Trends in Parasitology

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Salivary Prostaglandin E2: Role in Tick-Induced Allergy to Red Meat

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Tick-induced allergy to red meat is associated with anti-α-Gal IgE antibody levels. We propose that tick salivary prostaglandin E2 triggers antibody class switching in mature B cells, increasing the levels of anti-α-Gal IgE antibodies. Immune tolerance to α-Gal in blood type B individuals might reduce the risk to this allergy.

Tick-induced allergy to red meat is becoming a global problem with increasing prevalence in the USA, Australia, and Europe, and several tick species have been implicated in these disorders [1]. Remarkably, most of the patients that become allergic, had tolerated red meat for many years before being sensitized by tick bites [1]. This finding suggests that anti-Galα1-3Galβ1-(3)4GlcNAc-R (α-Gal) IgE antibodies induced by tick bites, break the oral tolerance to food allergens. This tick-induced immune response is antigen-specific and results in gut-related but not lung-related allergy.

Tick saliva is a complex mixture of pharmacologically active compounds. Tick saliva and/or tick salivary gland extracts were shown to inhibit almost every defensive mechanism and affect leukocyte populations through immunomodulatory, antihemostatic and anti-inflammatory molecules [2]. Transcriptomics studies of tick salivary glands discovered clusters of related proteins that are referred to as multigene families and usually contain tens or even hundreds of more or less similar proteins, with protease inhibitors being the most abundant group of tick salivary secreted proteins in *Ixodes scapularis* [2]. Interestingly, the genes coding for these proteins are usually expressed sequentially throughout tick feeding, bringing up the question of whether this phenomenon could be a form of antigenic variation [2].

Apart from proteins with immunomodulatory activity, ticks also produce nonprotein molecules such as prostaglandin E2 (PGE_2), which is synthetized in the tick salivary glands and secreted via the saliva into the feeding lesion [3]. Several tick species from major genera such as *Ammblyomma*, *Ixodes*, and *Rhipicephalus*, which have been involved in tick-induced allergies, were found to secrete PGE_2 in their saliva [3,4]. Tick salivary PGE_2 was reported to have an immunomodulatory effect [3,4]. In particular, PGE_2 inhibited cytokine production by inducing cyclic AMP-proteins kinase A (cAMP-PKA) signaling in dendritic cells [3]. While attention has been paid to the immunomodulatory effect of tick salivary PGE_2 on dendritic cells [3,4], the effect of PGE_2 on B cells has been overlooked. However, recent reports showed that PGE_2 has a major impact on B cell function with implications in allergy [5]. Specifically, PGE_2 induces class switch recombination (CSR) on mature B cells [5].

Anti-α-Gal IgM and IgG antibodies are exclusively produced in humans in response to antigenic stimulation by α-Gal antigens produced by gut microbiota [6]. Several gut-dwelling bacterial species were suggested to induce anti-α-Gal response. These include several

