The pertussis hypothesis: *Bordetella pertussis* colonization in the etiology of asthma and diseases of allergic sensitization

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**ABSTRACT**

Decades of peer reviewed evidence demonstrate that: 1) *Bordetella pertussis* and pertussis toxin are potent adjuvants, inducing asthma and allergic sensitization in animal models of human disease, 2) *Bordetella pertussis* often colonizes the human nasopharynx, and is well documented in highly pertussis-vaccinated populations and 3) in children, a history of whooping cough increases the risk of asthma and allergic sensitization disease. We build on these observations with six case studies and offer a pertussis-based explanation for the rapid rise in allergic disease in former East Germany following the fall of the Berlin Wall; the current asthma, peanut allergy, and anaphylaxis epidemics in the United States; the correlation between the risk of asthma and gross national income per capita by country; the lower risk of asthma and allergy in children raised on farms; and the reduced risk of atopy with increased family size and later sibling birth order. To organize the evidence for the pertussis hypothesis, we apply the Bradford Hill criteria to the association between *Bordetella pertussis* and asthma and allergic sensitization disease. We propose that, contrary to conventional wisdom that nasopharyngeal *Bordetella pertussis* colonizing infections are harmless, subclinical *Bordetella pertussis* colonization is an important cause of asthma and diseases of allergic sensitization.

**Introduction**

Asthma is a multifactorial heterogeneous disorder [1–4] with varied pathophysiologic mechanisms, or endotypes [1,4–6], characterized by reversible airway obstruction, airway inflammation, and associated Th2-IgE-mediated immunity in about half of patients [7,8]. Allergic disease here refers to hypersensitivity initiated by a specific, typically IgE-mediated, reaction to an environmental allergen, and includes atopic dermatitis, allergic rhinitis, and food allergy [2,9]. Asthma and allergic sensitization diseases are frequently comorbid [10,11], and the atopic (IgE-mediated) allergic phenotype may progress over time in an "atopic march." [11–14]

Heritability estimates for asthma and allergic sensitization diseases vary widely [15], and standard genome-wide association panels of asthmatics account for less than half of common variation [16,17], implicating the importance of environmental factors in disease onset. For example, a consortium-based genome-wide association study estimated the population attributable risk of the combined effect of all asthma-associated loci identified for child-onset asthma at 38% (95% CI 28–44%) [18], and a large Australian twin study of self-reported asthma and hay fever found respective disease correlation rates of .65 between monozygotic twins, and .25 between dizygotic twins [17], further reflecting both genetic and environmental contributions to allergic disease. As Berin and Sampson summarized for one allergy epidemic, "Food allergies are increasing in prevalence at a higher rate than can be explained by genetic factors, suggesting a role for as yet unidentified environmental factors." [19]

Asthma and allergic sensitization share an overlapping medical literature grounded in a common pathophysiology and epidemiology, and as we propose, may both be significantly attributable to the environmental pathogen *Bordetella pertussis* (BP), particularly nasopharyngeal Subclinical *Bordetella pertussis* Colonization (SCBPC) infections. SCBPC is defined here as a BP infection that acutely causes minimal or no overt clinical symptoms, and does not induce BP immunocompetence. An SCBPC may initially be asymptomatic or mildly symptomatic, presenting at most with non-specific symptoms such as those of a common cold. As such, SCBPCs are unlikely to be specifically identified as BP infections.

To the extent a host mounts an immune response to SCBPC, manifest for example by a modest increase in BP-directed antibodies, such a response is, by definition, insufficient to clinically prevent subsequent BP infections. As a result of lower exposure dose or by lower frequency of exposure, SCBPC, again by definition, does not lead to potent immunity. This lack of induction of a protective BP-directed host immune response by an SCBPC infection is critical to our hypothesis, as it allows for the unopposed biological activity of toxins secreted by BP colonizing infections that may lead to future host pathology. The pertussis hypothesis proposes that nasopharyngeal colonizing BP infections, SCBPC,

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while initially quiescent, may subsequently lead to diseases of allergy as presented in this review, as well as to autoimmunity [20,21], and other neuropathology [22].

In contrast, Subclinical BP Immunization, or SCBPI, is defined as a BP infection that acutely causes minimal or no symptoms but nevertheless induces a protective mucosal and systemic BP immune response. Such response is typically the result of a higher dose of BP exposure and/or a higher frequency of BP exposure. We propose that because SCBPI induces potent mucosal cellular and antibody responses against BP and BP toxins, toxins released by subsequent nasopharyngeal colonizing BP infections are neutralized prior to reaching cellular end-targets, including antigen presenting cells. As a result, SCBPs generally do not lead to the proposed allergic pathology that results from unopposed BP toxins, such as those released by SCBPC. In addition, SCBPI-induced mucosal BP immunocompetence generally leads to rapid clearance of subsequent nasopharyngeal colonizing BP infections, further reducing the risk of host exposure to unopposed BP toxins. In summary, as proposed by the pertussis hypothesis, new-onset subclinical BP infections can be either SCBPC or SCBPI infections, depending on the frequency and dose of BP exposure, and result in distinct immunologic profiles and clinical implications as shown in Table 1.

The existence of subclinical BP infections is supported by human data demonstrating nasopharyngeal BP PCR positivity in asymptomatic populations [23–25], and data obtained during a pertussis outbreak which identified a population of nasopharyngeal BP PCR positive subjects without cough whose serology remained negative (e.g. IgA, IgG, IgM) “due to a lack of a detectable BP immune response.” [26]. In addition, investigation of respiratory outbreaks attributed to BP have been deemed to be “mistaken” by the CDC in part due to the presence of nasopharyngeal BP PCR positive patients with negative BP serology [27]. We submit that it is plausible that these are documented examples of SCBPC.

SCBPC is proposed to occur more frequently in highly vaccinated populations with low BP prevalence, and infrequent minimally immunogenic nasopharyngeal BP exposure. In contrast, subclinical BP infections occurring in poorly vaccinated populations with high BP prevalence, and high frequency immunogenic nasopharyngeal BP exposure, are more typically SCBPI. What we define as SCBPI is also known as latent BP immunization, first recognized in British BP studies by Percy Stocks in the 1930s [28] and more recently supported by Lavine, who, in an age-structured model, found that a reduction in latent BP immunization can account for both the recent reemergence of BP despite high BP vaccination coverage and the shift in age-specific BP incidence in developed nations [29]. In our hypothesis, latent immunizing BP infections, or SCBPI, by inducing potently mucosal immunity, are proposed to reduce the risk and rate of future SCBPC infections.

Asthma affects an estimated 300 million people worldwide [30]. With epidemic and rising prevalence in many developed nations during the past three decades [31–34] (which may have recently leveled off in some countries with higher rates of disease [3]), a deeper understanding of how and why certain individuals develop asthma and allergic sensitization disease is more important than ever. Notably, asthmatics are at increased risk for respiratory and other infections including Bordetella pertussis [35,36], and show a trend (p = 0.063) for lower levels of anti-pertussis toxin IgG than non-asthmatics [36], consistent with a relative BP immunodeficiency in asthmatics compared with non-asthmatics, though immunosuppression from asthmatic treatment may explain some of this finding. The higher rate of BP in asthmatics may reflect genetic predisposition, airway remodeling with structural pathology and obstruction, or environmental factors such as lower levels of both BP exposure and latent BP immunization, resulting in mucosal BP immunodeficiency and an increased risk of SCBPC. We do not suggest that SCBPC is the only potential infectious cause of asthma and diseases of allergic sensitization, but that the pertussis hypothesis is consistent with current evidence and merits investigation.

The proposed pathogenic sensitizing agent Bordetella pertussis is a highly transmissible, Gram-negative bacterium that is typically acquired by inhalation of contaminated air from the cough, sneeze or exhalation of an infected individual. BP is the primary cause of acute clinical BP, better known as whooping cough, and BP secretes biologically active toxins, including the highly potent adjuvant pertussis toxin. Importantly, BP and pertussis toxin have been used for more than a half century to induce sensitization and pathology in multiple animal models of asthma and allergic disease [37–54]. To illustrate the relationship between BP infection and asthma and allergic sensitization, we first apply our hypothesis to six environmental observations.

