The red meat allergy syndrome in Sweden

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Summary

In the last decade, a novel type of food allergy presenting with severe allergic reactions several hours after consumption of red meat has been recognized. The allergic responses are due to IgE antibodies directed against the carbohydrate epitope galactose-α-1,3-galactose (α-Gal) found in mammalian meat. This review presents the red meat allergy syndrome in Sweden, discusses the features of the immune response to carbohydrates, and highlights the presence of heat stable α-Gal-containing proteins in meat.

The number of diagnosed red meat allergy cases in Sweden has increased significantly over the past few years. All patients have been tick bitten. Our recent work has shown that α-Gal is present in the European tick *Ixodes ricinus* (*I. ricinus*), thus potentially explaining the strong association between anti-α-Gal IgE and tick bites, with development of red meat allergy as a secondary phenomenon. Further studies using immunoproteomics have identified novel α-Gal-containing meat proteins that bound IgE from red meat allergic patients. Four of these proteins were stable to thermal processing pointing to the fact that the allergenicity of red meat proteins is preserved in cooked meat. In keeping with the fact that the α-Gal epitope is structurally related to the blood group B antigen, a positive association with the B-negative blood groups among our red meat allergic patients was noted. A selective IgE reactivity to the pure carbohydrate moiety was observed when investigating the specificity of the α-Gal immune response. IgE from red meat allergic patients does not recognize the other major mammalian carbohydrate, N-glycolylneuraminic acid (Neu5Gc), also present in high amounts in red meat. Furthermore, neither common cross-reactive carbohydrate determinants (CCDs) from plants nor venoms are targets of the IgE response in these patients.

Taken together, the α-Gal carbohydrate has shown to be a potentially clinically relevant allergen that should be taken into account in the diagnosis of food allergy. Many new findings in the field of red meat allergy have been obtained during the past years, but further efforts to understand the process of digestion, absorption, and delivery of α-Gal-containing molecules to the circulation are needed.

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α-Gal – carbohydrate – meat – tick bites – Sweden – beef processing

Introduction

Allergy to mammalian meat is rare even though meat is a main source of protein in Western societies. Initially, the identification of meat-derived allergens focused on protein antigens recognized by patients who reported allergic reactions occurring rapidly after exposure [1, 2]. However, during the

Abbrevations

α-Gal	Galactose-α-1,3-galactose
CCD	Cross-reactive carbohydrate determinant
Ig	Immunoglobulin
I. ricinus	Ixodes ricinus
B. taurus	Bos taurus
Neu5Gc	*N*-glycolylneuraminic acid

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last decade a novel type of meat allergy has been reported where patients have severe allergic reactions occurring several hours after red meat intake [3]. This unique presentation represents a challenge in linking ingestion to reactions for both patients and clinicians.

The allergic reactions were shown to be caused by IgE antibodies directed against a carbohydrate epitope, α-Gal [4, 5, 6], which is abundantly expressed on glycolipids and glycoproteins from non-primate mammals and some lower primates. In humans and higher primates, the gene encoding alpha-1,3-galactosyltransferase is not functional and no α-Gal is synthesized [7]. Although IgG antibodies to α-Gal are widely expressed in humans [8], presumably in response to continuous exposure to the α-Gal epitope via gut microorganisms [9], IgE antibodies to α-Gal are not. An IgE antibody response to α-Gal was first recognized when patients treated with the monoclonal antibody cetuximab experienced anaphylactic reactions upon the first injection [10]. Investigations revealed that the reactions were related to pre-existing IgE antibodies directed to the α-Gal epitope on the Fab portion of cetuximab.

Red meat allergic patients in Sweden

When the new syndrome of red meat allergy was reported in 2009 [4, 11], three cases with severe allergic reactions several hours after intake of red meat (beef, lamb, pork, or moose) were identified at the Allergy Clinic at Södersjukhuset in Stockholm. These cases all had IgE antibodies to α-Gal. Six years later, more than 200 cases have been diagnosed in Sweden. It soon became clear that the novel food allergy syndrome was not only present in USA, Australia and Sweden, but also in several other European countries [11, 12, 13, 14, 15, 16].

In the first Swedish report 39 adult patients with allergy to red meat were described [15]. All patients reported delayed allergic reactions to red meat and IgE antibodies to α-Gal. Nearly all patients displayed urticaria after red meat consumption and almost half of them reported anaphylactic reactions. When investigating their sensitization profile, all patients had IgE antibodies to beef and pork and many also to moose and other α-Gal-containing allergen sources such as cow's milk, dog, and cat.

