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The pertussis hypothesis: *Bordetella pertussis* colonization in the pathogenesis of Alzheimer's disease

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ABSTRACT

While a number of endogenous risk factors including age and genetics are established for Alzheimer's disease (AD), identification of acquired, potentially preventable or treatable causes, remains limited. In this paper, we review three epidemiologic case studies and present extensive biologic, immunologic and anatomic evidence to support a novel hypothesis that *Bordetella pertussis* (BP), the bacterium better known to cause whooping cough, is an important potential cause of AD. Cross-cultural documentation of nasopharyngeal subclinical BP colonization reflecting BP-specific mucosal immunodeficiency, proximate anatomy of intranasal mucosal surfaces to central nervous system (CNS) olfactory pathways, and mechanisms by which BP and BP toxin account for all hallmark pathology of AD are reviewed, substantiating biologic plausibility. Notably, respiratory BP infection and BP toxin secreted from subclinical BP colonization can account for the initiation and accumulation of amyloid β plaques and tau tangles. Additional mechanisms consistent with the immunobiologic effects of subclinical BP colonization include microglial activation and inflammation, atrophy and neurodegeneration, excitotoxicity, distinctive anatomic distribution and sequential spread of disease, impaired glucose utilization, and other characteristic CNS pathology of AD. We conclude by assessing the evidence for causation against the Bradford Hill criteria, and advocate for further investigation into the potential role of BP in the etiology of AD.

1. Introduction

First described in 1907 (Alzheimer, 1907), Alzheimer's disease (AD) is a neurodegenerative disorder characterized by slowly progressive cognitive and behavioral impairment in those with intracellular cerebral neurofibrillary tangles (NFTs) composed of abnormal tau protein, and extracellular plaques composed of amyloid-β (Aβ) peptides (Hyman et al., 2012; McKhann et al., 2011). Concordance rates for AD in monozygotic twins vary from 21 to 83% (Gatz et al., 2006; Breitner et al., 1995; Räihä et al., 1996), the wide range in part related to differences in study design and acquired risk factors (Gatz et al., 2006). Notably, when a genome-wide analysis of genetic loci associated with Alzheimer's disease was unable to expand the power of AD prediction models beyond age, sex and apolipoprotein E (APOE) status alone (Seshadri et al., 2010), a call was raised to shift the search for causes of AD "back to the environment" (Pedersen, 2010). In this respect, a role for infection in the pathogenesis of AD has been previously suggested (Honjo et al., 2009; MacDonald, 1988), and in a recent editorial, 33 AD researchers and clinicians "propose that further research on the role of infectious agents in AD causation. . .is justified"(Itzhaki et al., 2016).

Several neurotropic infections have been associated with AD, including *Chlamydia pneumoniae*, *Borrelia burgdorferi*, and *Toxoplasma gondii*, though not all investigators confirm these associations (Hammond et al., 2010; Nicolson, 2008; Kusbeci et al., 2011). It is plausible that chronic infections, particularly neurotropic infections, contribute to AD. For example, CNS Herpes Simplex Virus-1 (HSV-1) increases the risk of AD in carriers of the

Abbreviations: AD, Alzheimer's disease; BP, *Bordetella pertussis*; CNS, central nervous system; Aβ, amyloid β; NFT, neurofibrillary tangle; APOE, apolipoprotein E; HSV-1, herpes simplex virus-1; AβPP, Aβ precursor protein; BACE-1, β-site amyloid precursor protein-cleaving enzyme; US, United States; wPV, whole cell pertussis vaccine; aPV, acellular pertussis vaccine; SCBPC, subclinical *Bordetella pertussis* colonization; DPT, diphtheria pertussis and tetanus vaccine; PCR, polymerase chain reaction; CDC, Centers for Disease Control and Prevention; CVO, circumventricular organ; BBB, blood-brain barrier; kDa, kilodalton; CSF, cerebrospinal fluid; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; Th1, T helper 1; Th17, T helper 17; mRNA, messenger ribonucleic acid; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6; $GABA_B$, gama-aminobutyric acid B; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; sAPPα, soluble amyloid precursor protein α; GSK3β, glycogen synthase kinase 3β; ATP, adenosine triphosphate; NAD+, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; GLUT4, glucose transporter 4; GRK2, G-protein-coupled receptor kinase 2; GA, Golgi apparatus; ER, endoplasmic reticulum; PS, presenilin; MS, multiple sclerosis.

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APOEε4 allele (Itzhaki and Wozniak, 2008), and mouse brain cells infected with HSV-1 show increased levels of the Aβ precursor protein (AβPP) cleaving enzyme BACE-1 (β-site amyloid precursor protein-cleaving enzyme) and increased Aβ deposits (Wozniak et al., 2007). Still other investigations have demonstrated that Aβ has broad-spectrum antimicrobial properties, raising the possibility that this hallmark of AD is an evolutionary response to bacterial exposure (Soscia et al., 2010). *Corynebacterium diphtheriae*, which infects the human nasopharynx, has also been proposed to cause AD *via* the passage of diphtheria toxin from olfactory epithelium into the CNS (Merril, 2012), building on previous work suggesting that olfactory pathways may be conduits for microbes and toxins in the etiology of AD (Mann et al., 1988; Ferreyra-Moyano and Barragan, 1989).

