

Mycobacterium avium subspecies paratuberculosis—An environmental trigger of type 1 diabetes mellitus

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ABSTRACT

Type 1 diabetes mellitus (T1DM) is an autoimmune disease. The etiology of T1DM is incompletely understood but environmental agent(s) are thought to trigger T1DM in the genetically at-risk. Humans are widely exposed to *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a proven multi-host chronic enteric pathogen that is mostly studied in ruminant animals and causes the inflammatory disease paratuberculosis or Johne's disease. In humans, MAP is the putative cause of Crohn's disease and has been linked to sarcoidosis, autoimmune thyroiditis, multiple sclerosis and autoimmune diabetes. The role of MAP as a trigger for T1DM was first postulated in 2005; subsequent studies suggest a link. This article discusses MAP, human exposure to MAP, genetic susceptibility to MAP and MAP in human disease including T1DM.

Keywords: Autoimmune Diabetes; T1DM; Paratuberculosis; MAP; HSP65; Molecular Mimicry

1. INTRODUCTION

The etiology of T1DM is incompletely understood but environmental agent(s) are thought to trigger T1DM in the genetically at-risk. In the United States the prevalence of T1DM is increasing and is approximately 1 in 300 by 18 years of age. Research into risk factors for T1DM is an active area with attempts to identify genetic and environmental triggers that could potentially be targeted for intervention [1].

Evidence supports a critical role of exogenous factors in the development of T1DM: 1) less than 10% of individuals with HLA-conferred diabetes susceptibility progress to clinical disease; 2) pair-wise concordance of T1DM of less than 40% among monozygotic twins; 3) greater than 10-fold difference in the disease incidence

among Caucasians living in Europe, (annual rate Macedonia 3.2/100,000 vs. Finland 54/100,000); 4) several-fold increase in the incidence over the last 50 years; and 5) migration studies that indicate disease incidence increases in population groups who have moved from a low-incidence to a high-incidence region [2].

The postulate that MAP plays a causal role in T1DM was presented at the 2005 Colloquium on Paratuberculosis [3] and published in 2006 [4]. To understand the rationale of the hypothesis it is necessary to review MAP, the role MAP plays in animal disease and the proposed role MAP has in human disease.

2. MYCOBACTERIUM AVIUM SS. PARATUBERCULOSIS (MAP)

MAP is a gram-positive, acid-fast staining small rod-shaped bacterium. As with members of the Mycobacteriaceae genus, it has a unique cell wall structure rich in complex lipids. The thick and chemically distinctive cell wall of mycobacteria is responsible in large measure for the robust nature of these bacteria, both within the host cell and in the environment. The pathogenic potential of mycobacteria is correlated with their growth rate. Paradoxically, slow-growing mycobacteria are more virulent than fast-growing mycobacteria. With the exception of *Mycobacterium leprae* (the cause of leprosy in humans), which cannot be cultured *in vitro*, MAP has the slowest growth rate of pathogenic mycobacteria. After isolation from infected animals and grown under optimal conditions colonies of MAP are typically not visible for 3 months or more [5].

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes a chronic granulomatous inflammation of the intestines in ruminant animals called Johne's disease. Mostly studied in dairy cattle, goats and sheep, MAP also causes a chronic inflammation of the intestines in beef cattle and in a wide variety of other domestic and wild ruminants. MAP-induced enteric inflammation has been found in monogastric animals including dogs and pigs as

well as four different types of subhuman primates—macaques, baboons, gibbons and cotton-top tamarins” [6]. A majority of the dairy herds in the United States and Europe have infected animals within the herd [7].

MAP and Human Exposure

MAP is present in pasteurized milk [8,9], infant formula made from pasteurized milk [10], surface water [11, 12], soil [11], cow manure “lagoons” that leach into surface water, cow manure in both solid and liquid forms that is applied as fertilizer to agricultural land [13,14], and municipal tap water [5], providing multiple routes of transmission to humans. In a recent study in Ohio the DNA of MAP was detected in over 80% of domestic water samples [15].

3. MAP AND HUMAN DISEASE—INFLAMMATORY BOWEL DISEASE AND SARCOIDOSIS

In addition to Johne’s disease of animals, MAP is the putative cause of the striking similar Crohn’s disease of humans. Although there has been a century-long debate, the role of MAP in Crohn’s has evolved from controversial to compelling [16-18]. The major source of the debate is that conventional methods of detecting bacteria—namely, culture and stain—are largely ineffective in detecting MAP. However, with newer laboratory techniques, primarily PCR, evidence of MAP is readily found in Crohn’s tissues [19,20]. MAP can be identified within the granulomas by *in situ* hybridization [21]: and, with extreme care and patience, MAP can be grown from the gut and blood of Crohn’s patients [22-24].