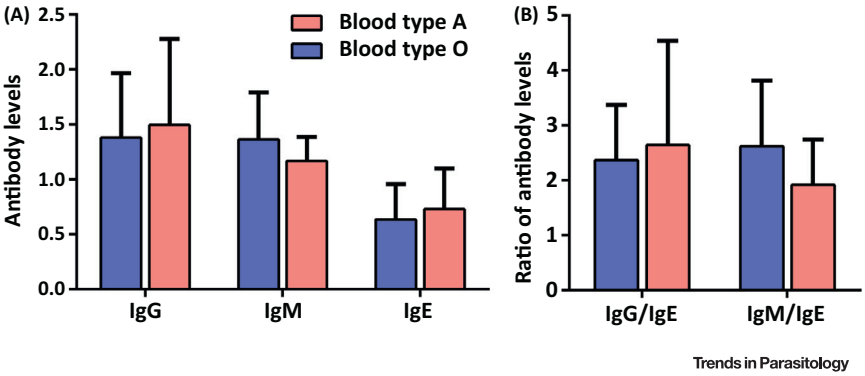

Figure 1. ABO Blood Types and Antibody Response to α-Gal: A Case Report. The ABO blood types are important self-antigens with implications in immune tolerance and xenotransplantation [6]. Humans have antibodies against missing A/B blood type antigens. As shown in a series of studies, the levels of anti-α-Gal antibodies are lower in individuals with blood type B [8]. This finding may be related to the fact that monoclonal and polyclonal anti-α-Gal antibodies show strong interaction with both galactose residues of α-Gal [11]. Therefore, the presence of galactose residues in blood type B antigen may be sufficient for the binding of anti-α-Gal autoantibodies to blood type B antigen, resulting in total or partial tolerance to Gal–Gal blocks in individuals with blood type B. The figure shows the analysis of anti-α-Gal IgG, IgM, and IgE ratios in healthy individuals with blood types O and A, which should have similar levels of anti-α-Gal antibodies. The dataset containing the anti-α-Gal antibody levels in healthy adults from the Iberian Peninsula was published by Cabezas-Cruz *et al.* [12]. (A) Anti-α-Gal IgG, IgM and IgE antibody levels (O.D. 450 nm) were determined by ELISA in sera from healthy individuals [12]. Anti-α-Gal IgE levels are lower than anti-α-Gal IgG and IgM levels in healthy individuals with blood types A and O. (B) Anti-α-Gal IgG/IgM/IgE ratio analysis shows that no significant differences ($P > 0.05$) exist between IgG/IgE and IgM/IgE ratios in healthy individuals with blood type A or O. These results support our hypothesis that tick bites may induce class switch recombination (CSR), which will result in an increase in anti-α-Gal IgE in individuals with blood types A and O. Subsequently, IgG/IgE and IgM/IgE ratios are expected to decrease below the values shown in the Figure (i.e., for blood type O individuals, IgG/IgE<2.36 with SD ± 1.0 and IgM/IgE<2.61 and SD ± 1.2, and for blood type A individuals, IgG/IgE<2.64 and SD ± 1.9 and IgM/IgE<1.92 and SD ± 0.8).

