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Short communication

The potential role of subclinical *Bordetella Pertussis* colonization in the etiology of multiple sclerosis

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ABSTRACT

It is established that (1) subclinical *Bordetella pertussis* colonization of the nasopharynx persists in highly vaccinated populations, and (2) *B. pertussis* toxin is a potent adjuvant that, when co-administered with neural antigens, induces neuropathology in experimental autoimmune encephalomyelitis, the principle animal model of multiple sclerosis. Building on these observations with supporting epidemiologic and biologic evidence, we propose that, contrary to conventional wisdom that subclinical pertussis infections are innocuous to hosts, *B. pertussis* colonization is an important cause of multiple sclerosis.

1. Introduction

For more than 50 years, *Bordetella pertussis* (BP) and BP toxin (BP_{TOX}) have served as laboratory adjuvants to induce sensitization to co-localized neural antigens in experimental autoimmune encephalomyelitis (EAE), the principle animal model of multiple sclerosis (MS) (Levine and Wenk, 1961). The use of bacterial adjuvants in EAE is described in thousands of publications and EAE recapitulates salient features of MS, including central nervous system (CNS) inflammation, demyelinated plaques, and disruption of the blood–brain barrier (BBB). Yet, a widely accepted environmental trigger for MS remains elusive. Given that nasopharyngeal subclinical BP colonization (SCBPC) is well-documented in highly BP-vaccinated populations (Zhang et al., 2014; Ward et al., 2005), we propose that BP and BP_{TOX}, beyond their role as adjuvants in animal models of MS, are actual human neuropathogens. We review epidemiologic and biologic evidence supporting the hypothesis that SCBPC is an important cause of MS.

MS is a heterogeneous autoimmune demyelinating disease of the CNS with concordance rates in monozygotic twins of 5.9–50% (Willer et al., 2003), indicating significant environmental causation. BP is a Gram-negative bacterium that secretes biologically active toxins and causes whooping cough (acute clinical BP). Subclinical BP colonizing infections are here defined as asymptomatic or mild infections (e.g., transient cough, rhinorrhea) that elicit minimal or no BP-directed host immunity, and thereby allow unopposed BP toxin activity in a host. To illustrate the relationship between BP colonization and MS, we first apply our hypothesis to three environmental observations.

2. Epidemiologic evidence

2.1. Case 1

The most striking MS-related epidemiologic phenomenon of the last century was the MS epidemic in the Faroe Islands during, and immediately after, World War II (WWII). This outbreak was elegantly investigated by Kurtzke, who speculated that "MS is the rare late outcome of a specific but unknown infectious disease of adolescence and young adulthood" (Kurtzke, 1993). While others have suggested an MS-BP link (Fiore, 2003), we propose that, more precisely, adjuvant-induced neural sensitization by co-localized

Abbreviations: SCBPC, subclinical *Bordetella pertussis* colonization; EAE, experimental autoimmune encephalomyelitis; BP, *Bordetella pertussis*; BP_{TOX}, *Bordetella pertussis* toxin; MS, multiple sclerosis; CNS, central nervous system; BBB, blood–brain barrier; wPV, whole-cell pertussis vaccine; aPV, acellular pertussis vaccine.

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nasopharyngeal BP colonization is the primary BP-mediated cause of MS.

Before 1920, Faroese pertussis outbreaks recurred every 5–6 years, stimulating BP-specific systemic and mucosal immunity, consistent with clinical studies (Long et al., 1990; Wendelboe et al., 2005), and primate studies demonstrating that acute clinical BP lowers mucosal BP colonization upon reexposure (Warfel et al., 2014). We suggest that the absence of documented MS in the Faroes during this period (Kurtzke, 1993) was due to repeated, likely unrecognized, BP infections which induced protective mucosal immunity (latent BP immunization) thereby limiting subclinical BP colonization. In addition, regular subclinical or high-dose BP and BP_{TOX} exposure may have suppressed autoimmunity by inducing T-regulatory cells (Tregs) and anti-inflammatory cytokines TGF-β and IL-10, as demonstrated in rodents (Weber et al., 2010). Notably, suppression of EAE has been demonstrated via pre-treatment with BP (Lehmann and Ben-Nun, 1992). Faroese BP epidemiology changed in the 1920s when whole-cell pertussis vaccination (wPV) studies immunized nearly 80% of those eligible (Madsen, 1933). These trials decreased BP disease rates, which we submit reduced individual BP exposure and latent immunization, thus diminishing Faroese mucosal BP immunity. Importantly, intramuscular wPV does not induce protective mucosal immunity (Warfel et al., 2014; Mills, 2001). By WWII, waning immunity, occurring at 4–12 years for wPV and 3.5–20 years for natural immunity (Wendelboe et al., 2005), would have raised the risk for BP colonization.

