



Mycobacterium avium ss. *paratuberculosis* Zoonosis – The Hundred Year War – Beyond Crohn’s Disease

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The factitive role of *Mycobacterium avium* ss. *paratuberculosis* (MAP) in Crohn’s disease has been debated for more than a century. The controversy is due to the fact that Crohn’s disease is so similar to a disease of MAP-infected ruminant animals, Johne’s disease; and, though MAP can be readily detected in the infected ruminants, it is much more difficult to detect in humans. Molecular techniques that can detect MAP in pathologic Crohn’s specimens as well as dedicated specialty labs successful in culturing MAP from Crohn’s patients have provided strong argument for MAP’s role in Crohn’s disease. Perhaps more incriminating for MAP as a zoonotic agent is the increasing number of diseases with which MAP has been related: Blau syndrome, type 1 diabetes, Hashimoto thyroiditis, and multiple sclerosis. In this article, we debate about genetic susceptibility to mycobacterial infection and human exposure to MAP; moreover, it suggests that molecular mimicry between protein epitopes of MAP and human proteins is a likely bridge between infection and these autoimmune disorders.

Keywords: paratuberculosis, MAP, Crohn’s, autoimmune, molecular mimicry, type 1 diabetes, autoimmune thyroiditis, multiple sclerosis

INTRODUCTION

In 1913, a concise description of what today is known as Crohn’s disease was offered by Scottish surgeon Kennedy Dalziel (1). Twenty years earlier, in 1895, German veterinary Johne H. A. described the cause of an incurable profuse diarrhea in cattle. He noted acid-fast bacteria (most often indicating the organism that causes tuberculosis) that, when transferred to a guinea pig, did not cause tuberculosis (2). Johne first labeled the disease “pseudotuberculosis” and it eventually became known as paratuberculosis.

Infected cow’s intestines had the same cobblestone aspect of Dalziel’s patient and microscopically, the patient’s and cattle’s diseased intestines were so alike that Dalziel wrote that the tissue characteristics were:

... so similar as to justify a proposition that the diseases may be the same (1).

He hypothesized that the disease in cattle and the disease in people shared the same cause. The disease in humans was later named after Dr. Crohn who described a series of patients in 1932 (3).

The heart of this 100-year controversy revolves around the fact that the usual diagnostic techniques to detect bacteria are commonly inefficient to detect *Mycobacterium avium* ss. *paratuberculosis* (MAP) in humans. A short explanation is that it is just very difficult to grow MAP from humans; and, MAP exists with a modified cell wall – the component of the bacterium that takes up the characteristic acid stain. In this state, the bacterium is no longer “acid fast” and cannot be detected microscopically. Recent work has identified the capacity of MAP to undergo a morphologic

change to become spore-like. The spore morphotype survives heat and other stressors and may lead to an increased persistence in hosts and the environment (4).

Understanding the difficulty in detection and appreciating the work of specialty labs that have shown MAP bacteremia in Crohn’s disease patients, there has been a warming to the association of MAP in Crohn’s (5).

MYCOBACTERIUM AVIUM ss. PARATUBERCULOSIS

Mycobacterium avium ss. *paratuberculosis* is an acid-fast staining small rod-shaped bacterium (6, 7). As with members of the Mycobacteriaceae genus, its cell wall structure rich in complex lipids is unique. The tough and peculiar cell wall of mycobacteria is, in large part, responsible for the persistence of these bacteria, both in the environment and inside the host. Paradoxically, the pathogenic potential of mycobacteria increases as their growth rate decrease. In fact, slow-growing mycobacteria are more pathogenic than fast growing mycobacteria. Except the uncultivable *Mycobacterium leprae* (the cause of leprosy in humans), MAP has the slowest growth rate among harmful mycobacteria. After inoculum of infected samples from infected animals and incubated under optimal conditions, MAP colonies usually appear not before 3 months or more (8).

MAP AND HUMAN EXPOSURE

Mycobacterium avium ss. *paratuberculosis* can be found in pasteurized milk (9, 10), milk powder for children (11), surface water (12–14), soil (12), cow manure that contaminates the soil and surface water, moreover cow manure is usually applied as fertilizer in different crops (15) and supply of drinking water (16) all

contributing to human exposure. Soil and plants in grazing areas retain MAP; its DNA can be detected in the upper greens of plants, their roots and in the soil below the roots to a depth of 80 cm (17, 18). MAP DNA was detected in over 80% of domestic water samples in Ohio (19). Chlorination and filtration may help to survive mycobacteria rather than eliminate these organisms by killing off their competitors (20). Moreover, mycobacteria organisms have been reported on tap water pipes (21) in biofilms (22) and plastic water bottles (23). One estimate is that mycobacteria could be present in drinking water in “massive numbers,” on the amount of up to 700,000 or 7×10^5 organisms per liter of water (22). A recent study reported testing infant formula for MAP in 65 samples from 18 countries: >40% tested positive for viable MAP (24).