Association with tick bites

Upon questioning the patients we noted that all had been tick bitten, which is in line with previous reports showing an association between tick bites from *Ixodes holocyclus* in Australia [17] and *Amblyomma americanum* in the US [18] and red meat allergy. In Europe, a history of tick bites had been reported in four of five patients from Northern Spain with IgE antibodies to α-Gal [12].

We investigated whether our patients were sensitized to *I. ricinus*, the tick species found in Sweden and Europe. We noted that all except two patients had IgE antibodies to *I. ricinus* and that the IgE levels correlated significantly with the α-Gal levels [15]. The finding was in line with our previous study where we provided the first direct evidence that α-Gal is present in the gastrointestinal tract of *I. ricinus*, thus potentially explaining the relationship between tick exposure and sensitization to α-Gal, leading to the development of red meat allergy [19]. The route of sensitization through the skin via tick bites seems to be of major importance for the induction of IgE antibodies against α-Gal since these antibodies are not found in serum of subjects living in the northern arctic part of Sweden where ticks are not present [18].

As Lyme disease is generally transmitted through tick bites, we analyzed 207 patients with Lyme disease for IgE to α-Gal and found that around one in five patients had IgE reactivity to α-Gal. However, their antibody levels were lower compared to the red meat allergic patients. We also investigated the frequency of sensitization to α-Gal in the general population and noted that 10 % of 143 healthy blood donors from the Stockholm area, where ticks are prevalent, had IgE to α-Gal. These results are in accordance with a report from Denmark and Spain [20]. The authors from that study concluded that the presence of α-Gal-specific IgE is associated with a history of tick bites, atopy, and cat ownership. The latter observation probably reflects sensitization to the cat allergen cat IgA (Fel d 5w) which we have previously shown to carry α-Gal [21]. Not only ticks but also parasites can give rise to α-Gal sensitization. This became clear when we investigated parasite-infected non-cat allergic patients from Africa who were sensitized to cat IgA [22].

Association with the B-negative blood groups

The α-Gal epitope is structurally closely related to the blood group B antigen [9]. Only the lack of a fucose residue on the glycan core distinguishes the structure of blood group B antigen from α-Gal [23]. Subjects with blood groups B and AB have been shown to produce natural anti-α-Gal antibodies that bind to α-Gal epitopes only; whereas subjects with blood groups A or O have antibodies that react to either α-Gal or B antigen [24, 25]. Natural antibodies against α-Gal moieties on non-primate mammalian tissues are often involved in acute organ rejection in xenotransplantation. With respect to the impact of the B-negative blood groups in the red meat allergy syndrome, we noticed for the first time that only two of our 39

red meat allergic patients (5 %) belonged to the B-positive blood group which is significantly less compared with the expected number in the Swedish population (18 %) [15]. The finding is in accordance with a recent report on the relationship between IgG and IgE responses to α-Gal and blood group B where IgE production to α-Gal was accompanied by low amounts of IgE to blood group B [26].

α-Gal-containing proteins in red meat

When the red meat allergy syndrome was recognized, efforts on identifying red meat proteins carrying the α-Gal moiety were initiated. Two high molecular weight α-Gal containing allergens, laminin γ-1 and the collagen α-1 (VI) chain (240 kDa and 140 kDa respectively) from *Bos taurus* were initially identified [27]. These findings were consistent with previous reports demonstrating cross-reactivity of red meat with gelatin, a derivative of collagen [28]. It is well known that heating or other treatments of proteins can modify IgE-binding properties by changing the conformation of epitopes. With respect to peanuts, frying and boiling has been shown to reduce the IgE-binding to the major peanut allergens, whereas roasting, which uses higher temperatures, increased the allergenicity [29].

