Published in final edited form as:

Hum Immunol. 2014 May ; 75(5): 440–451. doi:10.1016/j.humimm.2014.02.013.

Substantial gaps in knowledge of *Bordetella pertussis* antibody and T cell epitopes relevant for natural immunity and vaccine efficacy

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Abstract

The recent increase in whooping cough in vaccinated populations has been attributed to waning immunity associated with the acellular vaccine. The Immune Epitope Database (IEDB) is a repository of immune epitope data from the published literature and includes T cell and antibody epitopes for human pathogens. The IEDB conducted a review of the epitope literature, which revealed 300 *Bordetella pertussis*-related epitopes from 39 references. Epitope data are currently available for six virulence factors of *B. pertussis*: pertussis toxin, pertactin, fimbrial 2, fimbrial 3, adenylate cyclase and filamentous hemagglutinin. The majority of epitopes were defined for antibody reactivity; fewer T cell determinants were reported. Analysis of available protective correlates data revealed a number of candidate epitopes; however few are defined in humans and few have been shown to be protective. Moreover, there are a limited number of studies defining epitopes from natural infection versus whole cell or acellular/subunit vaccines. The relationship between epitope location and structural features, as well as antigenic drift (SNP analysis) was also investigated. We conclude that the cumulative data is yet insufficient to address many fundamental questions related to vaccine failure and this underscores the need for further investigation of *B. pertussis* immunity at the molecular level.

1. Introduction

Bordetella pertussis, the causative agent of whooping cough, has been a health concern since the Middle Ages when it was first described [1]. Before widespread vaccination, whooping cough was a universal disease, associated with significant morbidity and mortality [2]. Starting in the 1950s, wide use of the whole cell inactivated vaccine (wP) greatly reduced disease incidence in the U.S. However, mainly due to adverse effects associated with wP vaccination, starting in the 1990s [3], wP was widely substituted with the safer acellular (aP) subunit vaccine. Recently, the incidence of whooping cough in the U.S. has steadily increased, despite widespread vaccination coverage [4].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.humimm.2014.02.013>.

This increase has been attributed to several factors, including waning/insufficient immunity associated with the aP vaccine [5– 11], changes in the organism at the genomic level (antigenic drift) [12–19], and increase surveillance and detection [20,21]. It is possible that each of these factors may play a role, though to what extent is not clear from the current literature. The epidemiological evidence suggests that the increased incidence correlates with the switch from wP to aP in the mid 1990's [22], suggesting a significant role for waning immunity.

It is possible that the aP composition and/or formulation provides lower quality immunity of shorter duration than the whole cell vaccine (wP) or natural *B. pertussis* infection. Indeed, the wP vaccine consists of >3400 ORFs, whereas the aP vaccine formulations contain a small subset of antigens: 1–5 pertussis proteins. In addition, the pertussis toxin (PT) component of the aP vaccine is chemically detoxified, thus potentially altering proteins, affecting quality of immunity and therefore vaccine outcome [23,24]. This underscores the need for a more thorough investigation of human immunity at the antigen level, and more specifically to characterize the antigenic determinants that correlate with protection (T cell or antibody). While defining correlates of protection in human disease at the antigen level has been challenging (e.g. there is no clear serological correlate of protection defined) [11], identifying epitopes associated with protection and/or toxin neutralization/inhibition in human and animal studies may help highlight candidate antigenic regions for more in depth analysis. To date, no group has investigated the issue of waning immunity at the epitope level. Numerous studies have shown the importance of PT in protection [25–29] and have defined multiple antigenic determinants and/or regions of T and B cell recognition, but even this prominent antigen has not been characterized sufficiently at the molecular level (e.g., epitope mapping for all subunits in humans).

The Immune Epitope Database and Analysis Resource (IEDB) is a repository for immune epitope data for infectious disease, allergy, autoimmunity, as well as transplant-related disease. The IEDB captures T cell and B cell/antibody epitopes defined in humans, as well as animal models, in the context of the specific assays used to define them. Thus, it is possible to selectively query the data related to natural infection (by disease) versus immunization with a particular immunogen, or by host. It is also possible to search for data associated with specific assays, such as ELISA versus functional assays that define *in vitro* correlates of protection (neutralization or CTL).

We surveyed all epitope data related to *B. pertussis* to determine whether adequate data existed to address the leading hypotheses linked to waning vaccine efficacy, namely those related to breadth and quality of the immune response to active immunization, including epitope coverage per antigen, the distribution of response phenotypes, the balance of data among different host species (humans versus animal models), as well as a structure-function analysis of epitope location, and further explored whether the available data could be used to assess the contribution of pathogen escape (antigenic drift at the epitope level) on waning immunity.