From 1940 to 1945, 1000–7000 British troops occupied the Faroes (Kurtzke, 1993), an isolated archipelago with <30,000 inhabitants. Since Britain did not nationalize BP vaccination until 1957, a relatively high rate of BP carriage is expected among troops, themselves protected against BP colonization effects by acquired immunity from recurrent BP exposures at home. As asymptomatic BP carriage is transmissible (Long et al., 1990; Warfel et al., 2014), we propose that British soldiers re-introduced BP to a population rendered susceptible to BP colonization by lapsed mucosal BP immunity after the 1920s wPV trials. Without natural or vaccine-induced mucosal BP immunity, the Faroese were susceptible to BP transmission from British soldiers—a perfect storm for an SCPBC and MS epidemic.

The first recorded case of MS in a native-born Faroese resident occurred in 1943, heralding an epidemic peaking two years later (Kurtzke, 1993). Soldiers departed in 1945 and the incidence of MS rapidly dropped off by 1950. Faroese living closest to British barracks had the highest risk of MS (Kurtzke, 1993).

2.2. Case 2

Global MS rates exhibit an equatorial gradient: the further from the equator, the greater the MS risk. We propose that heterogeneous global risks for MS parallel heterogeneous global BP vaccination rates, as vaccination influences BP incidence, individual BP exposure, latent mucosal BP immunization, and thus BP colonization rates. In the WHO-defined African region, where initial pertussis vaccination series coverage was 5% in 1980, 52% in 2000 and 72% in 2012 (when US rates were 96%, 94% and 96% respectively) (WHO, 2014), MS incidence has been low. In several African nations from 1960 to 1986, the unweighted mean for MS per 100,000, derived from 8 prevalence studies, was 8 (range 3–15), while the mean from 9 US surveys from 1970 to 1985 was 72 (range 9–160) (Rosati, 2001). We suggest that low African BP vaccination rates lead to high individual BP exposure, high rates of latent immunization and mucosal BP immunocompetence among adolescents and adults, and therefore to lower BP colonization and MS rates. In contrast, high US vaccination rates lead to low individual rates of BP exposure, low rates of latent immunization and mucosal BP immunocompetence, and to higher BP colonization and MS rates. We propose that the equatorial MS gradient reflects a BP vaccination and SCBPC gradient.

The higher risk for MS in the US correlates with, but is not held to be directly caused by, wPV and acellular pertussis vaccination (aPV), which decrease acute clinical BP risk, but do not induce protective mucosal immunity, preclude subclinical BP infection (Zhang et al., 2014; Ward et al., 2005; Warfel et al., 2014) or prevent transmission (Long et al., 1990; Warfel et al., 2014). Subclinical BP colonization (not wPV, aPV or acute clinical BP) is proposed to directly increase MS risk.

2.3. Case 3

American schoolteachers and healthcare workers are at increased risk for symptomatic and asymptomatic BP infection (De Serres et al., 2000; Wright et al., 1999). Government data indicate increased MS mortality among elementary and secondary school teachers, compared with all other professional occupations (proportional MS mortality: 161%, $p < 0.0001$), which persists after adjustment for gender, race and age (Walsh and DeChello, 2001). Increased MS risk among health technicians and nurses' aides has also been demonstrated (Walsh and DeChello, 2001). Finally, the 2:1 female:male incidence ratio of MS mirrors the 2:1 female:male ratio of adult pertussis (De Serres et al., 2000).