We characterized the proteomic profile of different beef preparations (raw, medium rare, fried, and boiled) and investigated their potential allergenicity among Swedish red meat allergic patients. We noted that most of the red meat allergic patients recognized similar IgE-binding proteins of beef in a wide range of molecular weight (25–250 kDa) [30]. The IgE reactivity was stronger to raw and medium rare meat compared to fried and boiled meat. Using an immunoproteomic approach, we identified 18 proteins from *Bos taurus*, seven of them contained α-Gal (**Fig. 1)**. When examining different processed beef preparations, four IgE-binding α-Gal-containing proteins were shown to be stable to heat treatment (creatine kinase M-type, aspartate aminotransferase, β-enolase, and α-enolase) [30]. These are cytoplasmic proteins, playing essential roles in the metabolic pathways [31, 32]. Thus, the allergenicity of red meat proteins carrying the α-Gal epitope is preserved even upon thermal processing. These findings indicate that even thermal cooking of red meat may induce a severe delayed allergic reaction. Moreover, we noticed that red meat allergic patients also have IgE responses to non-α-Gal-containing beef proteins.

Features of the immune response to carbohydrates

Many food allergens are glycoproteins that contain one or several carbohydrates linked to the protein structure. Not only proteins but also carbohydrates can stimulate the production of IgE antibodies and be strong inducers of Th2 responses [33]. The current estimate is that 15–30 % of allergic patients generate specific anti-carbohydrate IgE antibodies [34], but this IgE response has very little clinical relevance [35].

At present, α-Gal is the only clinically relevant carbohydrate allergen from the mammalian kingdom. However, besides α-Gal, a sialic acid named Neu5Gc is the only mammalian carbohydrate present in high amounts in beef, lamb, pork, and cow's milk [36]. Similar to α-Gal, Neu5Gc is found in most mammals including primates [37] but not in humans due to a mutation in the gene encoding the enzyme responsible for Neu5Gc synthesis. IgG antibodies against Neu5Gc reach up to 0.25 % of total circulating IgG in some subjects which is similar to what is known regarding IgG antibodies to α-Gal, but the IgG levels to Neu5Gc and α-Gal have not been reported to correlate [36, 38]. In addition to α-Gal, carbohydrates from the plant kingdom and venoms have been shown to induce an IgE response.

We investigated whether the IgE responses to α-Gal targeted the glycoprotein or the carbohydrate structure only. Deglycosylation of an α-Gal containing protein, bovine thyroglobulin, significantly reduced the IgE response as compared to untreated thyroglobulin. In addition, the IgE levels against deglycosylated thyroglobulin were reduced more than hundredfold to below the cut-off (< 0.10 kU_A/l) in two thirds of patients tested. The results indicate that the anti-α-Gal IgE response of red meat allergic patients is directed against the pure α-Gal carbohydrate and is unrelated to the carrier protein itself. We furthermore scrutinized if red meat allergic patients also have an IgE response to Neu5Gc, carbohydrates from the plant kingdom or venoms. Our study revealed that red meat allergic patients neither had an IgE antibody response against Neu5Gc nor against CCDs from plant (nCup a 1, nArt v 1 and MUXF3) or venom (MUXF3) sources [39]. Taken together, IgE reactivity to carbohydrates from the animal and plant kingdom common in other allergic diseases is not of importance in the red meat allergy syndrome.

Concluding remarks

Red meat allergy syndrome is a novel type of food allergy which is increasingly recognized in Sweden. The diagnosis may be problematic as the allergic reactions are linked to red meat intake several hours before the clinical symptoms occur. The route of sensitization through the skin via tick bites seems to be of major importance for the induction of IgE antibodies. Characterization of the proteomic profile of different beef preparations has revealed that the α-Gal epitope is commonly present in IgE-reac-