MAP AND HUMAN DISEASES

In addition to Crohn's, MAP has been associated with multiple diseases: sarcoidosis and Blau syndrome (25), type 1 diabetes (26–32), Hashimoto's thyroiditis (33–36), and multiple sclerosis (MS) (37–49). In autoimmune diabetes, thyroiditis, and MS, MAP is thought to induce pathology due to molecular mimicry between protein elements of itself and the targeted organ elements of the host, e.g., MAP 3865c and Znt8 in autoimmune (type 1) diabetes and thyroiditis (31, 35, 36). **Figure 1** shows how MAP may trigger autoimmune diseases.

If humans are so readily exposed to MAP, why is there not pervasive Crohn's disease and the other diseases mentioned in this article?

GENETICS

CARD15

A good example about the interaction between the genetic susceptibility and microbial infection can be found in Crohn's

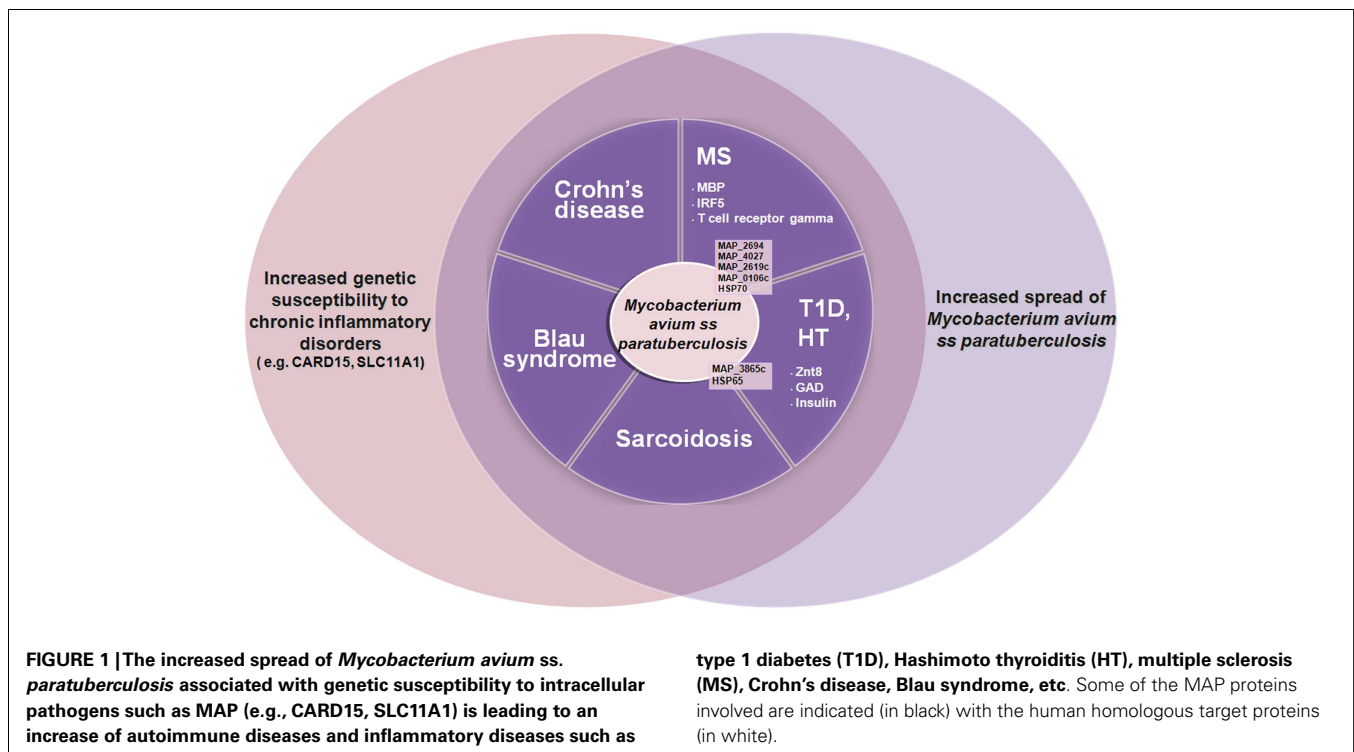
and Blau syndrome (50), both having polymorphisms within the CARD15 gene.