3. Biologic evidence

There is substantial biologic plausibility for subclinical BP colonization as a cause of MS. BP colonization is common in children, adolescents, and adults (Zhang et al., 2014; Ward et al., 2005). BP colonizes nasopharyngeal ciliated epithelium, co-localizing BP with neural antigens such as terminal olfactory nerves. Olfactory tract inflammation and demyelination are common in MS, supporting co-localized adjuvant-induced sensitization (De Luca et al., 2014).

Further, BP_{TOX} is a potent exotoxin adjuvant that promotes autoimmunity in EAE, inducing IL-6-dependent IL-17 producing T-cells, and reducing Foxp3 + CD4 + CD25 + Tregs, consistent with MS (Chen et al., 2006, 2007; Hofstetter and Forsthuber, 2002; Hofstetter et al., 2007). In EAE, neuroantigen-specific T cells are activated and propagated (Ben-Nun et al., 1981). BP_{TOX} stimulates clonal expansion of Th1 and Th17 neuroantigen-specific T cells, and increases production of immunoglobulins targeted to co-localized antigens (Hofstetter and Forsthuber, 2002; Hofstetter et al., 2007; Ryan et al., 1998). BP_{TOX} also activates antigen presenting cells (APCs) and effector CD8+ T cells, which are active in MS (Hofstetter and Forsthuber, 2002; Murphey et al., 2011). In EAE, BP_{TOX} recruits Th1 cells to the CNS, and, activated by myelin-derived peptide, induces adhesion of leukocytes to cerebrovascular endothelium while increasing BBB permeability (Kerfoot et al., 2004).

While spontaneous EAE may be induced in transgenic models that circumvent intrinsic autoimmune regulation, most models, including adoptive transfer EAE (Ben-Nun et al., 1981), require a bacterial adjuvant to activate and propagate the effector T cells that ultimately lead to neuropathology (Gold et al., 2006). The importance of BP_{TOX} in MS modeling is highlighted by the fact that when neural antigens are introduced without BP_{TOX} or other adjuvant, EAE is prevented, but when co-administered with BP_{TOX}, EAE is induced (Hofstetter and Forsthuber, 2002).

4. Discussion

We hypothesize that BP and BP_{TOX}, co-localized at the nasopharyngeal mucosa with neural antigens, and pathogens with epitope

homology to neural antigens (Epstein Barr Virus, human herpes virus 6) (Sospedra and Martin, 2006), facilitate host sensitization to neural tissue. Subsequent autoimmune responses to neural targets can then precipitate BP-mediated neuropathology consistent with MS. In addition, BP may promote EAE and MS through non-adjuvant effects. For example, BP_{TOX}-induced histamine sensitization, which can lead to compromised vascular endothelial integrity, is controlled by the Histamine receptor H1 (H1R) gene which has an established role in EAE susceptibility (Ma et al., 2002). Upregulation of H1R has been identified in chronic MS plaques in humans (Lock et al., 2002) and it is now established that the H1R gene is actually the *Bordetella pertussis histamine sensitization* (*Bphs*) gene.

Bradford Hill causality criteria (Hill, 1965) support subclinical BP colonization as a cause of MS. The SCPBC-MS association exhibits a biologic gradient: globally, the risk for BP colonization and MS reflects an equatorial BP vaccination gradient; locally, Faroese living closest to British soldiers were at higher MS risk. The association is strong with 161% proportional MS mortality in teachers, a group at increased risk for BP colonization. The correlation is consistent across populations and time such as the Faroes during WWII and the US and Africa in recent decades. The relationship is temporally logical as the introduction of BP preceded MS in the Faroes as it often does in EAE. Our hypothesis is analogous to disease models accepted as causal. For example, *Helicobacter pylori* causes peptic ulcer disease and parallels BP in MS: *H. pylori* also colonizes epithelium (gastric epithelium), causing disease through the release of toxins and by eliciting immune responses (Suerbaum and Michetti, 2002). There is evidence for biological coherence and plausibility, including the ability of BP_{TOX} to activate APCs, stimulate neuroantigen-specific T cells, and increase BBB permeability consistent with MS. Perhaps most striking, the hypothesis is supported by 50 years of EAE experiments: BP and BP_{TOX} are potent adjuvants used in the principle animal model of MS.