2. Methods

2.1. Script used to search PubMed

The following script was used to query PubMed for all epitope-references related to pertussis: ((epitope[TW] OR epitopes[TW] OR “epitopes”[MeSH Terms] OR mimotope[TW] OR ((MHC[tw] OR major histocompatibility complex[tw] OR HLA[tw]) AND (peptide[tw] OR peptides[tw])) OR TCR recognition[tw] OR (Class[tw] AND I motif[tw]) OR supermotif[tw] OR (peptide-based[tw] AND CTL[tw]) OR phage displa*[tw] OR antibody binding[tw] OR protective immune response[tw] OR antibody recog*[tw] OR cytotoxicity as-say[tw] OR new monoclonal[tw] OR novel antibody[tw] OR ((monoclonal antibod*[tw]) AND binding site[tw]) OR ((KA[tw] OR KD[tw]) AND (monoclonal[tw] OR mAb[tw])) OR neutralizing antibod*[tw] OR peptide vaccine[tw] OR peptide conjugate vac-cin/[tw] OR ((CD8[tw] OR CD4[tw]) AND T cell*[tw] AND (pep-tide[tw] OR peptides[tw])) OR antigenic repertoire[tw] OR ((peptide[tw] OR peptides[tw]) AND antibody reactivity[tw]) OR (Class II[tw] AND (binding [tw] OR bound[tw] OR peptide[tw] OR peptides[tw])) OR “immunogenic peptide”[tw] or hapten[TW] or haptens[TW] OR ((antigenic*[tw] OR immunogenic*[tw]) AND determinant*[tw]) or (monoclonal antibod*[tw] and peptide*[tw]) or (T cell*[tw] and peptide*[tw])) AND (hasabstract[text] AND English[Lang] AND (“1900”[PDat]:“2013/12/31”[PDat])) NOT (Re-view[PT] OR Editorial[PT] OR meta-Analysis[PT] OR Comment[PT] OR pmcbook[All Fields] or pubstatusaheadofprint[ALL])) AND pertussis[Title/Abstract].

2.2. IEDB data inclusion criteria

This analysis includes antibody and T cell epitopes associated with *B. pertussis* associated antigens. We followed the process of Davies et al. [30] to identify *B. pertussis* related data derived from peer-reviewed literature contained in PubMed. Epitope definitions (length and mass restrictions) and IEDB inclusion criteria can be found at (http://tools.immuneepitope.org/wiki/index.php/Curation_Manual2.0#Prevailing_Rules). For the purpose of this report, epitopes represent the specific molecular structures experimentally shown to react with a B cell or T cell receptor (the database only curates experimental data and does not include predictions).

2.3. Mapping epitopes onto 3D structures

The epitope data for PT S1 and S4 were first filtered for prominent regions using the Immunome Browser available on the Results Summary page for each query. Epitopes with the highest RFscores were selected and the sequences of these were mapped using the Homology mapping tool housed on the IEDB.

3. Results

3.1. Scope and limitations of the analysis

This study was designed to critically review the available data and as such does not provide new data on immunity to *B. pertussis* and vaccination. This work represents an analysis of epitope mapping studies that have been reported in the published literature and are captured in the IEDB. The study surveys the data from this database to determine whether the

available data is sufficient to characterize adaptive immune response to *B. pertussis*. The T and B cell/antibody epitopes that have been mapped are for the most part reflective of targeted studies, but we have found that few if any studies tackle the question of comprehensive T or B cell repertoires (e.g. by using overlapping peptides covering the entire proteins). Therefore the lack of defined T cell or antibody epitopes for any particular antigen reflects most likely the fact that a more complete mapping has not been performed, and not that these determinants do not exist.

A number of studies exist in the literature that provides data describing antibody recognition of rather large domains or fragments >50 amino acids in length. For this reason, they do not meet the inclusion criteria of the IEDB. There are also numerous papers that identify and characterize the functional capabilities of monoclonal antibodies (mAbs) or T cell clones, but do not report their sequence specificity (epitope). These data are also not included in the IEDB. The IEDB cannot capture data for which the epitope structure or sequence has not been defined or reported.

To ascertain whether any pertussis-related papers may have been missed and are therefore not included in the IEDB, we conducted a targeted survey of the published literature for any additional relevant pertussis papers. All resultant PubMed IDs (PMID) were compared to those currently in the IEDB reference tracking system. This survey identified seven outstanding papers which have now been incorporated in the IEDB. All additional *B. pertussis* papers were reassessed against the IEDB's inclusion criteria, and determined to have been excluded based on size criteria and/or lack of identified sequence (e.g. only aa positions reported). However, because this latter subset of references represents data relevant to the current analysis, a table of their respective details has been provided (Supplementary Table 1). This includes seminal papers related to PT, FHA and AC, monoclonal antibody or T cell clones shown to neutralize/inhibit and/or to protect in animal models of infection. The specificity of most clones (BCR or TCR) were broadly defined as regions (>50aa), N-versus C-termini or entire subunits (e.g. PT S1).