The gene was originally referred as the NOD2 gene and linkage studies have placed it on chromosome 16; now it is known as the CARD15 gene (51). The CARD15 gene is part of the ancestral innate immune system that recognizes bacteria peptidoglycan in particular mycobacterial glycolylated form of muramyl dipeptide MDP (52–54).

CARD15, BLAU SYNDROME, AND CROHN'S DISEASE

Insights into the consequence of genetic susceptibility to MAP infection may be observed in the rare inflammatory disease, Blau syndrome. This granulomatous inflammatory disorder is characterized by uveitis, arthritis, and dermatitis (50). Although rare, Blau syndrome has been of interest in recent medical literature because of the inherited or *de novo* mutation within the CARD15 gene, the same gene associated with Crohn's susceptibility (55, 56). However, Blau syndrome susceptibility component of the CARD15 gene is located at the nucleotide binding site domain (55, 56) whereas the Crohn's susceptibility can be found at the N-terminal leucine-rich repeat domain (57–59).

Blau syndrome shares the same clinical characteristics of juvenile sarcoidosis; in fact, new CARD15 mutations are consistently found in cases of sporadic juvenile sarcoidosis – Blau syndrome (60, 61). For these reasons – the clinical appearance of sarcoidosis and a shared genetic susceptibility with Crohn's – it was proposed that MAP could have a role in Blau syndrome. A series of Blau tissues comprised of skin, synovial samples as well as Blau granulomas of the liver and kidney were tested for the presence of MAP. Six tissues of five patients representing three different families were all found to have MAP present in the tissue granulomas (25).



The proposed etiopathology is that following MAP exposure, an individual genetically susceptible with mutations within the nucleotide binding domain of CARD15 will exhibit Blau syndrome whereas if the mutations are within the leucine-rich-repeat domain of the same gene, they may exhibit Crohn's disease. Moreover, it has been reported that CARD15 defects of the leucine-rich-repeat domain, are associated in an aggressive phenotype of Crohn's disease (62). Recent work has reviewed the susceptibility genes associated with Crohn's (63).

SLC11A1

An additional gene linked with Crohn's susceptibility is the solute carrier 11a1 (SLC11A1) gene (64). SLC11A1 was previously identified as natural resistance-associated macrophage protein 1 (NRAMP1) (65). Polymorphisms within this gene and its promoter are recognized as having a role in the susceptibility of humans and animals to a number of infections, in particular mycobacterial infections, and it has been related to the susceptibility to autoimmune and inflammatory disease as well (64, 65). The SLC11A1 gene, located on chromosome 2q35, is around 14 kb in length. It encodes an integral membrane protein of 550 amino acids that is localized within the acidic endosomal and lysosomal compartment of resting macrophages (65).

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 (66). As such, it plays a role in host innate immunity (67). Mutations of SLC11A1 may impair phagosome acidification yielding a permissive environment for the persistence of intracellular bacteria (68).

SLC11A1 IN INFECTIOUS AND AUTOIMMUNE DISEASE

Sarcoidosis, an other systemic disease associated with MAP, has been associated with polymorphisms of the SLC11A1 gene (69). Susceptibility to mycobacterial diseases, leprosy, and Buruli's ulcer were also associated with polymorphism of the SLC11A1 gene (70). Similar polymorphisms have been associated with Johne's disease (paratuberculosis) in cattle (71), goats (72), and sheep (73). A SLC11A1 defect mouse was created by researchers at the Belgium Pasteur Institute to develop a murine model for MAP infection (74).

Due to the capability of SLC11A1 to modulate 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 (75). In addition to leprosy (76) and tuberculosis (77), an association is found in rheumatoid arthritis (78), MS (39, 79), inflammatory bowel disease (80–82), and type 1 diabetes – all diseases associated with MAP (83, 84).

MOLECULAR MIMICRY

Molecular mimicry by a microorganisms has been hypothesized to initiate and exacerbate an autoimmune response through sequence or structural similarities with self-antigens (85, 86). Rheumatic fever is one of the best examples for molecular mimicry between group A streptococcus and host antigens leading to the glomerulonephritis and rheumatic heart disease (87, 88). The development of post-streptococcal sequelae is characterized by damage to the

heart, joints, and the central nervous system (Sydenham's chorea). Damage of the heart is the most critical effect and is present in 30–45% of the cases – mostly causing damage to the heart valves.