**Case studies**

**Asthma, allergies and the rise and fall of the Berlin Wall**

Prior to 1960, former East Germany (FEG) and former West Germany (FWG) had similar rates of atopic disease [55]. In 1961 the Berlin Wall was erected by FEG for geopolitical reasons, dividing Germany and isolating populations with similar genetic backgrounds and environments. It remained a prominent symbol of the cold war for nearly three decades until its removal in 1989. While partitioned, the two nations adopted sharply different BP vaccination policies, with FEG maintaining high BP vaccination rates and FWG rescinding their uniform childhood BP vaccination policy from 1974 to 1991 due to vaccine safety concerns [56].

During the 1980s, pertussis vaccination coverage for pre-school children was greater than 90% in FEG, but only 50–60% in southern FWG, and 2–20% in northern FWG [56]. Consequently, BP rates in FWG increased during the period of Germany’s separation and remained substantially higher in FWG than in FEG [57]. In addition, in FEG, travel and immigration were severely restricted, limiting importation of BP to an isolated FEG population. With the fall of the Berlin Wall in 1989 and subsequent German reunification, including a marked increase in socialization between FEG and FWG populations, BP rates in FEG began to rise [56,58] as shown in Table 2.

Reported rates of mortality from all bacterial disease in East Germany were unchanged from 1980 to 1997, so it is unlikely that reporting bias explains the rise in BP incidence during this period [60].

We propose that these circumstances, while successful in reducing acute clinical BP rates in FEG during separation, simultaneously attenuated mucosal BP immunity within the FEG population prior to 1989.

**Table 1**

Proposed Characteristics of SCBPC and SCBPI Nasopharyngeal Subclinical BP Infections.

<table>
<thead>
<tr>
<th>Subclinical* BP Infection (initial presentation)</th>
<th>Dose and Frequency of BP Exposure</th>
<th>More Common In</th>
<th>Induces Potent Mucosal and Systemic BP Immunity</th>
<th>Reduces the Risk of Future Nasopharyngeal Colonization</th>
<th>Reduces the Risk of Future Disease Due to Unopposed BP Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCBPC (Subclinical BP Colonization)</td>
<td>Low</td>
<td>Highly Vaccinated Populations</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>SCBPI (Subclinical BP Immunization)</td>
<td>High</td>
<td>Poorly Vaccinated Populations</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* A subclinical BP infection is defined as an asymptomatic or minimally symptomatic infection, at most leading to symptoms consistent with the common cold.
because whole cell pertussis vaccines (in use in FEG and FWG from 1961 to 1989) do not induce sterilizing mucosal immunity as demonstrated in non-human primates [61], and exposure to BP in FEG was infrequent, leading to low rates of latent BP immunization. This FEG population-wide mucosal BP immunodeficiency was inconsequential while BP rates remained low in FEG prior to German reunification, and exposure to BP in FEG was infrequent. However, we propose that mucosal BP immunodeficiency predisposed former East Germans to increased SCBPC rates post-reunification, as exposure to BP increased with sharp increases in FEG-FWG population interactions and the influx of BP into FEG. We submit that an increased incidence of nasopharyngeal SCBPC increased the risk for asthma and related allergic disease in post-unification FEG.

Consistent with the pertussis hypothesis, rates of allergic sensitization and asthma were low in FEG up to reunification [62,63] and substantially increased after the fall of the Berlin wall in 1989. Heinrich et al. analyzed data obtained in 1992–3, 1995–6 and 1998–9 from East German children aged 5–14 years [64]. After adjustment for gender, age, and study area, increases were seen for strong sensitization to any allergen with an odds ratio of 1.28 for the second vs. first survey disease increase, and 1.33 for the third vs. first survey disease increase (p-trend 0.02). For example, from 1992 to 1995 to 1999, rates for any allergy to food, drug, pollen, animal dander or fungus rose progressively from 0.02). For example, from 1992 to 1995 to 1999, rates for any allergy to food, drug, pollen, animal dander or fungus rose progressively from 0.02%. For example, from 1992 to 1995 to 1999, rates for any allergy to food, drug, pollen, animal dander or fungus rose progressively from 0.02% to 0.24%, and physician-diagnosed asthma rates rose from 2.6% to 3.5% to 4.8%. Statistically significant increases were also reported for clinically diagnosed asthma and eczema [64].

From another region of FEG, Frye et al. reported childhood asthma and allergy rates from 1992 to 1996 [65]. Bronchial hyperresponsiveness to cold air on pulmonary function testing corroborated Heinrich’s findings, increasing from 6.4% in 1992–1993 to 11.6% in 1995–1996 (odds ratio [OR]: 2.0, 95% confidence interval [CI] 1.3–3.0). In addition, in the decade following reunification, there was a fourfold greater increase in pollen sensitization (95% confidence interval 1.2–13.9) in FEG than in FWG [66].

We propose that high vaccination rates and social isolation suppressed BP prevalence in FEG and thereby reduced both latent BP immunization and mucosal BP immunocompetence in the FEG population. This, in turn, increased the risk of SCBPC in FEG individuals upon German reunification as BP was reintroduced into FEG from exposure to the more poorly vaccinated FWG population. We submit that, given the totality of evidence presented in this review, SCBPC is an unrecognized cause of both asthma and allergic sensitization, and that an increase in FWG-transmitted BP and FEG-acquired SCBPC infections is consistent with the significant rise in asthma and allergic disease in FEG after the fall of the Berlin Wall.

The asthma epidemic in the United States parallels the surge in Bordetella pertussis incidence

In recent decades, the incidence of asthma in the United States (US) has increased [31,67,68], correlating with the steady increase in BP incidence as tracked by the US Centers for Disease Control and Prevention (CDC) Surveillance and Reporting, 2013 [http://apps.who.int/iris/bitstream/10665/70149/1/WHO_IVB_2009_eng.pdf], and Centers for Disease Control and Prevention (CDC) Surveillance and Reporting, 2013 [https://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html]. In the US acellular BP vaccine trial completed in 1999, rates of undiagnosed BP infections for individuals age 15–65 years were estimated at 1 to 10 million cases per year, depending on case definition [71], in years when the CDC reported approximately 7000 US cases annually [https://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html]. The estimated unreported subclinical BP incidence in the figure is based on the quotient of these data, with an upper range ratio of 1:1400 for reported BP to unreported subclinical BP for a given year.
Prevention (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html). We submit that symptomatic BP infection rates are directly proportional to subclinical BP infection rates in relatively low BP incidence environments such as the US during the past several decades, when BP exposure levels are held to have been too low to induce widespread latent induction of BP mucosal immunity. That is, in populations where circulating rates of BP are low and mucosal BP immunocompetence is poor, such as that in the US with greater than 90% BP vaccination rates for decades (http://apps.who.int/immunization_monitoring/globalsummary/countries?countrycriteria%5Bcountry%5D%5B5%5D=USA), increasing rates of acute clinical BP correlate with increasing rates of SCBP because each additional BP exposure is more likely to lead to an SCBP. In contrast, in populations with poor vaccination rates and high rates of circulating BP, increasing acute clinical BP incidence correlates with an increasing rate of latent BP immunization and enhanced mucosal BP immunity, and thus a reduction in SCBP rates.

As seen in Fig. 1, US rates of both asthma [67,68] and estimated SCBP begin to rise in the 1990s as derived from WHO data (http://apps.who.int/iris/bitstream/10665/70149/1/WHO_IVB_2009_eng.pdf), coincident with the introduction of the acellular BP (aP) vaccine [69] which has been shown to provide less durable immunity than whole cell BP (wP) vaccines [70]. Notably, human-primate models demonstrate that neither wP nor aP vaccination induces sterilizing nasopharyngeal BP immunity or prevents colonization (in fact aP prolongs BP colonization), and further that aP does not prevent BP transmission [61]. In addition, human nasal swab PCR, culture and serologic data indicate that currently available BP vaccines decrease acute clinical BP risk but do not prevent asymptomatic or minimally symptomatic nasopharyngeal BP infection as demonstrated in multiple highly BP-vaccinated populations [23–26,71–74]. Human observational [75,76] and modeling data [77] also suggest that aP vaccines do not prevent BP transmission. Evidence substantiating the existence of SCBP, as defined by the pertussis hypothesis, includes Klement et al. who identified a population of nasopharyngeal BP PCR positive subjects without cough whose BP serology remained negative (e.g. IgA, IgG, IgM) [26], and Zhang et al. who documented BP PCR positivity in 4.8% of Chinese children. In the Zhang et al. study, “All children were asymptomatic when they entered the study. Children who reported symptoms of a respiratory infection such as cough, fever, or cataract, currently present or having occurred in the past 3 months, were excluded.” [23] Such BP colonized patients meet our definition of SCBP, which may include subjects who may have had minor respiratory symptoms that were not reported to investigators.