In addition to Crohn’s MAP has been reported as a candidate pathogen in the causation of irritable bowel syndrome [25] and some suspect that MAP causes the spectrum of inflammatory bowel disease including Crohn’s, ulcerative colitis and irritable bowel syndrome [26]. Irritable bowel syndrome is a widespread abdominal condition that affects about 10 to 15% of people in the industrialized economies of Europe, North America, Australasia, and Japan, with a rising prevalence among the populations in the developing economies of Asia. Some consider irritable bowel syndrome a *form fruste* of Crohn’s disease [27].

In addition to inflammatory bowel disease MAP has been historically linked is sarcoidosis; a multi-system inflammatory disease in which DNA evidence of MAP has been found (sporadically) in sarcoid granulomas [28].

4. GENETICS AND T1DM

Early studies indicated that the HLA region on chromosome 6p21 (commonly termed IDDM1, for insu-

lin-dependent diabetes mellitus locus) is a critical susceptibility locus for T1DM [29,30]. A comprehensive review of the genetics of T1DM is beyond the scope of this article; instead, we will focus on a gene that appears to be permissive to both MAP and T1DM (SLC11A1).

Natural resistance-associated macrophage protein 1 (NRAMP1) is now strictly referred to as SLC11A1 (solute carrier 11a1). The gene that encodes for this protein is recognized as having a role in the susceptibility of humans and animals to a number of infections, including mycobacterial infections, and is associated with a number of autoimmune diseases as well. In human beings, the SLC11A1 gene is located on chromosome 2q35. It encodes an integral membrane protein of 550 amino acids that is expressed exclusively in the lysosomal compartment of monocytes and macrophages [31].

The product of the SLC11A1 gene modulates the cellular environment in response to activation by intracellular pathogens by acidifying the phagosome thus killing the pathogen [32]. As such, it plays a role in host innate immunity [33]. Mutation of SLC11A1 impairs phagosome acidification yielding a permissive environment for the persistence of intracellular bacteria [34].

5. SLC11A1 IN INFECTIOUS AND AUTOIMMUNE DISEASE

Sarcoidosis, the previously mentioned systemic disease associated with MAP, is also associated with polymorphisms of the SLC11A1 gene [35]. Susceptibility to mycobacterial diseases tuberculosis, leprosy and Buruli’s ulcer are associated with polymorphism of the SLC11A1 gene [36]. Similar polymorphisms are associated with Johne’s disease (paratuberculosis) in cattle [37], goats [38], and sheep [39]. When researchers at the Belgium Pasteur Institute developed a murine model for MAP infection, they created an SLC11A1 defect mouse [40].

Given the pivotal roles that SLC11A1 plays in innate immunity, it is not surprising that the relationship between polymorphisms in SLC11A1 and a number of mycobacterial as well as autoimmune diseases has been explored [41]. Associations have been found with leprosy [42], tuberculosis [43], rheumatoid arthritis [44], visceral leishmaniasis [45], multiple sclerosis [46,47], inflammatory bowel disease [48-50], and type 1 diabetes mellitus [51,52].

6. MAP AND TYPE 1 DIABETES

Type 1 diabetes mellitus (T1DM) is an autoimmune disease manifest by progressive T cell-mediated autoimmune destruction of insulin-producing beta cells in the pancreatic islets of Langerhans [53]. In 2005 Dow postulated a causative role for MAP in the T1DM; Sechi in 2007 found the DNA of MAP in the blood of autoimmune

(type 1) patients but not non-autoimmune (type 2) diabetics [54-56]. Sechi also found an association of polymorphisms of the SLC11a1 gene and MAP in T1DM patients [51].

The link connecting MAP and T1DM comes from the concept of molecular mimicry: protein elements of the pathogen share sequence and/or conformational elements with the host to a degree that immune responses directed at the pathogen also attack the host. A proposed link is the mimicry of mycobacterial heat shock protein of MAP (HSP65) and pancreatic glutamic acid decarboxylase (GAD) [4].

7. MOLECULAR MIMICRY/HEAT SHOCK PROTEINS—HSP65

Molecular mimicry has long been implicated as a mechanism by which microbes can induce autoimmunity [57]. Rheumatic fever is the classic example for molecular mimicry between an infecting agent—*Streptococcus pyogenes* (group A streptococcus) and a related autoimmune disease in humans [58]. The disease is characterized by damage to the heart, joints, and the central nervous system (Sydenham's chorea). The activity of the host's immune system against the streptococcus generates a cross-recognition to human tissue causing an autoimmune reaction [59].

Heat shock proteins (HSPs) are produced in response to environmental stress. They act in a protective capacity helping cells survive stressful conditions and promoting recovery [60]. Mycobacterial HSPs have been found in several autoimmune diseases [61]. For example, the mycobacterial 65 kDa HSP has been implicated in the pathogenesis of rheumatoid arthritis [62], autoimmune hepatitis [63], primary biliary cirrhosis [64] and scleroderma [65]. HSP65 is implicated in multiple vasculitis-associated systemic autoimmune diseases such as Kawasaki disease [66], Behcet's disease [67] and Takayasu's arteritis [68].