A half-century ago Poskanzer suggested that "The epidemiological features of multiple sclerosis are compatible with the hypothesis that the clinical illness may be an occasional manifestation of a widespread subclinical infection" (Poskanzer et al., 1963). With documentation that BP, a potent adjuvant, frequently colonizes the human nasopharynx, particularly in highly vaccinated populations, the progression from BP-induced animal modeling of MS to investigation of the role of BP in human neuropathology seems warranted. EAE models using intranasal co-localization of BP and neural antigens for sensitization protocols, SCBPC surveillance of cohorts followed for MS onset, screening for olfactory tract BP-PCR in clinically isolated syndromes which frequently progress to MS, and BP colonization eradication studies with MS as an outcome measure, may one day test the hypothesis that subclinical BP colonization is an important, previously unidentified, cause of MS.

Conflict of interest

KR and SG are employed by ILiAD Biotechnologies, which is developing a vaccine for the prevention of *Bordetella pertussis*. KR has received salary and equity in ILiAD Biotechnologies and holds pending patents for an irrigation assembly. SG has an equity interest in ILiAD Biotechnologies.

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References

Ben-Nun, A., Wekerle, H., Cohen, I.R., 1981. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur. J. Immunol. 11, 195.
Chen, X., Winkler, R.T., Carbonetti, N.H., Ortaldo, J.R., Oppenheim, J.J., Howard, O.M., 2006. Pertussis toxin as an adjuvant suppresses the number and function of CD4+CD25+ T regulatory cells. Eur. J. Immunol. 36, 671.
Chen, X., Howard, O.M., Oppenheim, J.J., 2007. Pertussis toxin by inducing IL-6 promotes the generation of IL-17-producing CD4 cells. J. Immunol. 178, 6123.
De Luca, G., Joseph, A., George, J., Yates, R., Hamard, M., Esiri, M., 2014. Right under our noses: olfactory pathology in central nervous system demyelinating diseases (P6. 173). Neurology 82 (Suppl. P6. 173).
De Serres, G., Shadmani, R., Duval, B., Boulianne, N., Dery, P., Douville Fradet, M., Rochette, L., Halperin, S.A., 2000. Morbidity of pertussis in adolescents and adults. J. Infect. Dis. 182, 174.
Fiore, D., 2003. Diagnosis and treatment of multiple sclerosis and amyotrophic lateral sclerosis: neuropathies from *Bordetella pertussis*. Am. J. Ther. 10, 377.
Gold, R., Linington, C., Lassmann, H., 2006. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain 129, 1953.
Hill, A.B., 1965. The environment and disease: association or causation? Proc. R. Soc. Med. 58, 295.
Hofstetter, H.S., Shive, C.L., Forsthuber, T.G., 2002. *Pertussis* toxin modulates the immune response to neuroantigens injected in incomplete Freud's adjuvant: induction of TH1 cells and experimental autoimmune encephalomyelitis in the presence of high frequencies of Th2 cells. J. Immunol. 169, 117.
Hofstetter, H.G., Grau, C., Buttman, M., Forsthuber, G.T., Gaupp, S., Toyka, V.K., Gold, R., 2007. The PLPp-specific T-cell population promoted by pertussis toxin is characterized by high frequencies of IL-17-producing cells. Cytokine 40, 35.
Kerfoot, S.M., Long, E.M., Hickey, M.J., Andonegui, G., Lapointe, B.M., Zanardo, R.C., Bonder, C., James, W.G., Robbins, S.M., Kubes, P., 2004. TLR4 contributes to disease-inducing mechanisms resulting in central nervous system autoimmune disease. J. Immunol. 173, 7070.
Kurtzke, J.F., 1993. Epidemiological evidence for multiple sclerosis as an infection. Clin. Microbiol. Rev. 6, 382.