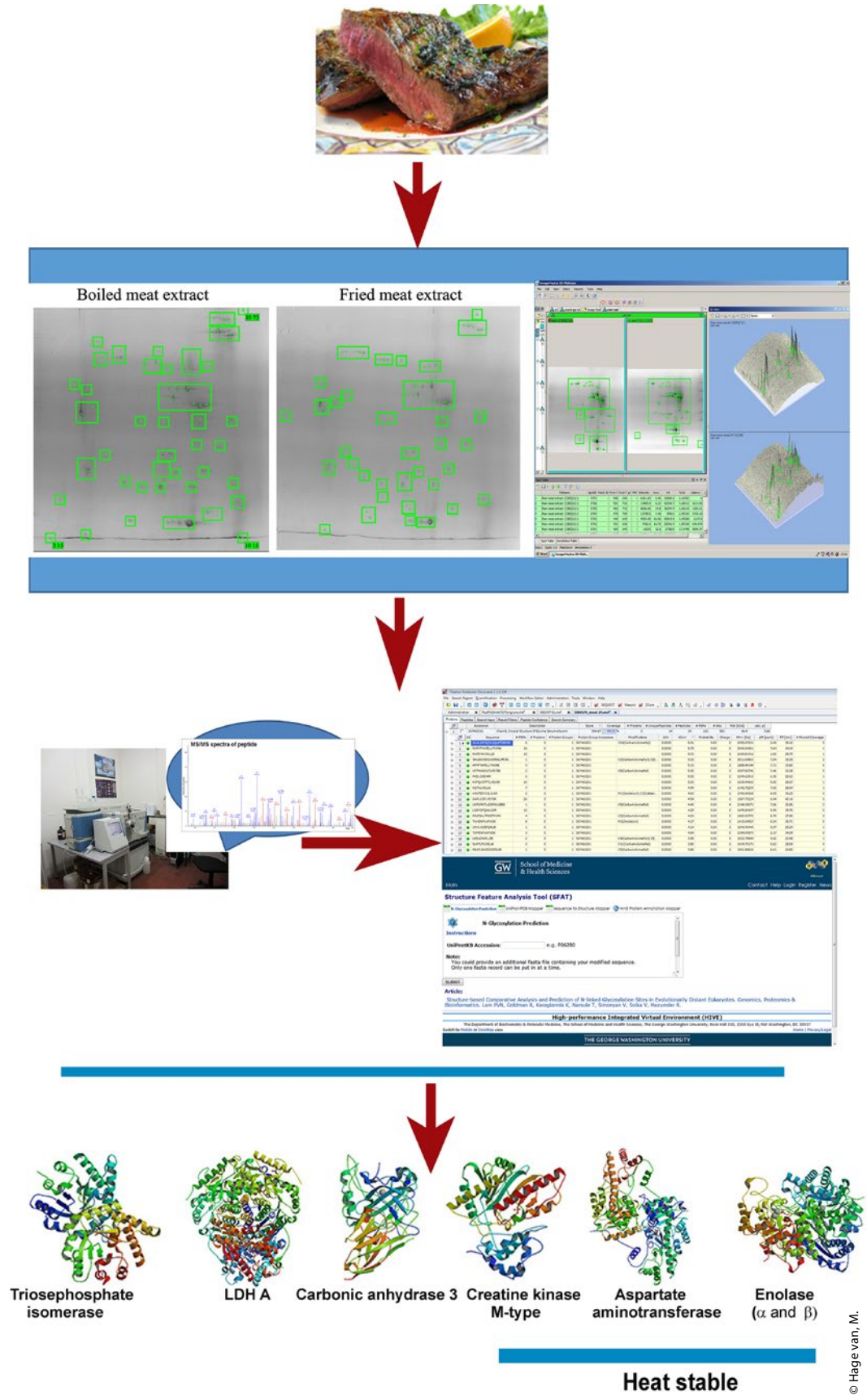

Fig. 1: Proteomic approach for identification of α-Gal-containing proteins. Protein extracts from different processed meat (boiled and fried) were subjected to high resolution 2D electrophoresis (upper panel, left side). Protein spots were examined and IgE-binding proteins were subjected to trypsin digestion (upper panel, right side) and analyzed with nLC-MS/MS (lower panel, left side). The seven novel α-Gal-containing proteins were identified using anti-α-Gal-antibody and bioinformatics (lower panel, right side).

tive beef proteins, some of which are stable to heat treatment. Consequently, even cooking meat at high temperatures fails to abolish the allergenicity of several red meat proteins. Furthermore, red meat allergic patients have a selective IgE response to the pure α-Gal carbohydrate and carbohydrates from plants and venoms are not an issue in the pathogenesis and diagnosis of red meat allergy.

Taken together, the α-Gal carbohydrate has shown to be a potentially clinically relevant allergen that should be taken into account in the diagnosis of food allergy. Moreover, exposure to *I. ricinus* has increased over the last decade in Sweden and ticks are extending to the north as well as to the west of the country where they have become prevalent (Public Health Agency of Sweden, www.folkhalsomyndigheten.se). This is probably due to expanding deer and rodent populations (major tick vectors) as well as climate changes. It implies that more people in Sweden will be at risk of developing red meat allergy. The many new findings obtained over the past years have significantly increased our knowledge in the area of red meat allergy. However, further studies are needed to elucidate the process of digestion, absorption, and delivery of α-Gal-containing molecules to the circulation.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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