

## **Report on Medium-term Research Fellowship**

# **Insights into the allergenic relationship between cow's milk and red meat**

<b>Awardee</b>	Marija Perusko, PhD student
<b>Home supervisor</b>	Professor Tanja Cirkovic Velickovic, PhD
<b>Home Institution</b>	University of Belgrade - Faculty of Chemistry
<b>Host supervisor</b>	Professor Marianne van Hage, MD, PhD
<b>Host Institution</b>	Karolinska Institutet, Department of Medicine Solna, Division of Immunology and Allergy, Stockholm, Sweden
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## **Background**

In the last decade a novel form of severe type of food allergy, red meat allergy, has been identified where nearly half of the patients experience anaphylactic reactions 3 to 6 hours after ingestion of mammalian meat (1). The reactions were shown to be caused by IgE antibodies directed against a carbohydrate epitope, galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal) (2). The  $\alpha$ -Gal epitope is abundantly expressed on glycolipids and glycoproteins from non-primate mammals and some lower primates. The number of diagnosed red meat allergy cases has increased significantly over the past few years and have been reported worldwide.

Many red meat allergic patients report allergic reactions after drinking cow's milk. Studies have reported that the majority of red meat allergic patients have IgE reactivity to cow's milk (1,3). However, only two studies have investigated the IgE reactivity to individual milk allergens, and the IgE responses were only elucidated against three milk allergens with no success to identify  $\alpha$ -Gal carrying milk glycoproteins (3,4).

The aim of this research project was to examine which of the different milk glycoproteins carry  $\alpha$ -Gal and if they are recognized by red meat allergic patients' IgE. Furthermore, we aimed to examine the allergenicity of milk glycoproteins and commercially available milk in basophil activation test among red meat allergic patients.

## **Methods**

Sera from 34 red meat allergic patients attending the Allergy Unit at Södersjukhuset, Stockholm, were used in the study. Serum from a healthy donor and one atopic patient were used as controls. Serum total IgE level and allergen-specific IgE levels to  $\alpha$ -Gal (bovine thyroglobulin), beef, milk,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, caseins,  $\gamma$ -globulins were analyzed by ImmunoCAP (Thermo Fisher, Uppsala, Sweden). Correlations between allergen-specific IgE levels were analyzed by Spearman rank test. Presence of the  $\alpha$ -Gal epitope in milk was tested by inhibition ELISA. Sera were pre-incubated with serial dilution of unprocessed or commercially available milk and residual IgE binding to plate-coupled  $\alpha$ -Gal epitope was determined. IgE-binding milk proteins were detected by immunoblotting using patients' sera. The involvement of the

carbohydrate epitope in the IgE binding to milk proteins was assessed by an inhibition assay with the rich  $\alpha$ -Gal carrier thyroglobulin. Individual milk proteins were directly tested for the presence of the  $\alpha$ -Gal epitope by immunoblot analysis using anti- $\alpha$ -Gal monoclonal antibody (M86, Enzo Life Science, Inc., Farmingdale, NY, USA). Allergenic activity of milk, major milk allergens, and  $\alpha$ -Gal containing milk proteins were tested in basophil activation test among 17 red meat allergic patients and 2 controls.

## **Results**

The vast majority of the recruited red meat allergic patients were IgE positive to milk, but the IgE levels to milk were lower than those to  $\alpha$ -Gal or beef. Significant correlations between IgE levels to milk and  $\alpha$ -Gal, as well as between milk and beef were observed. Dose-dependent inhibition of  $\alpha$ -Gal specific IgE binding by unprocessed or commercial milk showed presence of the  $\alpha$ -Gal epitope in bovine milk. Unprocessed milk exerted a slightly higher IgE inhibition than commercially available milk. IgE reactive milk proteins were detected by immunoblotting using patients' sera. The presence of  $\alpha$ -Gal carrying IgE binding milk proteins was confirmed by inhibition immunoblot using thyroglobulin. Immunoblot with anti- $\alpha$ -Gal antibody revealed presence of  $\alpha$ -Gal epitope on the surface of the IgE reactive proteins. Finally, activation of red meat allergic patient's basophils by  $\alpha$ -Gal carrying milk proteins and milk was demonstrated.

## **Conclusions**

Glycoproteins from bovine milk which are the major carriers of the  $\alpha$ -Gal epitope were identified. Recognition of  $\alpha$ -Gal positive milk proteins by red meat allergic patients' IgE antibodies was demonstrated and furthermore, these proteins activated basophils. Thus, this study highlights the importance of milk as allergenic food source among the meat allergic population.

The findings of this project will be presented at the upcoming EAACI Congress in Lisbon during poster discussion session. Manuscript entitled "Novel insights into the allergenic relationship between bovine milk and red meat" is under preparation.

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