We propose that the inability of aP to prevent asymptomatic nasopharyngeal BP colonization has contributed to the rise in acute clinical BP and SCBP in highly BP-vaccinated populations where rates of latent BP immunization are relatively low. In turn, we submit that it is plausible that the adjuvant effects of pertussis toxin secreted from SCBP, when co-localized with allergens such as pollen and certain foods, explains a significant part of the coincident rise in rates of pertussis and diseases of allergic sensitization, as exemplified in Fig. 1.

Of note, average estimated rates of US SCBP of 2–7% since 2000 (20/1000 in 2000, 70/1000 in 2013), derived from Ward et al. and presented in Fig. 1, were based on asymptomatic cases of serologically documented BP infection. These estimates approximate rates of nasopharyngeal PCR positivity in other highly vaccinated populations such as 4.8% of asymptomatic youth in China in 2014 [23], and 5.3% of infants in the control group of a German infant study in 1995–1997 [24].

The US rise in childhood peanut allergy and anaphylaxis parallels increasing US BP rates

Beyond asthma, the prevalence of other diseases of allergic sensitization, including peanut allergy [78] and anaphylaxis [79], is climbing in step with the US rise in BP rates as documented by the CDC (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html). Peanuts are commonly consumed as peanut butter, which persists in the oropharynx due to a combination of biochemical properties that confer its distinctive stickiness, and, as proposed herein, make it an ideal allergen by increasing its co-localization with colonizing pharyngeal BP infection and pertussis toxin adjuvant, increasing the risk of BP-mediated peanut sensitization. Peanut butter’s high fat content makes it hydrophobic, which impairs mixing with, and being washed away by, saliva, while commercial processing increases its adhesiveness [80] compared with unprocessed peanuts. We submit that these unusual properties offer an explanation for why childhood peanut allergy, among all food allergies, has sharply risen in recent decades [78] commensurate with rising US BP infection rates (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html), and the presence of BP in the nasopharynx in about 5% of children in highly vaccinated populations [23,24].

Sicherer et al. documented self-reported US peanut allergy prevalence rates, conducting three identical nationwide cross-sectional surveys at 5–6 year intervals. Survey-based prevalence rates more than tripled over 11 years of follow-up, rising from 0.4% in 1997, to 0.8% in 2002, to 1.4% in 2008 (p < .0001 for difference, 1997–2008) [78]. Rates of tree nut allergy rose as well during the same period, increasing from 0.2% in 1997 to 1.1% in 2008 (p < .0001). While the authors acknowledge the limitations of survey-based allergy self-report, they note that contemporaneous rates of peanut allergy in children outside the US were similar to their US 2008 estimate of 1.4%, with rates in Canada of 1.63% [81], in the United Kingdom of 1.2–1.85% [82,83], and in Australia of 1.15% [84], where studies relied on additional means of allergy validation including physician diagnosis, allergy testing, and food challenge.

Recent decades have also witnessed an increase in US anaphylaxis rates. Lin et al. reported a greater than four-fold jump in hospitalizations for anaphylaxis between 1990 and 2006 in New York residents < 20 years of age [79]. As might be expected, peanut allergy was the most common reason for food allergy anaphylaxis hospital admission, followed by “other specified foods”, tree nuts/seeds, fish and milk. In addition, the population incidence of anaphylaxis in Rochester, Minnesota increased from 46.9/100,000 persons to 58.9/100,000 (p = .03) between 1990 and 2000 [85].

Consistent with these findings, BP has been shown to “precipitate/enhance anaphylactic response capacity” in mammals [86]. For decades, animal models have demonstrated that pertussis toxin and administered BP possess “well-known anaphylactogenic effects” with the ability “to increase anaphylactic susceptibility of mice to a foreign antigen” [38]. Co-administration of BP and antigen can lead to anaphylaxis upon re-exposure to that antigen [87], and pertussis toxin, when co-administered with an antigen, can lead to an immediate type hypersensitivity reaction consistent with anaphylaxis upon antigen re-exposure [39].

Notably, pertussis toxin can also cause “drastic histological and histochemical changes” within the adrenal gland [88] of rats five days after injection with pertussis toxin, and more than a half-century ago Munoz noted “striking similarities between adrenalectomized and pertussis-treated mice (which) have led to the inference that pertussis... interfere[s] with adrenal function.” [89] While further clinical study is needed, it is tempting to speculate that SCBP-mediated impairment of adrenal function (due to SCBP-secreted pertussis toxin localizing to the adrenal gland via a hematogenous route), combined with nasopharyngeal SCBP-mediated host sensitization to peanut or other antigens could result in host anaphylaxis upon antigen re-exposure. That is, BP can not only induce host sensitization to an allergen via its inherent potent adjuvant effects, but BP can also lead to adrenal injury, compromising adrenergic production and release of epinephrine, a major anti-inflammatory mediator and vasoconstricting counter-regulatory hormone produced during a hyperimmune crisis which may
include circulatory collapse. That first line therapy for anaphylaxis to a sensitized antigen is the administration of exogenous epinephrine from a portable epinephrine auto-injector, is consistent with BP-mediated adrenal injury, suboptimal production and release of endogenous epinephrine, and the pertussis hypothesis.

Asthma and gross national income per capita (GNI) of country of birth

Rates of asthma and allergic sensitization tend to be lowest in the world’s least developed nations [90,91], where vaccination coverage has historically lagged and BP incidence is relatively high [57,92] (and from the WHO: http://apps.who.int/immunization_monitoring/global_summary/countries?countrycriteria%5Bcountry%5D%5B%5D=USA). A positive correlation between high economic status, as reflected by gross national product, and several allergic diseases, including asthma and atopy, has also been noted across the globe [91,93]. In addition, the CDC has reported that rates of pertussis vaccination are also directly proportional to GNI by successive quartile [94].

We propose that in low BP vaccination rate/high BP incidence nations with lower GNIs, increased frequency and degree of BP exposure and increased rates of community acquired latent BP immunization, SCBPI, tend to be high. High latent immunization rates in turn lead to potent mucosal BP immunity, reduced susceptibility to SCBPC, and reduced rates of SCBPC, asthma and related allergic disease. Conversely, we suggest that in highly BP vaccinated nations where GNI tends to be higher, reduced frequency and degree of BP exposure leads to lower rates of latent BP immunization, SCBPI, higher rates of mucosal BP immunodeficiency, greater susceptibility to SCBPC, and ultimately to higher rates of SCBPC, asthma and diseases of allergic sensitization. Importantly, while countries with higher GNI tend to have higher BP vaccination rates, wP and aP vaccines do not induce potent mucosal immunity [61] and so are not held to reduce SCBPC risk.

The potential importance of mucosal BP immunity in the etiology of asthma and allergic sensitization is supported by data on the risk of these diseases in those who relocate to an environment with a different rate of endemic BP exposure. Chang and Kelvin have shown that for immigrants, the risk of asthma persists in proportion to the duration one remains in the country of origin [95]. Cross-sectional survey data from 2009 from immigrants to New York City in the US indicate that the risk of asthma is lowest for those born in nations with a lower GNI, and rises with GNI of the country of origin [95]. Differences are clinically meaningful with asthma rates of 14% in those born in high GNI countries, 7% for those born in middle GNI countries, and 3.7% for those born in lower GNI countries (p < 0.001). After adjusting for more than a dozen potential confounding variables, differences remained statistically significant (p < .001). Consistent with these observations, the CDC has reported that higher rates of BP vaccination are directly proportional to GNI with the top quartile of nations achieving 98% administration of three doses of diphtheria tetanus pertussis vaccination (DTP3), the upper middle GNI quartile achieving 70% DTP3 rates, the lower middle quartile achieving 44% DTP3 rates, and the lowest quartile achieving 26% DTP3 rates [94].

Immigrants from low GNI countries who lived in the US for 5–9 years have a lower risk of asthma than those who lived in the US for more than 10 years (OR = 0.18, p = 0.02), consistent with the idea that a marked reduction in latent BP immunization enables the waning of mucosal BP immunity over time, commensurately increasing the risk and rate of SCBPC and allergic diseases such as asthma. These data, derived with a single methodology from immigrants to a single city, eliminate some of the ascertainment limitations and biases in studies spanning multiple countries.

