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Mycobacterium paratuberculosis and autism: Is this a trigger?

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ABSTRACT

Autism is a heterogeneous group of life-long neurologic problems that begin in childhood. Success in efforts to understand and treat autism has been mostly elusive. The role of autoimmunity in autism has gained recognition both for associated systemic autoimmune disease and the presence of brain autoantibodies in autistic children and their family members. There is an acknowledged genetic susceptibility to autism – most notably allotypes of complement C4. C4 defects are associated with several autoimmune diseases and also confer susceptibility to mycobacterial infections. *Mycobacterium avium* ss. *paratuberculosis* (MAP) causes an enteric inflammatory disease in ruminant animals (Johne's disease) and is the putative cause of the very similar Crohn's disease in humans. Humans are widely exposed to MAP in food and water. MAP has been also linked to ulcerative colitis, irritable bowel syndrome, sarcoidosis, Blau syndrome, autoimmune (Type 1) diabetes, Hashimoto's thyroiditis and multiple sclerosis. Environmental agents are thought to trigger autism in the genetically at risk. Molecular mimicry is the proposed mechanism by which MAP is thought to trigger autoantibodies. Autoantibodies to brain myelin basic protein (MBP) is a common feature of autism. This article considers the subset of autoimmunity-related autism patients and postulates that MAP, through molecular mimicry to its heat shock protein HSP65, triggers autism by stimulating antibodies that cross react with myelin basic protein (MBP).

Introduction

Autism is a devastating childhood developmental disorder that features language impairment, dysfunctional social interactions, and repetitive behavior patterns [1]. Autism spectrum disorders (ASD) are a collection of developmental neurobehavioral conditions within the pervasive developmental disorders that include autistic disorder, Asperger's disorder, and pervasive developmental disorder [2]. Autism appears to be on the rise in the United States [3,4], although some of the factors, including earlier-age diagnosis, could influence the reported frequency increases [5]. The Centers for Disease Control and Prevention (CDC) reports that about 1 in 150 children have a form of ASD [6]. It has been suggested that ASD arise from multiple causal pathways [7] and represent a multi-system disorder [8]. The current thought is that genetic background plays a role in autism [9]; and, prenatal and perinatal environmental factors pose risk and/or trigger the disease [10–13]. This paper proposes a causal link between infection with *Mycobacterium avium* ss. *paratuberculosis* (MAP) and autism.

Autism—genetic and environmental factors

Twin studies comparing concordance for autism in identical and fraternal twins have shown that autism has a genetic component with significant heritability indices [14]. The average concordance for identical twins is 64% vs. 9% for fraternal twins [15]. Family studies reported significant sib recurrence risks in large families; the latter sib recurrence risk was 8.6%, and for families with two or more affected children, the recurrence risk approached 35% [16]. An additional indicator that autism is genetically determined comes from finding that a comprehensive genetics evaluation can reveal a chromosomal or Mendelian cause or predisposition in 15–40% of children who fit ASD behavioral diagnostic criteria [17]. While genetic factors – mutations, deletions, and copy number variants – are clearly implicated in causation of autism, they account for a fraction of cases, and do not readily explain key clinical and epidemiological features. The increased prevalence of autism over the last 20 years and the incomplete concordance for autism in MZ twins has prompted the search for environmental triggers of autism [18].

The most powerful proof-of-concept evidence of environmental triggers comes from studies specifically linking autism to exposures in early pregnancy – thalidomide, misoprostol, and valproic acid; maternal rubella infection; and the organophosphate insecticide, chlorpyrifos [19]. Most notable of suspected environmental

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agents was thimerosal. Thimerosal, a preservative used in a number of childhood vaccines and the measles–mumps–rubella vaccine have been the major targets of interest. The original studies by Wakefield [20,21] that suggested an association between immunizations and autism have been disproved with the work retracted by *The Lancet* [22]. Although parental concern is still significant, multiple studies and lines of scientific evidence have identified no support for a relationship between immunizations and autism [23–26]. A tragedy resulting from fear of an autism epidemic has been the decreased use of childhood immunizations leading to outbreaks of measles and childhood deaths [27,28].