3.2. High level summary of *B. pertussis* immune epitope data

We surveyed *B. pertussis* epitope data captured in the IEDB as of 2013, enumerating the total number of pertussis-related references, and total number of epitopes, including positive and negative peptides. There are currently 300 epitopes reported from 39 different pertussis-related references. These data represent epitopes (molecular structures 50 amino acids) defined in the context of T cell responses (CD8⁺/class I and CD4⁺/class II), antibody responses (linear and conformational), as well as MHC binding, and include negative data (126 peptides for which only negative assays results have been reported). To date, few antigens have been investigated at the epitope level. These include: pertussis toxin (PT), adenylate cyclase (AC), pertactin (PRN), fimbrials 2 and 3 (fim2, fim3), and filamentous hemagglutinin (FHA). The majority of epitopes (~60%) are derived from PT, and more specifically from PT subunits 1 and 4. Anti-body/B cell epitopes (249) far outnumber T cell epitopes (47) and MHC binding peptides (43). Only one of the reported antibody epitopes is conformational in nature (mouse mAb against fim3). The majority of data were generated in murine hosts rather than human; of 27 monoclonal antibodies for which the epitope

recognized was reported, none was derived from humans. Table 1 provides a summary of the *B. pertussis* epitope data.

An analysis of the IEDB data with respect to host is shown in Tables 2a and b. At the high level we found that mice (39%), rabbits (32%), and humans (29%) make up the bulk of the data. Further, T cell epitopes have been defined in both humans and murine models, with the majority of these being CD4⁺/class II. Antibody responses have been defined in humans, mice, rabbits and goats, with the human data (65) considerably less than that from mice (98) and rabbits (99). When these data are considered in the context of aP vaccine-specific antigen components, the epitopes defined in humans were derived fairly equally among the four vaccine-specific pertussis proteins (18% fim2, 16% fim3, 44% PT and 22% PRN), in mouse and rabbit models the vast majority of epitope were derived from PT (76 and 73%, respectively) and far fewer epitopes were defined in other vaccine-specific antigens (Fig. 1). Somewhat surprising was the lack of defined epitopes for humans against FHA; most reactivity defined to date is limited to large regions of this antigen (Supplementary Table 1).

3.3. Assessment of epitope data in the context of potential correlates of protection, vaccine-related issues and MHC restriction

Next we sought to analyze the cumulative data in the context of the vaccine-related issues, namely what data are currently available at the epitope level with respect to assays related to immunity (protection and correlates of protection), and further, to what extent these data can be used to evaluate differences in epitope reactivity between natural infection and immunization (aP and wP). First we focused on those data that were generated with assays considered potential *in vitro* correlates of protection. With respect to “*in vitro* correlates,” there are several different toxin neutralization/inhibition assays that exist, each with its own advantages and limitations. These include the CHO cell clustering assay, leukocytosis-promoting (LP) activity, islet-activating (IA) activity, permeability-increasing (PI) activity, hemagglutinating activities and ADP-ribosyltransferase activity [31,32]. For T cell responses, the current epitope data has been generated using a small number of standard assays: proliferation assays (³H), radioimmunoassay (RIA), ELISPOT and ELISA assays to measure cytokine responses (IL-2 and IFN γ) and cytotoxicity assays (⁵¹Cr). We selected cytotoxicity and IFN γ assays as potential correlates of protection for this analysis, as proliferation and IL-2 production are less often used as *in vitro* correlates of protection. Table 3 provides details for all *in vitro* T cell and antibody assays captured to date for *B. pertussis* epitopes. These represent assays that demonstrate functional capacity for the epitope-specific effector cell, distinguishing these from all other assays that do not provide any insight in the functionality of responses (such as ELISA, WB, proliferation, etc.). In general, a relatively small portion (10%) of the overall data was found to define epitopes in the context of protective responses (*in vitro* correlates).

In terms of epitopes defined in the context of *in vivo* protection (challenge and survival assays), a mere five antibody epitopes were shown to be protective against challenge *in vivo*, all derived from PT subunit 1 and all in mouse models of lethal shock syndrome using toxin challenge (Table 4). When these same sequences were used to query the human data, we found that all were also recognized by human T cells and/or B cells. Overall, 60% of the

epitopes associated with *in vitro* correlates, as well as those protective *in vivo* (Tables 3 and 4) were shown to be recognized by human subjects, as either the same exact sequence or a closely related one.