Taken together, these data associate both the risk for asthma and the risk for pertussis (the latter inversely proportional to BP vaccination rate), with GNI by quartile. That is, since higher rates of BP vaccination are associated with higher GNI, and higher rates of BP vaccination are associated with lower rates of SCBPI and higher rates of SCBPC, we suggest that the asthma risk for country of origin GNI in Chang and Kelvin is a direct result, on a population level, of SCBPI and SCBPC rates in the country of origin.

Children raised on farms and in homes with pets have lower rates of asthma and allergic disease

In studies around the world, including North America, Europe, and Australia, growing up on a farm reduces the risk of asthma and allergic disease, lowering asthma risk by 30–50%, and early farm life is also associated with lower levels of IgE antibodies indicative of atopy [96–98]. In two large cross-sectional studies, children raised on farms were exposed to a greater variety of microorganisms than non-farm raised children, and exposure to Gram-negative rods reduced the odds ratio of atopy to 0.45 (95% CI, 0.27–0.76, p = 0.0003) [99]. An accompanying editorial noted, “Remarkably, atopy, which had the strongest inverse relationship with farm residence, was associated only with the broad category of ‘gram-negative rods’ [96].”

An inverse relationship has also been observed between exposure to endotoxin, a cell wall component of Gram-negative bacteria, and asthma, hay fever, and atopic sensitization [100]. More recently, a study comparing Amish and Hutterite children reported that prevalence rates of asthma and allergic sensitization were 4 and 6 times lower in the Amish, while LPS endotoxin (i.e., lipopolysaccharide from Gram-negative bacterial cell walls) levels in home dust samples were 6.8 times higher in Amish homes [101]. While the Amish and Hutterites are of similar European origin and lifestyle, including reproductive isolation, the Amish maintain traditional farming practices with small single-family farms “where children are reared in close proximity to farm animals and their sheds”, whereas Hutterites have adopted large-scale and highly industrialized practices [102], and “young Hutterite children typically do not spend time near farm animals or in the barns, which are much larger and located at much greater distances from their homes than are Amish barns” [103]. This study further demonstrated that in an adjuvant-mediated mouse model of allergic asthma, intranasal instillation of dust extracts from Amish, but not Hutterite, homes inhibited airway hyperreactivity and eosinophilia in wild type mice, but not in mice with compromised innate immunity [101], implicating the endotoxin-rich Amish environment as having a protective role in allergic asthma. Notably, Hutterite barn dust extract did inhibit airway hyperreactivity, bronchiolar lavage eosinophilia and house-dust mite-specific IgE as effectively as Amish barn-dust extract, suggesting that “an environment with robust asthma-protective properties appears to exist within the Hutterite colonies, but Hutterite children are not exposed to this environment in early life” [103].

Similarly, children raised in homes with pets such as cats and dogs are less likely to develop atopic disease [104–109] and asthma [108–111]. For example, current dog contact is inversely associated with hay fever (OR 0.26, 95% CI 0.1–0.57), asthma (OR 0.29 95% CI 0.12–0.71) and sensitization to cat allergen and grass pollen [108]. A prospective birth cohort study in Michigan demonstrated that “Exposure to 2 or more dogs or cats in the first year of life may reduce subsequent risk of allergic sensitization to multiple allergens during childhood” [105]. A literature review noted that while “findings have not been duplicated in all studies...data from recent years has shown that pet exposure in early childhood may actually prevent the development of allergic sensitization and allergic diseases including allergic rhinitis, asthma, and atopic dermatitis” [109]. Notably, a recent study of the microbiota of families and their pets showed that “humans tend to share more microbes with individuals, including their pets, with which they are in frequent contact” [112], increasing the likelihood of human exposure to B. bronchiseptica, a Gram-negative rod bacterium, and close relative of BP.

While induction of innate immunity by endotoxin may play a role in the protective effects of farm living and pet ownership on asthma and
allergic disease, an alternative explanation is that protective endotoxin exposure effects are but one aspect of the primary protective role of * Bordetella bronchiseptica* in the prevention of BP-mediated asthma and allergic disease in humans. *B. bronchiseptica* does not secrete the adjuvant pertussis toxin [113] and so is not expected to induce diseases of allergic sensitization, however *B. bronchiseptica* is a highly contagious respiratory pathogen that is better known to cause “kennel cough” in dogs and cats [114] and atrophic rhinitis in pigs [115]. Evidence supporting the role of *B. bronchiseptica* in reducing BP-mediated asthma and allergies includes the following: 1) *B. bronchiseptica*, a Gram-negative bacterium that synthesizes LPS endotoxin, is well known to infect domesticated and farm mammals such as dogs, cats, pigs, cows, sheep and horses [116,117], 2) *B. bronchiseptica* and BP share a close genetic relationship [118] and several key virulence factors including filamentous haemagglutinin (FHA) [119], pertactin [120], dermonecrotic toxin [121], and lipopolysaccharide [114], 3) living on a farm and having household animals increases human exposure to *B. bronchiseptica* as these infections are common in animals, e.g., 18.6% of lung samples from pigs with respiratory disease [122] and 10% of cats [114] test positive for *B. bronchiseptica*, 4) although not a primary host, and investigations are limited, humans can be infected and colonized by *B. bronchiseptica* [114,123–125], usually as a result of contact with animals [123,126], and perhaps most importantly, 5) by stimulating host TLR4 mucosal responses, *B. bronchiseptica* endotoxin “protects against Bordetella pertussis colonization” in rodents [127].

The pertussis hypothesis holds that given that BP exposure protects against *B. bronchiseptica* infection in mice [120], that *B. bronchiseptica* LPS endotoxin protects against BP colonization in mice [127], and given the substantial overlap in key virulence factors [119] and cross protection conferred by antigens such as FHA and pertactin [120], it is plausible and probable that *B. bronchiseptica* exposure reduces the risk of human BP infection, and SCBPC infections in particular. In summary, we propose that *B. bronchiseptica* transmitted from *B. bronchiseptica*-infected pets and farm animals to humans provides latent immunization, induces protective nasopharyngeal mucosal immunity to shared *Bordetella* virulence factors, and thereby reduces SCBPCs and BP-mediated asthma and allergic disease.

The hygiene hypothesis revisited: The inverse relationship between allergic disease and both family size and birth order is consistent with SCBPC risk

In 1989 Strachan proposed that atopic diseases such as hay fever, asthma and childhood eczema could possibly “…be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings.” [128] The hypothesis that early childhood infections protect individuals from allergic disease has come to be known as the hygiene hypothesis. Strachan initially supported this premise by noting the inverse correlation between both family size and sibling birth order with the risk for atopic disease [128]. Subsequently, multiple other investigators have found similar decreases in risk for asthma and atopic disease in those with a greater number of siblings [129–131].

Rather than protection from allergic disease conferred by an increased number of unspecified infections in those with larger families and a greater number of older siblings, we note that specifically, childhood BP exposure increases with both family size [132] and later rank in birth order (having more older siblings) [133]. In the pre-vaccine era, such increased BP exposure in families was noted to protect children from subsequent clinical BP infection [134,135]. In 1933, Stocks proposed to explain this unexpected observation “by the greater immunization of children by subclinical infections where the population is denser,” coining the term “latent immunization.” [28] We propose that recurrent BP exposure from older siblings leads to latent BP immunization and increased mucosal BP immunity, thereby reducing the risk of SCBPC, which in turn decreases the risk of asthma and atopic disease.

The hypothesis that recurrent, often subclinical, BP infections induce latent BP immunization which in turn reduces allergy risk is supported by studies of BP transmission and immunity within families demonstrating that (1) BP is highly infectious within households [76,135], with 80–90% attack rates for exposed non-immune contacts [136], (2) two-thirds of BP infections transmitted within households are transient and subclinical [76], and (3) household BP transmission induces effective immunity to acute clinical BP, and may do so through subclinical infections which confer “latent immunity” [28,76,135]. Given these data, we propose that recurrent childhood BP exposure in larger families proportionately reduces the risk for SCBPC, and thus the risk for co-localization of allergens with SCBPC, which in turn reduces the risk for asthma and related allergic disease. We suggest that an alternate explanation for Strachan’s observations is that members of larger families and later born siblings gain protection from allergic disease through latent BP immunization which confers a decreased risk for SCBPC, thus lowering asthma and atopy risk.