Autoimmunity and autism

The connection between autoimmunity and ASD was first recognized by Money [29]. Children with ASD were found to be more likely to have a family member with an autoimmune disease than normal controls. Immune dysfunction is a common occurrence among cases of autism and ASDs [30–34]. Hertz-Picciotto [35] noted the probable relationship between autism and the immune dysfunction associated with autism. The presence of brain autoantibodies in a significant number of autistic children [36–40] suggests the pathogenic role of autoimmunity in those autistic patients. This is true not just for children with autism, but also for their family members [41].

C4 allotypes-shared susceptibility to autism, autoimmune disease and mycobacterial infection

Complement component C4 is an important protein of the classical pathway of complement activation. C4 plays an important role in innate defense against microbes and, as such, it is an adjunct or "complement" to humoral immunity [42–44]. The frequency of C4B null allele is significantly higher in autistic children. In addition, a family history of autoimmunity imparts a significant risk for association with C4B null allele in autistic children [45–46].

Increased prevalence of C4 null alleles is a common feature of autoimmune diseases [47–50]. The shared association of C4B null allele with both autoimmune disease and autism suggests that autoimmunity has a role in autism [51]. Additionally, complement C4 defects increase susceptibility to mycobacterial infections of *Mycobacterium leprae*, *Mycobacterium tuberculosis* and *Mycobacterium avium* [52–54].

Mycobacterium avium ss. *paratuberculosis* (MAP)

MAP is a gram-positive, acid-fast staining small rod-shaped bacterium. The thick and chemically distinctive cell wall of mycobacteria is responsible in large measure for the robust nature of these bacteria. With the exception of *M. 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 [55].

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 Johne's disease 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" [56]. A majority of the dairy herds in the United States and Europe have Johne's infected animals within the herd [57].

MAP and human exposure

MAP is present in pasteurized milk [58,59], infant formula made from pasteurized milk [60] surface water [61–63], soil [61], cow manure "lagoons" that can leach into surface water, cow manure in both solid and liquid forms that is applied as fertilizer to agricultural land [64], and municipal tap water [55], 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 [65].

Normal water treatment processes such as filtration and chlorination enables mycobacteria organisms by killing off their competitors [66]. Mycobacteria organisms grow on tap water pipes [67] in biofilms [68] and on plastic water bottles [69].

MAP and human disease

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 [70–72]. 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 [73,74]; it can be visualized within the granulomas by in situ hybridization [75]: and, with extreme care and patience, MAP can be grown from the gut and blood of Crohn's patients [76–78].

MAP is the suspected cause of the whole spectrum of inflammatory bowel disease – Crohn's, ulcerative colitis and irritable bowel syndrome [79–80]. Irritable bowel syndrome is a widespread abdominal condition that affects about 10–15% 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 [81].

MAP has also historically been linked to sarcoidosis [82]. More recently, MAP has been associated with autoimmune (Type 1) diabetes [83,84], autoimmune thyroiditis [85,86], Blau syndrome [87] and multiple sclerosis [88]. 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; it is broader divide to assign a role for MAP in autoimmune diabetes, thyroiditis and autism. The proposed link connecting MAP and these diseases comes from the concept of molecular mimicry: protein elements of the pathogen share sequence and/or conformational elements of the host to a degree that immune responses directed at the pathogen also attack the host.

Mimicry/heat shock proteins – HSP65

Molecular mimicry has long been implicated as a mechanism by which microbes can induce autoimmunity [89,90].

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 [91]. Mycobacterial HSPs have been found in a several autoimmune diseases [92]. For example, the mycobacterial 65 kDa HSP has been implicated in the pathogenesis of rheumatoid arthritis [93–95] autoimmune hepatitis [96], primary biliary cirrhosis [97] and scleroderma [98]. HSP65 is implicated in multiple vasculitis-associated systemic autoimmune diseases such as Kawasaki disease [99], Behcet's disease [100] and Takayasu's arteritis [101] and Type 1 diabetes [102].

