EDITORIAL

Control of vector-borne infectious diseases by human immunity against α-Gal

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The World Health Organization estimates that 1 billion individuals suffer from vector-borne diseases (VBDs), accounting for 17% of all infectious diseases worldwide, and 1 million of these individuals die annually due to VBDs. In addition, recent reports highlight a disturbing picture regarding the current situation of VBDs in the continental Europe, UK, and China. These diseases are caused by pathogens transmitted by arthropod vectors such as ticks (e.g. Lyme disease caused by Borrelia burgdorferi, human granulocytic anaplasmosis caused by Anaplasma phagocytophilum, and tick-borne encephalitis (TBE) caused by TBE virus), mosquitoes (e.g. malaria caused by Plasmodium spp. and dengue fever caused by dengue virus), phlebotomine sand flies (e.g. various forms of human leishmaniasis caused by Leishmania spp.), tsetse flies (e.g. sleeping sickness caused by Trypanosoma brucei), and Triatomine bugs (e.g. Chagas disease caused by Trypanosoma cruzi). Effective vaccination strategies to control most of the VBDs have not been successfully developed or implemented, and the use of insecticides and/or chemotherapy has resulted in an increasing number of insecticide-resistant vectors and drug-resistant pathogens. Therefore, alternative strategies for control of VBDs are urgently needed. During evolution, hominid primates and humans lost the gene encoding the enzyme to synthesize the carbohydrate Galactose-alpha-1,3-galactose (α-Gal) that resulted in an almost unique capacity to produce high antibody titers against α-Gal. This process may be viewed as a major evolutionary adaptation for protection against VBDs. Other mammals do not produce anti-α-Gal antibodies because they express α-Gal, which is recognized as a self-antigen resulting in tolerogenic immune responses. Carbohydrate-based vaccines have shown promising results against various infectious pathogens such as Neisseria meningitidis, Haemophilus influenzae type b, and Salmonella typhi. Recently, Yilmaz et al. found that bacteria expressing α-Gal in the gut microbiota of mice with knockout α-Gal pathway trigger a systemic antibody response that protects against transmission of malaria-causing Plasmodium parasites by Anopheles mosquitoes. The sterile protection against malaria transmission is achieved by α-Gal-specific IgM and IgG antibodies that neutralize Plasmodium sporozoites via a mechanism involving the binding of these immunoglobulins to the α-Gal moieties on the surface of the parasite that activates the classical complement pathway. This was a groundbreaking finding that may greatly impact the control of human VBDs. Notably, when α-Gal is used for immunization, IgM and IgG responses are also induced and have a protective role. In sharp contrast to these findings, anti-α-Gal IgE response induced by tick bites has been associated with anaphylactic reactions with still unknown implications for susceptibility to tick-borne pathogens. These results suggest that the immune response to α-Gal may be highly influenced by the context in which this antigen is presented to the immune system. In addition to Plasmodium, trypanosomatid protozoan parasites such as Trypanosoma spp. and Leishmania spp. are probably vector-borne pathogens that also contain α-Gal on their surface, suggesting that the protective effect of anti-α-Gal immune response may extend to a larger number of pathogens. However, the challenge is to implement effective and easy-to-deliver prevention therapies using α-Gal. One possible strategy is the use of probiotics (defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit to the host') containing α-Gal-producing bacteria such as Lactobacillus spp. as dietary supplements. This approach has the advantage that, in general, probiotic-based products are safe, easy to distribute, well received by the public, and with a well-established regulatory body. In addition, probiotic-based vaccines may have short-term and long-term beneficial effects to mucosal and systemic immunity. In fact, probiotics were proposed as an alternative to antibiotics and show antimicrobial effects that have been used to control infectious and parasitic agents such as Candida spp., Helicobacter pylori, Clostridium difficile, and Toxocara spp.