In sum, high BP transmissibility and induction of latent BP immunity through repeated BP exposures offers a more specific alternative explanation for the epidemiologic associations for atopic risk observed by Strachan. Given the breadth of the evidence presented in this review, including the unusual ability of BP and pertussis toxin to induce asthma and allergic disease in multiple animal models [41–44], we propose that the inverse association between allergic disease and both family size and sibling rank, previously attributed to the hygiene hypothesis, is better and more precisely explained by the pertussis hypothesis.

Assessing causation of SCBPC in asthma and allergic sensitization with the Bradford Hill guidelines

To assess causation from observational data without the benefit of randomized controlled trials, Hill offered several features of an association to weigh when making a judgment on potential causation, including strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy [137]. Hill’s construct is used to organize our analysis of the evidence for SCBPC as a cause of asthma and allergic sensitization, and draws from the case studies presented and biologic data from the medical literature.

Strength: The association corresponds with an increased relative risk (RR) of disease in those with the associated risk factor compared with those without

In a 1990s Swedish BP vaccine trial measuring the incidence of childhood asthma over time, the RR of asthma by age 2.5 years of age was 2.1 for those with a history of BP compared to those without (absolute risk 19% vs. 9%, p = .03) [138]. It is possible that bronchial hyperreactivity after pertussis infection confounded asthma diagnosis and biased detection of asthma. However, we propose that beyond SCBPC-induced asthma by nasopharyngeal antigen sensitization and respiratory antigen exposure, acute clinical BP may be a direct cause of asthma in a minority of acute clinical BP cases, with infants and young children at greatest risk of acute clinical BP-mediated asthma.

Lung development is dynamic and most rapid in this age group, and likely more susceptible to structural aberration and long term pulmonary pathology (see plausibility section below regarding BP and BP toxins in pulmonary remodeling) than fully developed airways (which remain susceptible to pathology from BP-mediated antigen sensitization and hyperimmune responses upon antigen re-exposure as reviewed in Figs. 2 and 3). Further consistent with this perspective, while a confirmed asthma diagnosis was not made, a cohort of children in the Netherlands with laboratory-confirmed acute clinical BP before six months of age was found to have a RR of 2.8 (95% CI: 1.1–7.0) for asthma symptoms between the ages of 1–3 years, compared with previously BP-uninfected children [139].
We emphasize that these data correlate asthma risk with documented BP infection, not a history of BP vaccination. Given the inability of currently available pertussis vaccines to prevent SCBPC and transmission, and the proposed critical role of SCBPC in the etiology of asthma and allergies, it is not surprising that there has been no credible direct relationship established between BP vaccination history and the development of asthma and allergic sensitization [140–142]. Anecdotes, uncontrolled studies, and stories on the internet and in news media suggesting that vaccines directly cause asthma and allergic disease have been shown to be flawed, as reviewed by Offit and Hackett [142], who further showed that well-controlled large epidemiologic studies substantiate no causal relationship.

Consistency: The association persists across populations and circumstances such as location and time

Consistency across human studies

The association between BP and asthma is supported in multiple study designs, regions, and times. Beyond the BP vaccine trial from Sweden demonstrating an increased incidence of asthma in children with a verified history of whooping cough compared with those without (19% vs. 9%, p = .03) [138], cross-sectional surveys from Turkey in 1997 and 2004 [143] demonstrate significantly higher reported rates of allergy in those with a history of BP vs. those with no history of BP (1997: 22.3% vs. 6.6%, p = .001) and (2004: 8.8% vs. 4.5%, p = .035), and higher rates of asthma (1997: 37.6% vs. 7.4%, p = .001) and (2004: 26.2% vs. 5.0%, p = .001) in the first and second surveys, respectively. Further, while it is possible that some whooping cough was misdiagnosed as asthma [144], a cohort study from the Netherlands in 2009 reported that toddlers with a history of laboratory confirmed pertussis were more likely to have reported asthma symptoms (obtained with a questionnaire adapted from the International Study of Asthma and Allergies in Childhood) than age-matched controls (RR 2.8, 95% CI 1.1–7.0) [139]. A British national cohort study following over 8,800 children born in 1958 similarly found an increased risk of asthma in those with a history of whooping cough (RR 1.2–1.4, varying with age at the time of infection, p < .001) [145].

We note the observations of prior generations of physicians suggesting a relationship between childhood acute clinical BP and both asthma and allergies, including Heaton in 1933 ("asthma may be initiated by whooping-cough") [146], Stevenet in 1961 ("whooping cough appears to be an important factor in the formation of hypersecretion respiratory allergies of the child") [147], and notably, the American Academy of Pediatrics in 1937 ("pertussis in children is often followed by asthma") [148]. Additionally, over 60 years ago, Feingold cited six pre-vaccine era publications linking BP to asthma, summarizing what appears to have been a generally accepted association: "Practically every modern text on either pediatrics or allergy cites the association between BP and asthma is supported in multiple epidemiologic observations. The highly BP-vaccinated FEG population of the late 1980s, with its low BP incidence, low rate of latent BP immunization, and proposed weak mucosal BP immunity, experienced a sudden rise in BP exposure after German reunification in 1990. Rates of asthma and allergic sensitization disease in FEG likewise climbed after the fall of the Berlin Wall, following an increase in BP exposure in FEG due to both a reduction in BP vaccination rates [56] and socialization with the higher BP incidence population of FWG – ultimately leading to the proposed commensurate rise of SCBPC in FEG.

In the United Kingdom, the National Cohort Study tracking over 8800 children reported that the risk of developing asthma or wheezing between the ages of 11 and 16 was significantly increased in those with a history of whooping cough (RR 1.2–1.4, varying with age at the time of infection, p < .001) [145]. We acknowledge that because whooping cough and asthma may bias the other’s diagnosis, confounding is possible.

Temporality is further supported by the previously noted cohort studies in the Netherlands and Sweden wherein an increased risk of asthma symptoms followed BP infection in the first 6 months of life [139] and first 2.5 years of life [138], respectively. While acute clinical BP during infancy or early childhood may cause a subset of asthma due to structural anatomic injury to rapidly developing lungs (see plausibility section on BP and BP toxins in pulmonary remodeling), we propose that the majority of BP-mediated asthma results from intranasal mucosal sensitization to a variety of antigens via co-localization with subclinical BP colonizing infections, and subsequent pulmonary pathology from hyperimmune responses to these antigens when they are inhaled into the airways and localize to bronchi and bronchioles.

Beyond human temporal evidence, multiple preclinical studies have utilized BP or pertussis toxin as an adjuvant to induce asthma and allergic sensitization. In these animal studies, BP exposure precedes allergen sensitization models of asthma. Controlled studies have quantified BP-induced biologic responses consistent with asthma pathology across multiple dimensions, including bronchial hyperresponsiveness [41,47,50], broncho-alveolar lavage (BAL) allergen-specific IgE [42], BAL eosinophilia [42,46], and BAL IL-4 and IL-13 [50], with BP and pertussis toxin inducing and exacerbating hallmark pathologies of asthma.

In a study using intranasal wild-type BP and pertussis toxin deficient BP to evaluate the impact of pertussis toxin, not only was the presence of pertussis toxin “associated with exacerbated host airway responses during peak B. pertussis infection” but also pertussis toxin “…inhibit[ed] host mechanisms of attenuating and resolving inflammation in the airways,” [43] consistent with pathologic airway inflammation observed in asthma.

As noted by Krug and Rabe, “Most animal models do not naturally and spontaneously develop asthma. Therefore an allergic asthmatic reaction has to be artificially induced by active immunization, using adjuvants like… Bordetella pertussis." [51]

Biological gradient: The association has a positive dose-response relationship

In East Germany during the 1990s, the risk of asthma and allergic sensitization disease increased with the risk of SCBPC. Note in Table 2 the rise in estimated annual BP cases/100,000 from < 1 in the 1980s, to 3.4 in 1994, to 20.5 in 2000, commensurate with allergy rates to various food and environmental allergens in East German children, which increased significantly from 13.7% in 1992–3, to 19.6% in 1995–6, to 24.3% in 1998–9 [64]. Corresponding asthma rates were 2.6%, 3.5% and 4.8%, also a significant progressive rise (p values for each successive rise < .001).

A biologic gradient between estimated subclinical BP infection and asthma in the US from 1980 to 2007 is also evident (see Fig. 1).
Plausibility: The association is consistent with accepted biologic knowledge

The evidence below is divided into sections on human and animal data. Special attention is given to evidence that BP may act as an adjuvant to sensitize a host to other infectious agents.

