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In vitro immunogenicity of various native and thermally processed bovine milk proteins and their mixtures

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ABSTRACT

In vitro immunogenicity of various native and thermally processed (72°C/15 s and 100°C/30 s) bovine milk protein fractions, their mixtures, whey, and skim milk, was studied by analyzing the immune response of T helper (Th) cells in human peripheral blood mononuclear cells. The secretion of Th type cytokines induced by the protein stimulants was quantified while determining the heat-induced protein denaturation. Purified whey proteins, caseins and whey fraction, and skim milk provoked substantial immune responses at various degrees, indicating their potent immunogenicity. The protein mixtures prepared using the fractionated whey proteins with or without caseins appeared less immunogenic in both native and heat-treated forms, implying their potential of producing less immunogenic dairy products. The 100°C/30 s treatment significantly altered the immunogenicity of most of the potent protein stimulants, which mostly coincided with their levels of protein denaturation. The 72°C/15 s treatment caused the least protein denaturation but altered the immunogenicity of several protein stimulants notably, including heat-stable caseins and α-lactalbumin.
Key words: bovine milk protein, thermal processing, protein denaturation, immunogenicity

INTRODUCTION

Bovine milk provides high-quality proteins to fulfil the AA requirements of humans, and usually it is the first source of foreign proteins ingested by infants in large quantities (Caira et al., 2012). However, the infant's intestinal system is insufficiently developed to digest bovine milk proteins, and their immune system frequently reacts to milk proteins (Caira et al., 2012). The presence of bovine milk proteins in the breast milk of lactating women and bovine milk protein-specific antibodies in the cord blood demonstrate the early exposure of neonates to bovine milk proteins (Høst et al., 1999).

Immunogenicity is the ability of a substance to elicit an immune response or capacity to provoke a detectable immune response (Bier et al., 1981; Actor, 2014). Generally, different proteins demonstrate either potent or weak immunogenicity. The molecular size and other characteristics, including the nature of AA, which make up immunogenic epitopes and the accessibility to those epitopes, contribute to immunogenic capacity of a specific protein (Bier et al., 1981). Epitopes are the portions of immunogenic molecule, mostly proteins, that can bind with the complementary sites of an antibody or T/B cells (Bogahawaththa et al., 2017a). Moreover, the immunogenicity of a protein fraction can alter when it is alone or in combination with other proteins or in the original source of protein (Cross and Gill, 2000).

Bovine milk proteins, more broadly, are immunogens (e.g., antigens and allergens), which can provoke immune response in human immune system through modulating the functions of immune cells including T cells or binding with antibodies such as IgG or IgE (Cross and Gill, 2000). The bovine β-LG and BSA were reported to induce the activation and proliferation of T helper (**Th**) cells in human peripheral blood mononuclear cells (**PBMC**) and secretion of associated cytokines, where β-LG was more potent than BSA (Vocca et al., 2011). The PBMC from a specific group of infants (suffered from necrotizing enterocolitis) showed significantly elevated production of Th type cytokines in response to bovine β-LG and caseins, where β-LG was more immunogenic than the caseins (Chuang et al., 2009). The Th cells, including Th1 and Th2 subsets, play a key role in mediating the immune defense through the cell- and antibody-mediated immune response, respectively.

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Moreover, the balance between Th1 and Th2 type cytokines is believed to determine the appropriateness of the immune response. The overexpression of Th2 type cytokines may contribute to production of IgE antibodies, which can elicit an immediate allergic response, for instance classical milk protein allergy reaction, in an immunocompromised subject (Rengarajan et al., 2000; Donkor et al., 2012).

Most bovine milk proteins can potentially bind with protein-specific antibodies. The ability of protein antigens to bind with IgG antibodies is termed antigenicity, whereas binding with IgE antibodies mostly result in allergenicity in humans. The bovine or cow milk protein allergy is the most prevalent food allergy among infants (2–6%). The caseins and β-LG usually act as the major allergens, whereas other milk proteins such as α-LA, BSA, and immunoglobulins can also be involved in milk protein allergy; however, their degree of involvement differs mostly from one protein fraction to the other (Bogahawaththa et al., 2017a). It is also well established that various heat treatments applied in the thermal processing of milk in the dairy industry can potentially denature native milk proteins, including unfolding and aggregation of native structure, which could subsequently modify the immunogenic epitopes and or their accessibility leading to altered immunogenicity (Bogahawaththa et al., 2017a).