Individuals at-risk for T1DM produce anti-GAD antibodies. HSP65 was first associated with T1DM via GAD in 1990 [102].

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Mycobacteria produce HSP65 in response to stress. Epitope homology between mycobacterial HSP65 and pancreatic glutamic acid decarboxylase (GAD) likely triggers the anti-GAD antibodies that secondarily destroy the pancreas [103]. It has been proposed that MAP provides the mycobacterial HSP65 [104].

Mycobacterial HSP65 and anti-brain myelin basic protein

There are behavioral and neurologic diseases that are driven by autoantibodies. Microbial-prompted molecular mimicry occurs in group A streptococcus when antibodies cross-react with host neural elements and produce the neuropsychiatric and movement disorders of Sydenham chorea. Additionally, pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS) is a new, but well characterized, obsessive–compulsive disorder also due to antibody-directed neurologic-targeted autoimmune inflammation [105]. Autoimmunity to brain antigens is a common feature of autism. Of all brain autoantibodies, the most prevalent is that to CNS myelin-derived myelin basic protein (MBP) [106,107].

A major characteristic of autoimmune diseases is an infiltration of mononuclear cells into tissues that otherwise exhibit a paucity of immune cell types. Under normal circumstances, T cells confer immunity by the specific recognition of foreign antigen. Autoimmunity occurs when these T cells also target host tissue that has epitope homology with the foreign antigen. Such is the case with homology between mycobacterial HSP65 (aa3-13) and MBP (aa84-102) [108]. The homology between these proteins is the crux of this article.

Vitamin D and autism

Vitamin D deficiency has become a major health concern where topical sunblock and indoor activities have limited sun exposure for children and dietary sources cannot make up the difference. Vitamin D is a potent modulator of the immune system. There is a recognized contribution of vitamin D deficiency to the development of autoimmune diseases. Epidemiological studies present evidence linking vitamin D deficiency with autoimmune diseases, such as rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus [109–111].

Vitamin D deficiency is also postulated to have a role in autism [112]. Case-controlled cross-sectional study clearly shows that circulating vitamin D levels are significantly lower in children with autism than in healthy controls suggests that vitamin D insufficiency may play a role in the etiology of autism [113].

Vitamin D also has a role in fighting mycobacterial infection. Niels Ryberg Finsen was awarded the Nobel Prize in 1903 for the treatment of mycobacterium infections with UV-B light [114]. There have been several studies looking at various doses of vitamin D in treatment of mycobacteria. Sun exposure results in the production of 10–20 000 IU of vitamin D in a relatively short period of time [115]. In a series studying individuals with tuberculosis, the use of 10 000 IU of vitamin D3 daily in addition to the antibiotics resulted in 100% sputum conversion rates as compared 77% in those using the antibiotics alone of *Mycobacterium tuberculosis* [116]. The mechanism for this remained unclear until the publication of a study by Liu et al. showing that the vitamin D-induced anti-mycobacterial affect is dependent upon the induction of cathelicidin [117].

The striking male:female ratio (4:1) known with autism may be explained by vitamin D.

Estrogen and testosterone appear to have markedly different effects on vitamin D metabolism. Studies have found a positive effect of estrogen on calcitriol levels [118] but not so with testosterone. If estrogen increases calcitriol, but testosterone does not; such differences may mean that estrogen shields females from calcitriol deficiencies, while testosterone does not for males.

Summary

Although autism was first described more than 60 years ago by American psychiatrist Leo Kanner, autism still remains poorly understood [119]. This article postulates a parsimonious pathway linking autism to MAP. Innate immune dysfunction of C4 allotypes – associated with autism – allows for mycobacterial infection (MAP). The persistent presence of MAP results in its production of mycobacterial HSP65, the response to HSP65 results in peripheral blood autoantibodies to myelin basic protein, MBP. The inflammation associated with anti-MBP antibodies may cause the language, social and behavioral patterns of autism.

Conflict of interest statement

Author attests to no conflicts of interest.

Grants

None.

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