Subclinical nasopharyngeal Bordetella pertussis colonization is underreported and common

Subclinical BP infection rates are under-recognized, and SCBPC has been documented across the age spectrum and in widely varying epidemiologic settings. In a highly (99%) vaccinated population in China in 2011, during a period without a proximate BP outbreak, 4.8% of 629 asymptomatic children tested positive for BP by a single nasopharyngeal swab PCR [23]. A cross sectional study in 2015 reported 7.1% of 70 asymptomatic Iranian healthcare workers screened positive by nasopharyngeal culture for BP [25]. In a German sudden infant death study conducted from 1995 to 1997, 5.3% of 441 subjects in the control group were colonized with BP, as documented by nasopharyngeal swab PCR [24]. The annual incidence of BP infection in a Dutch study conducted between 1995 and 1996, as determined by serology in 3–79 year olds, was 6.6%. In contrast, the yearly incidence of notified Dutch BP cases was 0.01% [72], a ratio of estimated total cases to total reported cases of 660 to 1. In a separate study in the Netherlands in 2006–2007 that screened for serologic evidence of BP infection, 9.3% of asymptomatic subjects age 9 years and older had significantly increased levels of anti-pertussis toxin IgG, suggesting BP infection during the past year [73]. An Italian study documented serologic evidence of recent BP infection with rates that increased from 9.3% (95% CI 7.5–11.1%) in 1997 to 14.1% (95% CI 11.4–16.8%) in 2013 [74], when rates of reported BP infection had been well below 1% in all age groups, and approximately .001% in those over age 15 [150]. Finally, in the 1999 US acellular BP vaccine trial, estimated rates of undocumented BP infections for those age 15–65 years totaled 1 to 10 million cases annually, depending on case definition [71] when the CDC reported approximately 7000 US cases each year (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html), for a ratio of reported to unreported BP cases of 1:140. Study data not presented, but reported in Ward et al., indicated that the number of asymptomatic BP patients with seroconversion surpassed the number of symptomatic patients by a factor of up to 10, for a ratio of reported to unreported BP cases of 1:1400. In summary, a diverse medical literature from multiple populations documents unreported symptomatic and SCBPC rates far in excess of reported BP infection rates, and includes evidence from highly vaccinated populations.

Human plausibility data in asthma and allergic sensitization disease

A history of BP infection correlates with increases in total serum IgE in children compared with age-matched controls, particularly in children 3–12 years of age [151,152]. A statistically significant increase in positive skin prick test results has been observed 8–14 months after laboratory-confirmed acute clinical BP infection [152]. In addition, we note again the long history of prior authors’ case series suggesting that respiratory infection, including whooping cough, causes asthma [146–149,153].

Consistent with plausibility, BP can cause “widespread mucus plugging and extensive mucosal damage” as seen in postmortem studies of infants with acute clinical BP, and lead to the production of viscous mucus [117] — findings commonly observed in patients with asthma [154]. Further, in an analysis of international cross-sectional studies from 1995 to 2005 involving 54,943 schoolchildren aged 8–12 years, acute clinical BP infection was associated with wheeze (adjusted OR 1.68; 95% CI 1.44–1.97), the classic finding of asthma [155]. Additionally, in a Finnish study of induced sputum or pharyngeal swabs of asymptomatic adults with asthma, 28% of mild asthmatics and 20% of moderate asthmatics tested PCR positive for BP, and BP positive subjects had a lower ratio of forced expiratory volume in the first second/forced vital capacity (FEV1/FVC) (77.1% vs. 80.7%, p = 0.012) and more asthma symptoms (66% vs. 47% with symptom scores above median, p = 0.053) than BP negative cases [156]. Finally, using data from the British National Child Development Study, a history of acute clinical BP was associated with a 6 percent lower unadjusted mean FEV1 and FVC compared to controls without BP [157].

BP-mediated host sensitization to co-localized infection and a proposed role for SCBPC in type 1 diabetes

In preclinical models, BP and pertussis toxin sensitize hosts to intranasally introduced viruses such as respiratory syncytial virus [158,159]. Similarly, we propose that in humans, BP may serve as an adjuvant for a range of nasopharyngeal co-localized antigens, including infectious organisms such as rhinovirus [160], an established and common trigger of asthmatic exacerbations [161–164]. Childhood BP infection is coincident with an additional respiratory pathogen in 28–58% of cases [160,165] and in a prospective study of children with at least one week of cough, the most frequent mixed infection occurred between BP and rhinovirus, observed in 10% of patients [160].

Compared with other viral wheezing illnesses, rhinovirus infection in young high-risk children predicts the subsequent development of asthma [166,167]. Noting co-localization of BP infection with rhinovirus [160] and the ability of rhinovirus to infect both the upper and lower respiratory tract [168], we propose that SCBPC and SCBPC-secreted pertussis toxin act as adjuvants, sensitizing hosts to co-infecting agents such as rhinovirus in the nasopharynx, leading to pulmonary pathology consistent with asthma upon viral re-exposure in the lower respiratory tract. In essence, we suggest that microbes to which a host has been sensitized by BP or pertussis toxin, become aeroallergens and trigger a hyperimmune host response in the airways once the aeroallergen is subsequently inhaled.

Noting a 47% higher incidence of asthma in patients with type 1 diabetes (T1D) than controls in a nationwide Taiwan population-based cohort study of 3545 T1D patients and 14,180 controls (adjusted hazard ratio for asthma of 1.34 in T1D patients [95% CI = 1.11–1.62]) [169], we suggest that disease from nasopharyngeal BP-mediated sensitization to a virus and viral re-exposure is not limited to respiratory infections and pulmonary pathology. For example, we propose that BP-mediated sensitization to co-localized coxsackievirus in the nasopharynx, subsequent coxsackievirus re-exposure and localization to pancreatic beta islets, and a host hyperimmune response to islet-localized coxsackievirus leads to beta islet cell-specific inflammation and pathology consistent with T1D as supported in greater detail below.

Given that both BP and coxsackievirus reside in the nasopharynx [117,170], that coxsackievirus is pancreotropic [171], that human pancreatic beta cells can harbor persistent coxsackievirus infections [172], that coxsackievirus has been isolated from pancreatic beta islet cells of children with acute-onset diabetes [173,174], and given that despite this, several large-scale studies (which did not control for the presence of SCBPC) have been inconsistent with respect to a causal link between coxsackievirus and T1D [175], the pertussis hypothesis suggests that mucosal SCBPC-mediated sensitization to coxsackievirus and a subsequent hyperimmune host response to beta cell-localized coxsackievirus provides a plausible explanation for the pathogenesis of coxsackievirus-associated T1D. More specifically, we suggest that research to understand the host-pathogen relationship in coxsackievirus-associated T1D may need to control for the impact of nasopharyngeal SCBPC-mediated host sensitization to coxsackievirus.

Similarly, we propose that pharyngeal co-localization of SCBPC with insulin, such as in cows’ milk formula [176], provides an opportunity for BP-mediated host sensitization to insulin resulting in...
inflammation specific to beta islet cells, where insulin is produced and secreted. Adjuvant-mediated sensitization to insulin, including oral insulin, and the subsequent onset of insulitis is well-established in animal models [177,178]. As Toreson wrote nearly 50 years ago, “Islet lesions in the diabetic rabbits immunized with insulin-adjuvant distinctly resemble the reported lesions of juvenile acute-onset diabetes in man” [178].

In short, asthma and T1D comorbidity may be the result of a common sensitizing agent, SCBPC. Like asthma, T1D rates have been rapidly increasing in many developed nations in recent decades [179], and we suggest that this rise is due, at least in part, to a concurrent increase in SCBPC rates.

**BP and pertussis toxin induce an immunologic profile seen in asthma and allergic sensitization disease**

Most asthma, including early-onset-allergic, exercise-induced, and late-onset eosinophilic, is associated with CD4 + Th2 activation and increased IL-4 and/or IL-5 production [4], and IL-13 production [180]. Pertussis toxin can likewise activate Th2 host immune responses characterized by increased production of IL-4, IL-5, IL-13, and immunoglobulins [50,181].

In addition, BP may induce a Th17-driven immune response [182-184] characteristic of the mucosal immune response to bacterial infection [185,186], and an emerging subset of neutrophilic asthma [4,187-190]. BP adenylate cyclase toxin [183], pertussis toxin [184], and BP lipopolysaccharide [182] can each independently induce a Th17 immune response.