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 (89). Sechi in 2008 found the DNA of MAP in the blood of autoimmune (type 1) patients (32) but not non-autoimmune (type 2) diabetes (27, 28). Sechi also found an association of polymorphisms of the SLC11a1 gene and MAP in T1DM patients (59, 64, 82).

While it may be intuitive to envision an occult presence of MAP as an infective agent producing a granulomatous lesion of Crohn's or sarcoidosis (Table 1A); it may be more difficult to assign a role for MAP in T1DM. The link connecting MAP and T1DM is molecular mimicry: protein elements of the pathogen "look like" elements of the host's endocrine pancreas; and immune responses directed at the pathogen sometimes may attack the host (Table 1B). Childhood exposure to cows milk-based infant formula is a strong risk factor for juvenile autoimmune diabetes (30)

Table 1 | (A) Map-related granulomatous diseases. (B) Map-associated autoimmune diseases.

(A) MAP-RELATED GRANULOMATOUS DISEASES		
Disease	Shared genetic susceptibility	Reference
Crohn's	CARD15, SLC11A1	(8, 51, 52, 57, 59, 62, 64)
Sarcoidosis	SLC11A1	(54, 69)
Blau syndrome	CARD15	(52–56, 60)

(B) MAP-ASSOCIATED AUTOIMMUNE DISEASES		
Disease	Mimicking elements	Reference
Autoimmune diabetes	HSP65/GAD MAP3865c/ZnT8 – pancreatic	(31, 86–88, 90–94)
Autoimmune thyroiditis	MAP3865c/ZnT8 – thyroid	(35, 36)
Multiple sclerosis	HSP70, MAP_2694, MAP4027, MAP_2619c 352-61, MAP_0106c protein 121–132	(37–49)

These granulomatous diseases are ones where evidence of MAP can be found in the granuloma. CARD15, caspase recruitment domain gene 15; SLC11a1, solute carrier 11a1 gene.

These autoimmune diseases have autoantibodies. There are share molecular elements between MAP proteins and host organs. HSP65, heat shock protein 65; GAD, glutamic acid decarboxylase; MAP3865c, M. paratuberculosis protein 3865c; ZnT8, zinc transporter 8; HSP70, heat shock protein 70; MAP-0106c, M. paratuberculosis 0106c protein (aa. 121–132); MBP85-98, myelin basic protein (aa. 85–98).

and, as mentioned in the “exposure to MAP” section of this paper, viable MAP is found in infant formula (24).

The proposed links is the mimicry of mycobacterial protein MAP3865c and the human homolog Znt8 (31, 35) along with the heat shock protein of MAP (HSP65) and pancreatic glutamic acid decarboxylase (GAD) (30). Different islet autoantibodies (aAbs) may characterize the period preceding T1D clinical onset, aAbs against islets antigens such as insulin, glutamic acid decarboxylase (GAD65), insulinoma associated protein-2, and zinc transporter 8 (ZnT8) may be detectable for months up to years before disease onset. Sechi et al., for example, reported that anti-MAP and anti-ZnT8 antibodies (Abs) targeting homologous membrane-spanning sequences are cross-reactive and capable of eliciting strong immune responses in T1D adult patients (91). One of the sequences was also able to elicit a T cell response (95). An association between MAP and T1D in children was demonstrated by Cossu et al. (96). Additional evidence of the involvement of MAP in the early phases at T1D onset appear from two studies (91, 92) where an association between Abs positive for ZnT8 and MAP homolog epitopes in Sardinian and Italian children at T1D onset was demonstrated. Moreover, Sechi et al. (93) reported a similar high antibody response against insulin epitopes and its MAP homologous peptides in children; those both at risk for T1D and at T1D onset. A review on the topic was previously reported (94).