Identified in 1906 (Bordet and Gengou, 1906), *Bordetella pertussis* (BP) is a Gram-negative bacterium that secretes biologically active toxins and causes whooping cough (acute clinical BP). Both subclinical and symptomatic BP infections persist in highly vaccinated populations (Ward et al., 2005; Zhang et al., 2014; de Melker et al., 2006; Hallander et al., 2011; van Boven et al., 2000), with escalation of reported whooping cough cases in the past two decades in the United States (US) (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html) and many other industrialized nations (Chiappini et al., 2013). In 2012, it was noted that "the prevalence of *B. pertussis* infection is high in [US] adults and increasing at a significant rate, especially in individuals more than 65 years of age" (Weston et al., 2012). In an American study of individuals over age 65, BP serology elevations consistent with infection were detected in 10% of subjects over a three year period, and one-half to one-third were asymptomatic (Hodder et al., 2000). Multiple linear regression analysis suggests that between 2006 and 2010 there were 465,000 cases of pertussis infection in US adults age 65 and older, though only 6369 cases were actually reported (Masseria and Krishnarajah, 2015). In short, *Bordetella pertussis* insidiously infects large numbers of adults, including the elderly, and pertussis rates have been rising in recent decades.

Multiple factors are held to play a role in rising US BP rates, including a reduction in vaccine efficacy when the US switched from whole cell pertussis vaccines (wPV) to acellular pertussis vaccines (aPV) in the late-1990s due to wPV safety concerns (Winter et al., 2014). Acellular pertussis vaccines generally contain three or four purified, inactivated components of BP, compared to the broad antigenic BP profile of wPV prepared from heat or chemically inactivated whole bacteria, and it is generally agreed that the transition from wPV to aPV led to less durable immunity (Winter et al., 2014). In addition, aPV is unable to protect against BP nasopharyngeal colonization as demonstrated by Warfel et al. in a non-human primate model, and colonized baboons are able to transmit BP infections to naive baboons in close proximity (Warfel et al., 2014). As such, the authors concluded: "that aP [vaccines]. . .fails to prevent colonization or transmission provides a plausible explanation for the [US] resurgence of pertussis." Complimenting these data, recent phylodynamic analysis and mathematical modeling studies by Althouse and Scarpino indicate that while "waning immunity plays a role in the epidemiology of pertussis', "asymptomatic transmission is the most parsimonious explanation for many of the observations surrounding the resurgence of *B. pertussis* in the US" (Althouse and Scarpino, 2015).

Subclinical BP colonization (SCBPC) infections are here defined as asymptomatic or mild infections (*e.g.* transient cough or rhinorrhea) that elicit minimal or no BP-directed host immunity, thereby allowing unopposed BP toxin activity in a host. To illustrate the relationship between BP infection and AD, we first apply our hypothesis to three environmental observations. Evidence of anatomic and biological plausibility follow in the third section, and immunobiologic mechanisms are discussed in the fourth section of this review.

2. Epidemiologic case studies

2.1. Case 1: the concurrent age-adjusted rise of AD and BP in the US

Increasing death from AD (Xu et al., 2010) and age-adjusted risk for AD (Akushevich et al., 2013) in the US since the early 1990s coincide with the commensurate upsurge in BP (Clark, 2014), the only vaccine-preventable disease in the US increasing in incidence in recent decades (Brooks and Clover, 2006). While limited to clinically identified AD cases, and though part of the overall rise in AD burden in recent decades is due to increased longevity and a diagnostic shift from diseases with improved survival, we propose, given the totality of evidence presented below, that the age-adjusted rise in AD is a true independent increase, and may be substantially due to escalating BP infection rates.

Others have proposed that systemic inflammatory disease may drive neurodegeneration in AD (Perry et al., 2007). We suggest that systemic inflammatory diseases may contribute to the progression of AD without being sufficient cause for AD alone. In fact, many common diseases with an inflammatory component, including stroke, myocardial infarction, colon cancer, breast cancer, and rheumatoid arthritis (Akushevich et al., 2013), have decreased in age-adjusted incidence and mortality as AD incidence has risen. Additional potential sources of systemic inflammation, such as smoking (CDC, 2011) and periodontal disease (Hugoson et al., 2008), have also dropped over the same period. In summary, we submit that generalized inflammation alone is insufficient to explain the increase in AD in recent decades, distinguishing BP as a specific potential cause that has risen with AD during the same period.

2.2. Case 2: vaccination and reduced AD risk

Diphtheria and tetanus vaccines typically, though not exclusively, are co-administered with BP antigens. In a cohort of 3682 non-demented Canadians 65 years of age and older, self-reported vaccination for diphtheria or tetanus, "considered together, as they are usually given together in vaccination programs," correlated with a statistically significant 60% (95% CI 35–75%) lower risk for the development of AD over five years during the 1990s (Verreault et al., 2001). No significant association was found between vaccination for influenza and AD, making it unlikely that a boosted nonspecific immune or vaccine response alone is AD-protective. The model adjusted for age, sex, education, alcohol consumption, family history of dementia, measures of activities of daily living, antecedents of chronic diseases, and perceived health status. The cohort percentage that received BP vaccination bundled with diphtheria or tetanus vaccination was not reported, however it is likely that a high proportion had simultaneously received pertussis vaccination because: (1) combination diphtheria, pertussis and tetanus vaccines (DPT), pioneered in Canada, were readily adopted after introduction in 1943 (Varughese, 1985), (2) DPT administration has been routine for school vaccination programs in Canada since 1948 (Verreault et al., 2001), and (3) currently, 9 of 11 available preparations of tetanus toxoid-containing vaccines in Canada are combined with BP antigens (Canadian Immunization Guide, 2016).

A second independent Canadian study of 694 non-demented individuals age 65 and over observed for the onset of AD corroborated the same association between vaccination and reduced AD risk. In this study, a history of vaccination for tetanus or diphtheria, and thus likely BP, was associated with a significantly reduced risk