Perhaps most notably, intranasal colocalization of pertussis toxin and respiratory syncytial virus (RSV) leads to increased RSV-specific IgG1, increased total serum IgE, elevated IL-4 in lung supernatants, and augmented IL-4 production by splenic lymphocytes exposed to pertussis toxin and RSV recall antigens. Noted the authors, “…coadministration of PT [pertussis toxin] with RSV at the lung epithelium augments PT’s adjuvant effects and suggests a PT-induced effect on the local cytokine milieu leading to the altered composition of the RSV-specific immune response...These results suggest that PT can influence the local production of IL-4 to alter the humoral and cellular immune responses to viral infection as well as to coadministered antigens.” [159]. In a subsequent editorial, the authors remarked, “In separate experiments, we showed that simultaneous intranasal delivery of PT and RSV produced a robust type 2 cytokine response in the lung…” [191], corresponding to the cytokine profile of many allergic diseases [192].

**BP and PT toxins induce pulmonary remodeling in humans and animals**

Lung remodeling in asthmatics includes airway wall thickening with smooth muscle hypertrophy and hyperplasia, epithelial hypertrophy, and subepithelial fibrosis [193,194] and a subgroup of human asthematics manifests irreversible airway defects [194,195]. Although understanding of the relationship between pulmonary inflammation and remodeling is developing and controversial, chronic airway inflammation may induce asthmatic bronchial remodeling in preclinical studies [196], and it is well established that BP and pertussis toxin induce inflammation, even in nanogram quantities [117,197]. Human clinical support for BP inducing permanent structural lung change includes evidence from a cohort of adults (with no excess of physician-diagnosed asthma) with a history of acute clinical pertussis by age seven. These individuals have a statistically significant 6% decrease in forced vital capacity compared with those without an acute clinical BP history (p = .04), documenting the correlation between a fixed pulmonary structural change and a history of acute clinical pertussis. Differences between groups persist after treatment with albuterol, as expected for fixed defects. Acute clinical pertussis-associated pulmonary remodeling is to be distinguished from the potential for nasopharyngeal SCBPC to induce allergen specific sensitization and resulting reversible allergic airway disease manifested as asthmatic bronchospasm [157]. In sum, we propose that, in addition to the reversible obstructive airway disease due to allergic sensitization seen in asthma, BP may also be responsible for the fixed structural remodeling reported in asthmatics.

In animal models of asthma, BP infection prior to sensitization leads to ‘increased inflammation of bronchiolar walls with accompanying hyperplasia and mucus metaplasia of lining epithelia” [198], similar to asthmatic histopathology. Toxins released by BP induce marked respiratory histologic changes, including extrusion and destruction of ciliated respiratory epithelium by BP tracheal cytotoxin [199] and not only is BP toxin associated with airway hyperresponsiveness, but it also inhibits host mechanisms that attenuate airway inflammation [43]. At the cellular level, pertussis toxin has been shown, *in vivo* and *in vitro*, to induce cytoskeletal reorganization with increases in endothelial permeability, loss of integrity of cell-cell junctions, and “profound actin cytoskeletal rearrangement and stress fiber formation” [200], while BP tracheal cytotoxin in synergy with endotoxin inhibits DNA synthesis in cultured tracheal epithelium [201]. Finally, *in vitro* studies demonstrate that Wnt signaling modulates lung development, lung fibrosis, and the proliferation and differentiation of multiple pulmonary cell types [202]. Since pertussis toxin blocks several forms of Wnt signaling [203-205], localized pertussis toxin from BP infection may contribute to asthma via compromise of Wnt pathways.

Taken together, clinical, histologic, and cellular evidence demonstrates that BP and BP toxins induce respiratory tissue injury and remodeling consistent with asthma.

**Animal plausibility data for asthma and allergic sensitization**

**BP infection and asthma and allergic sensitization**

BP and pertussis toxin are such potent adjuvants that sensitization and anaphylaxis to ragweed pollen may inadvertently occur in mice administered BP in late summer, when pollen is “in the air” (without experimentally introducing antigen, but by unintentional exposure to pollen in unfiltered air) [45]. In their landmark study, Chang and Gottshall observed that mice that were intranasally administered BP and then, 8 or 9 days later, exposed to concentrated aerosolized pollen became sensitized to pollen (“windows wide open”), became sensitized to pollen. The investigators warned that “when using pertussis organisms as an adjuvant for sensitizing mice, precautions must sometimes be necessary to avoid producing antibodies against inadvertently administered airborne antigens.”

An earlier murine study by the same investigators similarly demonstrated that inhalation of aerosolized albumin after an established BP colonization infection led to albumin sensitization and to pathology upon albumin re-exposure [49]. The pertussis hypothesis asserts that above and beyond laboratory experiments, this is precisely what initiates a substantial number of human asthma and allergic rhinitis cases. That is, as illustrated in Fig. 2, an individual infected with nasopharyngeal SCBPC becomes sensitized to airborne antigens such as pollen when that antigen is co-localized with SCBPC and SCBPC-secreted toxins. Once sensitization has occurred, the antigen becomes an allergen to the sensitized host such that allergen re-exposure upon inhalation leads to a hyperimmune host airway response, with attendant intranasal inflammatory pathology in the case of allergic rhinitis, and bronchiolar inflammatory pathology in the case of allergic asthma as illustrated in Fig. 3.

In addition to laboratory experiments using pertussis toxin as an adjuvant to induce asthma and allergic sensitization disease [41,42,45,46,52,53], inhaled virulent BP has been shown to significantly exacerbate adjuvant-mediated allergen-induced asthma in mice [198]. In a rodent model of allergic asthma, aerosolized virulent BP increased asthmatic histopathology and airway hyperreactivity after ovalbumin (OVA) sensitization, compared with uninfected control
mice. OVA sensitization was achieved through intraperitoneal OVA injection with an aluminum-based (non-BP) adjuvant. BP infection-associated changes included increased peribronchiolar leukocyte infiltration, epithelial hyperplasia, mucus metaplasia, and increases in BAL fluid IL-10 and IL-13 [198]. These findings demonstrate that, beyond induction of sensitization, virulent BP may exacerbate airway pathology in allergic asthma.

Using the same model, investigators later demonstrated that both whole-cell pertussis (wP) vaccination [47] and acellular pertussis (aP) vaccination [48] protect against BP infection-induced exacerbation of OVA-induced asthma, decreasing immunopathologic changes and airway hyperactivity, compared with controls. Of note, while both aP and wP vaccines may induce protective systemic immunity to reduce pulmonary injury during virulent BP exposure, neither aP nor wP induce potent mucosal immunity [61,76] and are therefore not anticipated to prevent BP-mediated asthma secondary to SCBPC (i.e. allergic asthma from nasopharyngeal SCBPC-mediated sensitization to co-localized antigens and subsequent re-exposure to those antigens upon inhalation).

In sum, sensitization models utilizing BP and pertussis toxin spanning over four decades demonstrate pertussis-induced sensitization to inhaled allergens [45,49], bronchial hyperresponsiveness [41,47,50], broncho-alveolar lavage (BAL) allergen specific IgE [42], BAL eosinophilia [42,46], and BAL IL-4 and IL-13 [50], all changes which are characteristic of asthma [180,206,207].

Overview of plausibility

A summary of the pertussis hypothesis is illustrated in Figs. 2 and 3.

Specificity: The cause is associated with a single type of disease in a defined population

We propose that multiple diseases are associated with SCBPC, and have previously proposed the potential role of BP-mediated sensitization in multiple sclerosis [20] and celiac disease [21]. The specific form of BP-associated disease (e.g. asthma, multiple sclerosis, celiac disease) is dependent not only on the presence of SCBPC, but on the presence of additional necessary causes, such as genetics and the co-localization of nasopharyngeal SCBPC with particular allergens such as pollen or gluten. In real-world multifactorial disease, the heterogeneous distribution of disease risk factors attenuates Hill’s proposed specificity of effect, and we do not expect that SCBPC risk will exclusively correlate with allergic sensitization disease risk. Specificity is predicted to the degree that only the necessary causes of a particular disease are present. Conversely, we propose and the evidence presented supports, that there are circumstances when more than one set of sufficient causes for different BP-associated diseases are comorbid, such as atopy and asthma, celiac disease and asthma [208], and multiple sclerosis and asthma [209].