MAP AND AUTOIMMUNE THYROIDITIS

The most common autoimmune disease associated to T1D is autoimmune thyroid disease, its frequency is estimated at >90% among patients with T1D and autoimmune diseases (97). Different articles associate MAP to autoimmune (Hashimoto's) thyroiditis (HT) (33, 34). The same molecular mimicry principle is suggested as the link between MAP and Znt8, one of the organ-specific autoantigens of thyroiditis (33–36). Though ZnT8 is primarily expressed in pancreatic islet cells, it is also expressed in the follicular and para-follicular epithelial cells of the thyroid gland. In view of the evidence accounting for a cross-recognition of MAP3865c/ZnT8 homologs sequences in T1D subjects, and applying the theory which proposes MAP as an HT environmental trigger (acting through a molecular mimicry mechanism) (35, 36), it is natural to consider MAP for a causal role in HT. Moreover, it has been reported that the occurrence of islet aAbs (especially ZnT8) was associated with a positive titer of thyroid peroxidase antibodies (ATPO) in newly diagnosed adult-onset autoimmune diabetic patients (98).

HEAT SHOCK PROTEINS

Heat shock proteins (HSPs) are expressed at high level in response to environmental stress. They stabilize proteins and are involved in the folding of denatured proteins helping cells survive stressful conditions and promoting recovery (99). HSPs are synthesized to respond to the presence of invading pathogens. However, pathogens may also produce their HSPs. The increased expression of both self and infective stress proteins and the extensive sequence homology between microbial and human HSP (50–80% amino acid homology of mycobacterial HSP65 and human HSP60) have led to the concept that HSPs are involved in the

etiology and pathogenesis of many immune-mediated disorders (100). Antibodies to mycobacterial HSPs have been found in various autoimmune diseases (101). Just to mention some, the mycobacterial 65 kDa HSP has been associated to rheumatoid arthritis (102–104), autoimmune hepatitis (105), primary biliary cirrhosis (106), and systemic sclerosis (107). HSP65 has been reported in different vasculitis-associated systemic autoimmune diseases such as Kawasaki disease (108), Behcet's disease (109) Takayasu's arteritis (110), moreover, Hsp70 has also been associated with MS (90).

MAP AND MULTIPLE SCLEROSIS

Sechi et al. have published studies implicating MAP in MS (37–39, 41–49). Molecular mimicry and SLC11A1 associations are central to this association as well (40, 41). MAP has been associated with Epstein–Barr virus (EBV – thought to be one of the triggers of MS) (44): peptides of each microorganism (MAP and EBV) cross react with anti-myelin basic protein (MBP) (43) and interferon regulatory factor 5 (IRF5) in MS patients (48). Interferon-beta therapy influence antibody response against MAP (49). An extensive review on the topic has been previously published (46).

THE FUTURE – MAP AND HUMAN DISEASE

The role of MAP in Crohn's disease has progressed from controversial to conspicuous to compelling. The century-old striking similarities existing between Johne's and Crohn's diseases on a tissue level are now validated at cellular and molecular levels (90). There is an increasing awareness and call for resolution (111, 112). Improved testing strategies for ruminant herds such as metabolomic profiling (113) will aid in the public health approach to animal disease and sources of human exposure. On a limited basis, Crohn's disease has been treated successfully with antibiotics (114, 115). As the MAP/Crohn's debate resolves and as more diseases are linked to MAP, there will likely be a major shift in the public health approach to MAP and human disease. Early indications of such a shift are two clinical trials employing anti-mycobacterial drugs: clarithromycin, rifabutin, and clofazimine. One is a 60-center trial in Crohn's disease (116) and another is the same treatment for MS (117). Positive outcomes from efforts like these – curing Crohn's disease and MS with anti-mycobacterial medication as well as prevent autoimmune diabetes and thyroiditis – will further solidify the role of MAP as a zoonotic agent in human disease and, perhaps after more than a century, will resolve this medical controversy.

ACKNOWLEDGMENTS

The authors wish to acknowledge Magdalena Niegowska-Conforti for creating **Figure 1**. The research has been possible thanks to the following funds: Fondazione Banco di Sardegna year 2014; Regione Autonoma della Sardegna LR7, year 2010 and Grant supported by FISM entitled GEOEPIDEMIOLOGY OF MULTIPLE SCLEROSIS: THE ENVIRONMENTAL RISK FACTORS, Year 2012.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 November 2014; accepted: 18 February 2015; published online: 04 March 2015.

Citation: Sechi LA and Dow CT (2015) *Mycobacterium avium* ss. *paratuberculosis* Zoonosis – The Hundred Year War – Beyond Crohn's Disease. *Front. Immunol.* **6**:96. doi: 10.3389/fimmu.2015.00096

This article was submitted to *Mucosal Immunity*, a section of the journal *Frontiers in Immunology*.

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