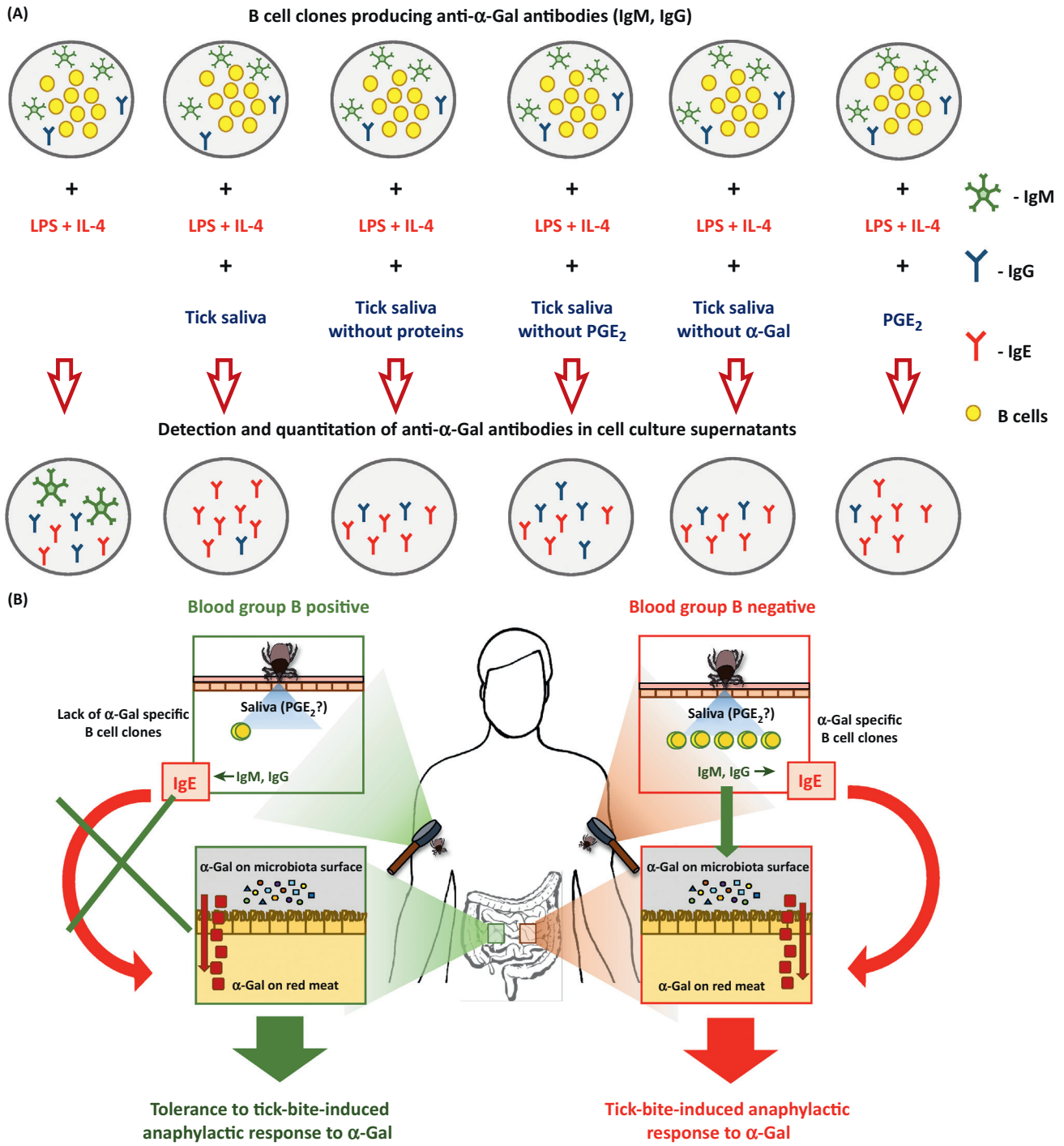

Figure 2. Immunological Basis of Tick-Induced Allergy to Red Meat. (A) Our hypothesis is that tick salivary PGE_2 induces class switch recombination (CSR) towards IgE production on pre-existing anti-α-Gal IgM- and/or IgG-specific mature B cell clones, and blood type B-negative individuals will be more susceptible to develop α-Gal-related allergy to red meat after tick bites. To test this hypothesis, tick saliva can be added to B cell culture in combination with other established isotype switch inducers such as lipopolysaccharides (LPS) and IL-4. PGE_2 would serve as a positive control, as it was shown to enhance the quantity of IgE produced by LPS/IL-4-stimulated B cells [5]. To exclude the influence of tick salivary proteins, saliva could be dialyzed against a 3 kDa semipermeable membrane. To confirm the role of α-Gal, tick saliva could be depleted of α-Gal by removing the galactose epitope by α-galactosidase enzyme and added with and without synthetic α-Gal. To support the conclusions of this experiment, several genetic tools such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9

(Figure legend continued on the bottom of the next page.)

strains of *Escherichia coli*, and *Klebsiella*, *Serratia*, and *Salmonella* species that produce α-Gal and were isolated from normal human stool [6]. In fact, gut colonization by *E. coli* strain O86 resembles the etiology of anti-α-Gal antibodies production in alpha-1,3-galactosyltransferase gene knockout mice and primates [6]. Additionally, anti-blood type A or B agglutinins (immunoglobulins) have also been proposed to be formed as a consequence of antigenic stimulation by gut microbiota [7]. The origin of anti-blood type A response is still controversial, but a consensus exists that anti-blood type B agglutinins are associated with the immunity to components of the gut microbiota [7]. A critical question emerges here: if α-Gal antigens are constantly exposed on the surface of gut bacteria, why do allergic episodes that follow tick bites happen only when α-Gal is exposed on nonmicrobial proteins (i.e., from red meat and cetuximab)? One possible explanation is that α-Gal associated with microbial proteins never leaves the gastrointestinal tract, while α-Gal associated with food proteins may reach the general circulation via food absorption processes. This fact may explain the delayed nature of tick-induced allergy to red meat, which appears usually 2–6 h (but can also occur from 0.25 to 12 h) after red meat consumption [1].