Coherence: The association does not conflict with accepted biologic knowledge

Hill’s inclusion of both plausibility and coherence acknowledge that both consistent and inconsistent data may exist for a hypothesis.

Anticipated concerns

BP may be assumed to be too uncommon to cause asthma and allergic sensitization

Questions regarding coherence may arise from an under-appreciation of the well-documented incidence of subclinical BP infection [26,71,156,210–212]. As previously noted, using subclinical BP rates from a large US vaccine efficacy trial in the late 1990s [71] (wherein biases are minimized by the prospective study design incorporating scheduled clinical and biologic assessments) and commensurate reported BP rates from the CDC (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html), the ratio of reported BP infection to unreported subclinical BP in the US was estimated to be 1:140 to 1:1400. Therefore, current increases in reported BP, while garnering attention as a threat to public health for the burdens of whooping cough [213], likely denotes a far greater rise in SCBPC and, as proposed, SCBPC-associated disease risk. The causal connection between BP and the rise in asthma as seen in Fig. 1 has not previously...
been suggested, and is incompatible with the conventional perception of acute clinical BP infections. As recently as 2005, it was written that “Clinically, the full spectrum of disease due to B. pertussis infection is now understood.” [117] We propose that incoherence is resolved with an appreciation of the magnitude of SCBPC, the established role of BP in sensitization models including experimental animal models of asthma, and the potential etiopathogenic role of SCBPC in asthma and allergic sensitization.

We embrace a multicausal model of disease and do not propose that all disease phenotypically grouped with asthma and related allergic disease requires BP. Some proportion likely requires other microbes or exogenous causes for disease. For example, various animal models of disease require BP. Some proportion likely requires other microbes or all disease phenotypically grouped with asthma and related allergic sensitization.

BP rates do not always correlate with asthma and allergic sensitization disease rates

Coherence may also be questioned if SCBPC is scrutinized as the only necessary cause for a BP-associated disease, ignoring other component causes distinct from BP. The population variance of the full complement of necessary causes, including exposure to environmental antigens, dictates the population incidence of allergic sensitization disease. Further, in a multicausal model, diseases may have multiple sets of sufficient cause. In sum, incoherence may be anticipated with univariate analysis of SCBPC as the only necessary cause of multicausal diseases such as asthma and allergic sensitization.

Finally, the relationship between BP incidence and proposed BP-associated disease is non-linear, which may confound initial expectations. While BP incidence positively correlates with asthma and allergy rates in lower BP incidence environments where BP vaccination rates are relatively high like the US, asthma and allergic sensitization disease rates inversely correlate with BP incidence in environments where BP incidence is high enough to facilitate latent BP immunization. That is, we propose that in populations where vaccination rates are high and circulating BP rates are low, each additional exposure to BP increases the risk of SCBPC. Conversely, in populations where vaccination rates are low and circulating BP rates are high, each additional BP exposure, rather than leading to SCBPC, is more likely to lead to latent immunization with attendant enhancement of mucosal and systemic BP immunity (a “boosting” effect). Latent immunization, in turn, is held to reduce the risk of SCBPC and allergic sensitization, consistent with the lower rates of asthma found in immigrants to New York City from lower GNI nations [95] (see related case study above). Apparent incoherence is resolved with appreciation of the differentiated impact of environmental BP exposure, state of mucosal BP immunity, and risk of SCBPC, a proposed major cause of asthma and allergic sensitization disease.

Experiment: The association can be altered by manipulation of the cause

Human experimental data

As previously noted, in the randomized controlled human trial investigating the effect of BP vaccines on atopic disease, Nilsson et al. observed a statistically significant risk increase in asthma by 2.5 years of age in children with a history of acute clinical BP, compared with those without a history of acute clinical BP (19% vs. 9%, p = .03) [138].

Further, as reviewed in the first case study above, a malevolent political decision in the latter decades of the twentieth century led to a large-scale unintended human experiment with the rise and fall of the Berlin wall. The wall divided a German population with common genetic and cultural ancestry into regions that adopted divergent BP vaccination, immigration, emigration and visitation policies. By
maintaining higher BP vaccination rates and limiting BP importation compared with FWG, FEG experienced low rates of BP prior to the fall of the Berlin Wall, followed by higher rates of acute clinical BP (and as proposed in detail above, SCBPC) with the influx of BP upon German reunification in 1990. Serial data obtained over the next decade from East German children documented statistically significant progressive increases in asthma (both clinically diagnosed and as documented by pulmonary function testing), as well as increases in eczema and strong sensitization to any allergen [64,65].

Animal experimental data

As noted earlier, multiple animal studies have used BP in sensitization models of asthma [41,42,46]. BP and pertussis toxin may also serve as adjuvants in allergic disease models, including anaphylaxis [52,54,87,217,218]; ovalbumin [46–49], house dust mite [42,50] and pollen sensitization [37,45]; and food allergy [40,53,219,220].

Analogy: The association is analogous to another association that is accepted as causal

It is widely recognized that subclinical human infections may lead to a variety of chronic immune- and toxin-mediated disease. For example, *H. pylori* can cause peptic ulcer disease [221]. The role of *H. pylori* in peptic ulcer disease is analogous to BP in asthma in that *H. pylori* colonizes the mucus layer (in this case, of gastric mucosa as opposed to respiratory epithelium) [222], may adhere to the epithelium [223], and causes disease through the release of enzymes [224] and toxins [225], or by eliciting an immune response [226].

Conclusion

With the pertussis hypothesis, we propose that SCBPC may be an important cause of asthma and related allergic disease, consistent with epidemiologic phenomena such as the rise in rates of asthma and allergic sensitization in FEG after the fall of the Berlin Wall; the increase in the incidence of asthma, peanut allergy, and anaphylaxis in the US in recent decades; the correlation between rates of asthma and per capita GNI of one’s country of birth; the lower risk of asthma and allergy in children raised with pets and on farms; and the reduced risk of atopy with increased family size and later sibling birth order. Using the Bradford Hill criteria to assess causation from association, we find the relationship between SCBPC and allergic sensitization disease is strong, as measured by the increased relative risk of asthma in young children with a history of BP infection, consistent across varied populations and time, temporally logical in both human and animal data, and exhibits a biologic gradient as reviewed in data from East Germany and the US. Furthermore, a role for SCBPC in asthma and other allergic disease is biologically plausible and consistent with current knowledge given not only the widespread documentation of SCBPC particularly in highly BP-vaccinated populations, but also the potent adjuvant sensitizing effects of BP and pertussis toxin, widely documented and supported by dozens of animal and human experiments as reviewed herein. Finally, the SCBPC-asthma/allergy disease model is analogous to other models widely accepted as causal, such as the role of *H. pylori* in peptic ulcer disease.

Ultimately, our review of the evidence for the pertussis hypothesis is a call for further study. Notably, a controlled BP colonization and immunity study, including intranasal swab and nasal wash assays has recently been approved for human investigation [227]. Further prospective investigation may begin with (1) longitudinal surveillance with nasal swab or nasal aspiration PCR and serum antibody studies for SCBPC starting in infancy or early childhood, with cohorts followed for the development of physician-diagnosed asthma and allergic sensitization, testing all patients prospectively on a predefined schedule, (2) randomized controlled trials of regular nasopharyngeal irrigation, which is generally regarded as safe and effective for acute and chronic rhinosinusitis [228], to minimize SCBPC and reduce the risk of allergic sensitization disease, and (3) ascertainment of BP and BP markers in nasopharyngeal (e.g. PCR) and serum (e.g. pertussis toxin IgG) samples from patients with new-onset anaphylaxis, with patient and microbial controls.

We suggest that reducing SCBPC-mediated disease, including asthma and allergic sensitization, will optimally require the development of next generation BP vaccines that induce potent mucosal BP immunity and thereby prevent subclinical BP colonization. With the implementation of such vaccines, randomized controlled trials are likely to definitively determine the role of SCBPC in the pathogenesis of asthma and allergic sensitization disease. Until then, it is our hope that the evidence presented herein encourages further exploration of the pertussis hypothesis and the potential role of subclinical BP colonizing infections in the onset of human disease.

Conflict of interest statement

KR and SG are employed by and hold an equity interest in IliAD Biotechnologies, which is developing a vaccine for the prevention of *Bordetella pertussis*.

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Medical Hypotheses 120 (2018) xxx-xxx


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