The immunogenicity of native bovine milk proteins, in terms of their ability to provoke T cell-mediated immune response in human, has been studied to a certain extent using some individual protein fractions (e.g., β-LG, BSA; Vocca et al., 2011) and milk protein sources (e.g., skim milk; Opatha Vithana, 2012). However, the immunogenicity of various protein mixtures (e.g., β-LG and α-LA) appears to be unknown. Although several studies reported on the effect of thermal processing on altered antigenicity and allergenicity of bovine milk proteins (Bu et al., 2013), it is largely unknown how thermal processing affects the ability of various bovine milk proteins and their mixtures to provoke T cell-mediated immune responses in relation to protein denaturation. Thus, the present study aimed to examine the immunogenicity (in the form of ability and capacity of provoking Th cell-mediated immune response in vitro) of various native and thermally processed bovine milk protein stimulants (at their natural concentrations), such as fractionated major proteins, their mixtures, native whey, and skim milk, using human PBMC and analyzing the secretion of associated Th type cytokines. We also expected to be able to identify possible associations between the altered immunogenicity affected by thermal processing and the level of protein denaturation.

MATERIALS AND METHODS

Materials and Preparation of Protein Samples

Skim Milk and Native Whey. Murray Goulburn Co-operative (Laverton North, Victoria, Australia) provided raw bovine milk on 2 separate occasions. Upon arrival, the raw milk was skimmed by centrifugation (Avanti J-26XP, Beckman Instrument Australia Pty. Ltd., Gladesville, New South Wales, Australia) at 3,500 × *g* for 20 min at 20°C. An aliquot of skim milk was set aside (−20°C) for further experiments (S1 in Figure 1), and the remaining portion of the skim milk was divided into 2 parts used to prepare the native whey and caseins separately. The outline of the steps followed for the preparation of various fractionated proteins and protein mixtures is shown in Figure 1. The pH of the skim milk was adjusted to 4.6 using 0.1 *M* HCl and then the precipitated caseins were separated from the whey by centrifugation (Avanti J-26XP centrifuge, Beckman Instrument Australia Pty Ltd.) at 30,000 × *g* for 2 h at 20°C as explained previously (Bogahawaththa et al., 2017b). The pH of the resultant whey was readjusted to 6.7 using 0.1 *M* NaOH, which was the pH of the fresh raw milk. An aliquot of purified native whey was stored (−20°C) for further experiments (S2 in Figure 1), whereas the rest was used for the fractionation of native whey proteins as per our previous work (Bogahawaththa et al., 2017b).

Fractionation of Whey Proteins. In brief, 100 μL of the native whey was injected at a time with mobile phase (0.05 *M* sodium phosphate buffer at pH 7, including 0.3 *M* sodium chloride) into a size exclusion chromatography (SEC) column (Biosep SEC-s2000, Phenomenex Australia Ply Ltd., Lane Cove West, New South Wales, Australia), which was mounted to a fast protein liquid chromatography (FPLC) system (GE Healthcare Australia Pty. Ltd., Parramatta, New South Wales, Australia). The different whey proteins, such as IgG, BSA, β-LG, and α-LA, were eluted at different retention times with mobile phase and collected separately using a Frac-950 fraction collector (GE Healthcare Australia Pty. Ltd.). An RVC 2–18 rotational vacuum concentrator (John Morris Scientific, Deepdene, Victoria, Australia) was then used to concentrate the fractionated proteins by evaporating the mobile phase at 30°C.

Caseins. The other portion of skim milk was used to separate the caseins from the whey by ultracentrifugation (Beckman L-70 ultracentrifuge, Beckman Instrument Australia Pty Ltd.) at 100,000 × *g* for 1 h at 22°C without adjusting the pH (O'Mahony and Fox, 2013). The casein pellet was removed from the centri-