The antimicrobial mechanisms of action of probiotics are diverse and include immunologic and non-immunologic responses, and also direct effects on the pathogens. A dilemma posed to probiotic-based vaccines is that some species of microbes have beneficial, but also pathogenic strains, and therefore, the use of the beneficial strains will raise safety concerns. For instance, Enterococcus faecalis strain CECT 7121 has a direct larvicide effect on T. canis larvae but regulatory bodies may not accept a probiotic comprising E. faecalis because many pathogenic strains are known for this bacterium. Likewise, the protective role of α-Gal against malaria was related to an Escherichia coli strain for which pathogenic strains also exist. Nevertheless, probiotics include several microorganisms, mostly within Lactobacillus or Bifidobacterium genera. Interestingly, gut colonization by the human gut pathobiont E. coli O86:B7, but not by the probiotic L. casei, induce an increase in the levels of anti-α-Gal-specific IgG and IgM. Apparently, both E. coli O86:B7 and L. casei contain α-Gal on their surfaces. In addition, Dahl et al. showed that even when the α-Gal epitope is highly represented in different enterobacteria of the gut microbiota of α-Gal knockout mice, no response to this antigen was induced without previous immunization. This raises the question whether all probiotic bacteria expressing α-Gal are able to induce a protective anti- α-Gal antibody response. Importantly, the expression of α-Gal by members of the family Enterobacteriaceae (Gram-negative bacteria, e.g. E. coli spp.) is mainly associated with the bacterial capsule and cell wall glycoproteins, as well as with carbohydrate units of bacterial lipopolysaccharide (LPS). However, Lactobacillus spp. are Gram-positive bacteria and do not produce LPS. Likewise, other E. coli spp. such as E. coli K2 strain may not produce α-Gal. The association of α-Gal to highly immunogenic components such as LPS in the membrane of pathogenic bacteria like E. coli O86:B7 may have a major impact in the immune response elicited against this antigen. To overcome this difficulty, beneficial probiotic species expressing α-Gal may be combined with adjuvants such as toll-like receptors (TLRs). TLRs are pattern recognition receptors expressed by various cells of the gastrointestinal track, including epithelial and immune cells. Differential activation of TLRs by microbiota or invading pathogens may contribute to intestinal homeostasis or to the initiation of inflammatory responses, respectively. It is well known that LPS is a specific activator of TLR4. TLR4 agonists such as LPS induce the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling, which elicit a pro-inflammatory cytokine production. TLR4 activation is also associated to increased intestinal permeability and enhanced bacterial translocation to inner lamina. In contrast, activation of TLR9 by unmethylated CpG motifs in intestinal epithelial cells suppresses NF-kB-induced proinflammatory cytokine production and contributes to intestinal homeostasis. Lactobacillus spp. DNA, which is rich in unmethylated CpG motifs, was shown to activate TLR9, inducing a tolerogenic and anti-allergic immune response in the gut. Although not necessarily related to the anti-α-Gal immune response, recently Villarino et al. showed that the composition of the gut microbiota is a risk factor for severe malaria. They provided evidence that abundance increase of Lactobacillus and Bifidobacterium in the gut flora is associated with resistance to malaria in mice and proposed the modulation of the gut microbiota using probiotics as a potential intervention to reduce malaria severity. In the context of the increasing burden of emerging VBDs worldwide, we propose the use of probiotics composed of bacteria producing the carbohydrate α-Gal as dietary supplements to control VBDs. Although previous studies have shown that anti-α-Gal antibodies in human serum do not induce 100% killing of Plasmodium, the use of probiotics containing bacteria-producing α-Gal may alter host natural response to this carbohydrate resulting in increased protection to parasite infection. Considering the results of previous studies, while probiotics such as Lactobacillus spp. may constitute the appropriate carrier of α-Gal in probiotic-based vaccines against vector-borne pathogens, the combination with TLR4 agonists may be needed to develop a potent and protective immune response against this carbohydrate. Future studies should focus on the characterization of the mechanisms involved in the immune response to α-Gal. This antibody response may be effective against different vector-borne pathogens that contain α-Gal on their surface.

Therefore, the probiotics-based vaccines exploiting this major evolutionary adaptation may constitute an effective strategy to reduce the impact of VBDs on human health. Although the road to probiotic-based vaccines appears to be challenging, the rational design of vaccines exploiting the special immunity of human to α-Gal may be our best strategic move to win our battle against VBDs.

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