These facts support the hypothesis that blood type B-negative individuals are more susceptible to developing allergy to red meat after tick bites, because they do not display any tolerance to blood type B and related antigens such as α-Gal and gal2 (Figure 1). In fact, two studies have shown that red meat allergy after tick bites is strongly associated with individuals lacking the blood type B [8,9]. The study by Rispens *et al.* [8] showed that none of the individuals in Virginia with blood type B produced IgE antibodies to α-Gal [8]. *Amblyomma americanum* is highly prevalent in Virginia. Therefore, these individuals with blood type B were potentially exposed to tick bites, but they did not develop any anti-α-Gal IgE response. The study by Hamsten *et al.* [9] showed that, out of 39 allergic patients, only one had blood type B. This evidence led us to suggest that tick bite sensitization breaks peripheral tolerance to red meat allergens in the gastrointestinal tract, but not the central tolerance to blood antigen B. The role of tick bites in the induction of anti-α-Gal IgE, which seems to be a 'prerequisite' for developing tick-induced allergic reactions to red meat consumption, has been well established. However, major questions remain to be answered. What is the genetic basis of the susceptibility to tick-induced red meat allergy? Which components of tick saliva are responsible for IgE isotype switch induction or enhancement? Do patients susceptible to tick-induced allergy to red meat have pre-existing (i.e., before tick bites) anti-α-Gal IgM or IgG antibodies? Are individuals negative for blood type B more susceptible to tick-induced allergy to red meat? Furthermore, establishing the tick saliva components that induce the anti-α-Gal IgE response is critical towards the effective treatment and prevention of this type of allergy.

Our hypothesis is that, in addition to the evidence supporting the role of α-Gal-containing tick salivary proteins, tick salivary PGE_2 induces CSR, which leads to an increase in the frequency of B cell producing anti-α-Gal IgE in the blood. To test this hypothesis, we propose experiments to be conducted *in vitro* with the stimulation of B cells with LPS, IL-4, and tick saliva under different conditions for the quantitation of anti-α-Gal IgM, IgG, and IgE antibody responses (Figure 2A). Furthermore, we propose that central B cell tolerance to blood type B and related antigens such as α-Gal provides a protective effect that prevents the formation of clinically relevant levels of anti-α-Gal IgE. The '*dual exposure hypothesis*' of food allergy [10] may explain tick-induced allergy to red meat (Figure 2B). Two possible mechanisms explain the production of anti-α-Gal IgE antibodies after tick bites in the skin. The first mechanism proposes that the α-Gal antigen on tick salivary proteins is presented to antigen-presenting cells (APCs) and B lymphocytes in the context of Th2 cell-mediated immunity induced by tick saliva. This mechanism leads to the elevation of the anti-α-Gal IgE response. The second mechanism is based on the possibility that tick saliva contains factors that induce CSR to anti-α-Gal IgE-producing B cells of pre-existing B cell clones producing anti-α-Gal IgM and/or IgG. While the first mechanism is straightforward, and is supported by our current understanding of the immune modulation by tick saliva [2], the second one is less obvious and needs to be studied. The possibility cannot be excluded that both Th2-induced response and PGE_2-induced CSR may contribute to the development of high levels of anti-α-Gal IgE after tick bites.

(CRISPR/Cas9) and RNA interference can be used to deplete B cells from PGE_2 receptors. By using isotype-specific antibodies, two described pathways of CSR to IgE can be detected by evaluating the IgM/IgG/IgE ratio, a direct pathway from the IgM to the IgE isotype, and a sequential pathway from IgM to an IgG1 intermediate and then to IgE. (B) The '*dual exposure hypothesis*' of food allergy proposes that antigen exposure through the skin promotes sensitization, while early exposure through the gut is tolerogenic [10]. In blood type B-negative individuals, B cell clones producing IgM and IgG antibodies against α-Gal are present at high levels due to the presence of α-Gal on the gut microbiota bacterial surface. By contrast, individuals with blood type B produce these antibodies at lower levels due to immunotolerance developed against blood type B antigen, which is similar to α-Gal. After tick bite, and in response to components in tick saliva in individuals without blood type B, α-Gal specific B cell clones undergo CSR towards IgE. These IgE antibodies then form complexes with food antigens containing α-Gal (e.g., red meat) and induce anaphylactic reactions due to mast cells and basophils activation. Individuals with blood type B do not possess α-Gal-specific B cell clones and antibodies due to autotolerance to self blood type B antigen, therefore no isotype switch can occur and anaphylactic reaction is avoided. The cross reactivity between α-Gal and blood type B antigen is the key prerequisite for this effect.

In summary, the data currently available seem to suggest that there are three main risk factors for developing tick-induced allergy to red meat: (i) the presence of α-Gal-producing bacteria within the gut microbiota, (ii) the absence of blood type B, and (iii) the exposition to tick salivary PGE_2 after tick bites.

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