8. MOLECULAR MIMICRY AND TYPE 1 DIABETES MELLITUS

Individuals at-risk for T1DM produce anti-GAD antibodies. HSP65 was first associated with T1DM via GAD in 1990 [69]. Mycobacteria produce HSP65 in response to stress. Epitope homology between MAP/human HSP60/65 and pancreatic glutamic acid decarboxylase (GAD) likely triggers the anti-GAD antibodies that secondarily destroy the pancreas [70].

9. T1DM AND MILK

Several studies indicate an association between early exposure to dietary cow's milk and an increased risk of T1DM [71-73]. These studies were prompted by the ob-

ervation that children at risk for T1DM who were breast fed exclusively for more than six months were less likely to have T1DM later in life than similar risk children who were weaned onto cow's milk-based formula at an earlier age. This observation spawned a large study, the TRIGR study: Trial to Reduce IDDM in the Genetically at Risk [74]. The postulate is that there is something about cow's milk protein that is an immunologic trigger for T1DM and that breaking the protein with hydrolysis may eliminate the trigger. The TRIGR study is an ongoing, 17-country study enlisting 6200 infants who are genetically at risk to develop T1DM. Children weaned early from breastfeeding are randomized into two groups; one receiving traditional cow's milk-based formula and the other receiving formula in which the protein has been hydrolyzed. A recent, smaller but somewhat parallel study shows that exposure to the hydrolyzed infant formula resulted in lessened incidence of T1DM [75]. Antibodies against specific MAP proteins were found in Sardinian children involved in the TRIGR study (Sechi, personal communication) as previously mentioned, viable MAP has been found in infant formula powder [10].

10. MAP AND OTHER AUTOIMMUNE DISEASE—THYROIDITIS AND MULTIPLE SCLEROSIS

Two recent articles link MAP to autoimmune (Hashimoto's) thyroiditis. The same molecular mimicry principle is suggested as the link between MAP (HSP65) and the organ-specific autoantigens of thyroiditis [76,77]. Also, a recent article implicate MAP in multiple sclerosis [78]. Molecular mimicry and SLC11A1 associations are germane here as well [79].

11. MAP AND T1DM—THE FUTURE

While evidence mounts that MAP is, indeed, a zoonotic agent, what policies and interventions might be employed to address curtailing MAP and the effects of its persistence in individuals? Presently, sound farm management practices and stringent culling are considered the best means to reduce the spread of MAP from animal to animal, as well as from farm to farm [80]. However, because such practices have yet to eliminate MAP from food animals, other preventive or curative measures are needed.

TASF is a Swiss-based international forum for Transmissible Animal Diseases and Food Safety. TASF acknowledges the uncertainties of the zoonotic potential of MAP for Crohn's disease. TASF suggests that

“... a decision by food safety regulators to exercise the “precautionary principle”, label MAP as a potential zoonotic agent, and adopt measures to limit as much as possible the levels of MAP contamination of raw milk and meat would go far to protect the coming generations of

children from MAP exposure, possible infection, and potentially Crohn's disease." [81].

Preliminary studies with a probiotic of the *Dietzia* species have been shown effective in treating clinically ill adult cows with Johne's disease and in preventing Johne's in calves [82,83]. The use of *Dietzia* has also been suggested for individuals with inflammatory bowel disease [84]. Vaccines are effective in reducing the incidence of clinical Johne's disease [85,86] and attenuate pre-existing infection [87]. However, such whole killed vaccines do not eliminate subclinical MAP infection or its persistence in the gut. Additionally, about half of the animals receiving whole killed MAP vaccines become false positive using the conventional tuberculin skin test diagnostic for bovine tuberculosis [88] DNA vaccination may have an interesting application: [89] showed that lambs vaccinated with plasmids encoding mycobacterial antigens produced a Th1 immune response similar to that generated by natural infection by MAP. Lambs vaccinated with DNA mycobacterial antigens (HSP65) were protected against MAP infection. Unfolding knowledge regarding susceptibility polymorphism of genes such as the SLC11a1 gene described in this article may lead to breeding practices that would limit MAP infection in breeding lines thus keeping it from the human food chain.

In humans with MAP-associated disease recognition of both the need to treat as an infectious disease as well as the need to avoid further exposure is paramount. Aggressive anti-mycobacterial treatment has had beneficial effect in those who can tolerate the treatment [90,91]. Vaccines against MAP for use in humans are being advocated and prototypes are being developed [92].

12. CONCLUSION

The controversy regarding MAP and human disease has been going for a century and will likely continue for a long time. T1DM has only recently been added to the discussion and controversy. In addition to the human toll to individuals with T1DM, the dollar cost is extreme; the burden is passed to all of society in the form of higher insurance premiums and taxes, reduced earnings, and reduced standard of living [93]. These issues should elevate the discussion, draw resources and bring a sense of urgency to the MAP/T1DM connection.

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