniques. In vivo testing on sensitized laboratory animals such as rats (Atkinson and Miller 1994), mice (van Esch et al., 2011; van Esch et al., 2013), or guinea pigs (Kitagawa et al., 1995) is also used to evaluate antigenicity. Since guinea pigs can be sensitized orally without adjuvants, the guinea pig anaphylaxis model has been used frequently (Boner et al., 1992; Ceballos et al., 2009; Lee, 1992; McLaughlan et al., 1981; Piacentini et al., 2003). Although these in vivo techniques are useful, methods utilizing animal models are cumbersome, necessitating the development of appropriate alternative methods.

In clinical practice, the basophil activation test (BAT) is highly valued for its utility in the diagnosis of IgE-mediated allergic disease (Sato et al., 2010). BAT involves in vitro testing using the patient's peripheral basophils that are sensitized to specific IgE. Allergen-induced basophil activation is estimated by flow cytometric analysis of CD63 or CD203c up-regulation. Recently, we established a BAT system in the mouse model of milk allergy (Iwamoto et al., 2015). Unlike in human basophils, cell activation in mouse basophils results in the up-regulation of CD200R1 and down-regulation of CD200R3.

In this study, we evaluated the antigenicity of hydrolyzed formulas by murine BAT system, and showed that this in vitro test may be used as a substitute for the in vivo systemic anaphylaxis test.

2. Materials and methods

2.1. Milk formulas

Powdered milk formulas evaluated in this study are listed in Table 1. All of these are manufactured by Morinaga Milk Industry Co., Ltd. and available in the market.

The amounts of casein (CN) and β-lactoglobulin (β-LG) in each milk formula was determined by commercial ELISA-kits (FASPEK II, Morinaga Institute of Biological Science, Kanagawa, Japan) according to the manufacturer's instructions.

Molecular weight distribution of formulas is shown in Fig. 1. Defatted formula samples were applied to HPLC (LC-20AD, Shimazu, Tokyo, Japan) with poly-hydroxyethyl aspartamide column (PolyLC, Columbia, MD, USA). Immunoglobulin G (FW: 160,000, Sigma–Aldrich, Saint Louis, MO, USA), lactoperoxidase (FW: 93,000, Sigma–Aldrich), ovalbumin (FW: 43,000, Taiyo Kagaku, Tokyo, Japan), chymotrypsinogen A (FW: 25,000, Wako Pure Chemical Industries, Osaka, Japan), ribonuclease A (FW: 13,700, GE Healthcare, Uppsala, Sweden), bovine insulin (FW: 5740, Wako Pure Chemical Industries), basitrasin (FW: 1427, Sigma–Aldrich), oxytocin (FW: 1007, BACHEM, Bubendorf,

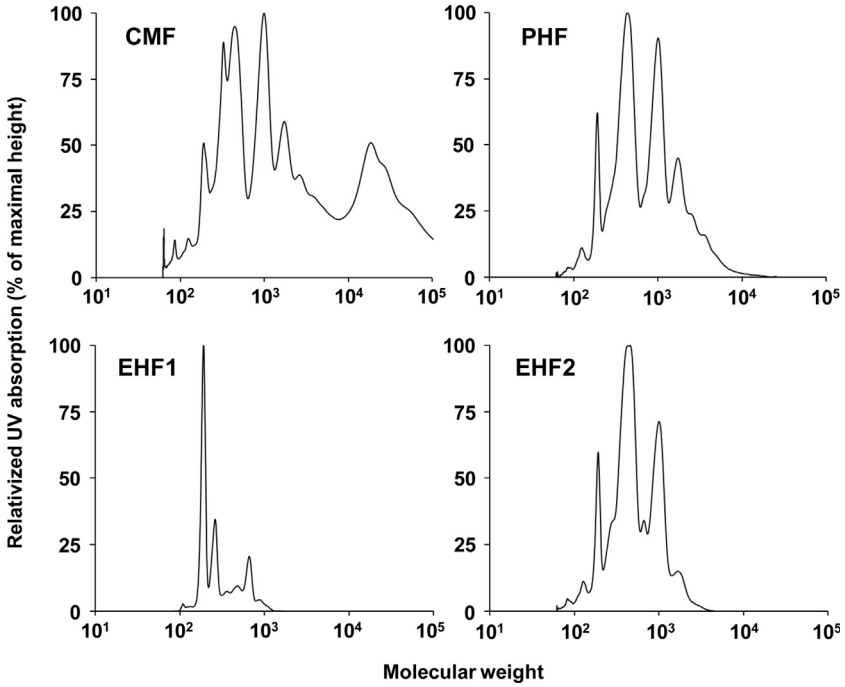

Fig. 1. Molecular weight distribution of milk formulas. Defatted formula samples were analyzed by HPLC. Vertical axis indicates the percentage of maximal peak height of UV 215 nm absorption.