Lehmann, D., Ben-Nun, A., 1992. Bacterial agents protect against autoimmune disease. I. Mice pre-exposed to *Bordetella pertussis* or *Mycobacterium tuberculosis* are highly refractory to induction of experimental autoimmune encephalomyelitis. J. Autoimmun. 5, 675.
Levine, S., Wenk, E.J., 1961. Studies on the mechanism of altered susceptibility to experimental allergic encephalomyelitis. Am. J. Pathol. 39, 419.
Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S.J., Oksenberg, J.R., Raine, C.S., Heller, R., Steinman, L., 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat. Med. 8, 500.
Long, S., Welkon, C.J., Clark, J.L., 1990. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J. Infect. Dis. 161, 480.
Ma, R.Z., Gao, J., Meeker, N.D., Fillmore, P.D., Tung, K.S., Watanabe, T., Zachary, J.F., Offner, H., Blankenhorn, E.P., Teuscher, C., 2002. Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science 297, 620.
Madsen, T., 1933. Vaccination against whooping cough. JAMA 101, 187.
Mills, K.H., 2001. Immunity to *Bordetella pertussis*. Microb. Infect. 3, 655.
Murphey, C., Chang, S., Zhang, X., Arulanandam, B., Forsthuber, T.G., 2011. Induction of polyclonal CD8+ T cell activation and effector function by *pertussis* toxin. Cell. Immunol. 267, 50.
Poskanzer, C.D., Schapira, K., Miller, H., 1963. Multiple sclerosis and poliomyelitis. Lancet 282, 917.
Rosati, G., 2001. The prevalence of multiple sclerosis in the world: an update. Neurol. Sci. 22, 117.
Ryan, M.M., McCarthy, L., Rappuoli, R., Mahon, B., Mills, K.H., 1998. Pertussis toxin potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD 28. Int. Immunol. 10, 651.
Sospedra, M., Martin, R., 2006. Molecular mimicry in multiple sclerosis. Autoimmunity 39, 3.
Suerbaum, S., Michetti, P., 2002. Helicobacter pylori infection. N. Engl. J. Med. 347, 1175.
Walsh, S.J., DeChello, L.M., 2001. Excess autoimmune disease mortality among school teachers. J. Rheumatol. 28, 1537.
Ward, J., Cherry, J.D., Chang, S., Partridge, S., Lee, H., Treanol, J., Greenberg, D., Keitel, W., Barenkamp, S., Bernstein, D., Edelman, R., Edwards, K., 2005. Efficacy of an acellular pertussis vaccine among adolescents and adults. N. Engl. J. Med. 353, 1555.
Warfel, J.M., Zimmerman, L.I., Merkel, T.J., 2014. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc. Natl. Acad. Sci. 111, 787.
Weber, M.S., Benkhoucha, M., Lehmann-Horn, K., Hertzenberg, D., Sellner, J., Santiago-Raber, M.L., Chofflon, M., Hemmer, B., Zamvil, S.S., Lalive, P.H., 2010. Repetitive pertussis toxin promotes development of regulatory T cells and prevents central nervous system autoimmune disease. PLoS One 5, e16009.
Wendelboe, A.M., Van Rie, A., Salmaso, S., Englund, J.A., 2005. Duration of immunity against pertussis after natural infection or vaccination. Pediatr. Infect. Dis. J. 24, S58.

Willer, C., Dyment, D., Risch, N., Sadovnick, A., Ebers, G., Group, C.C.S., 2003. Twin concordance and sibling recurrence rates in multiple sclerosis. Proc. Natl. Acad. Sci. 100, 12877.

WHO, 2014. Immunization: Diphtheria Tetanus Toxoid and Pertussis (DTP3) by WHO Region. WHO.

Wright, S.W., Decker, M.D., Edwards, K.M., 1999. Incidence of pertussis infection in healthcare workers. Infect. Control Hosp. Epidemiol. 20, 120.

Zhang, Q., Yin, Z., Li, Y., Luo, H., Shao, Z., Gao, Y., Xu, L., Kan, B., Lu, S., Zhang, Y., 2014. Prevalence of asymptomatic *Bordetella pertussis* and *Bordetella parapertussis* infections among school children in China as determined by pooled real-time PCR: a cross-sectional study. Scand. J. Infect. Dis. 46, 280.