We then examined potential difference among epitopes recognized following immunization versus natural infection in humans, since antibody and T cell response patterns of natural infection and the wP or aP subunit/vaccines may differ qualitatively. Table 5a provides a breakdown of the human data by these three categories for antibody and T cell epitope records. The vast majority of the human epitopes have been defined in the context of natural infection, most of this reflecting antibody reactivity, followed closely by epitope derived from wP-vaccinated subjects. Fewer data are available for those immunized with aP or other subunit formulations, and none of the acellular vaccine data reported to date used aP vaccines licensed for use in the U.S. (Daptacel, Infanrix, Tripedia, etc.), though one study did utilize the Swedish vaccine, JN1H-3 (contains PT and FHA). Moreover, there is a relative imbalance in epitope mapping (overall coverage) for the antigens comparable between natural infection and the vaccine formulations (Table 5b). For example, in the case of natural infection, fimbrial and pertactin-specific data far outnumbered PT-specific data. The important role of anti-fimbrial antibodies in immunity has been well-established [34–36]. However, while PRN and Fim are components of the aP vaccine (e.g. Sanofi Pasteur), they are not present in all formulations, whereas PT is universally included, and considered to contain major protective epitopes.

Finally, the database was specifically queried to identify all cases in which the MHC restriction of T cell epitopes was defined, either by *in vivo/in vitro* assay or by MHC binding. This information can be useful for the development of tetramer staining reagents to further characterize T cell responses to *B. pertussis*. Table 6 lists the number of epitopes restricted by various class I and II alleles reported to date. These include human HLA-A2 (A/02:01), HLA-DR (DRB1/01:01 and DRB1/11:01) and HLA-DQ, as well as murine H-2-IA^d, H-2-D^b and H-2-K^b. To date, very minimal data are available defining restriction in the context of disease or immunization, and, to the best of our knowledge, no tetramers have been reported thus far.

3.4. Structure and functional analysis with respect to epitope location

All of the antigens for which epitopes have been defined to date represent major virulence factors of *B. pertussis* pathogenesis and can be separated into two functional categories, exotoxins (PT and AC) and adhesins (fim2, fim3 PRN, and FHA). An analysis of epitope location with respect to the structure and function of these antigens may be of interest as it relates to the extent to which protective or immunodominant antibody and T cell reactivity is directed towards key regions responsible for attachment or enzymatic activity. Studies to date have identified critical regions on adhesins, such as heparin binding domains (HBD), RGD motifs and the carbohydrate recognition (CRD), that function in the attachment of the pathogen to host cells within the respiratory epithelium [37]. For the A–B exotoxins like PT and AC, critical function sites include the catalytic site on the A subunit and the receptor binding domains on the B subunit. Upon release in the infected host, subunit A of PT is activated and catalyzes the ADP-ribosylation of the alpha subunit of G protein on cells

within the respiratory tract (macrophages and DCs), increasing intracellular cAMP and thereby interfering with signal transduction and hampering immune responsiveness [38–40]. The B domain binds the whole toxin to host cells prior to invasion.

To analyze the relationship between structural or functional features and epitope location we utilized the Immunome Browser (IB) accessible through the IEDB query interface. The IB is a tool developed to plot response frequency scores [RFscores] (# respondents/# tested) along the length of an antigen per amino acid residue. In this way, the IB maps the epitope data onto the antigen allowing visualization of T cell and antibody response patterns and the RFscores serve to provide insight into the relative prominence of any epitope/region in a quantifiable manner. We therefore generated response frequency data for both antibody and T cell responses to all 5 antigens. We then identified all functional sites based on amino acid position corresponding to these epitopes/epitopic regions. Eighty-two antibody and T cells epitope were found to have residues that corresponded to functional domains. These included catalytic sites, cleavage sites, heparin binding domains, carbohydrate recognition domains, immune evasion, bacterial adherence, membrane insertion, NAD glycohydrolase activity, ATP-binding sites and toxin secretion. Table 7 provides details for each epitope, highlighting the residues involved in the functional site/region.

The highest RFscores for antibody responses were generated against PT subunit 1 (PT S1) which represents the catalytic A domain of the toxin and the fimbrials (fim2/3). Interestingly, while notable RFscore were also noted for T cell responses against PT S1, high T cell associated RFscores were observed for subunit 4 (S4) as well. S4 is part of the B domain of PT and is the receptor-binding domain (RBD) of the toxin. Here we observed that S4 epitope residues corresponded with the ATP-binding domain within the RBD. To further visualize their location relative to the overall structure, epitopes with high RFscores were mapped onto the 3D structure of PT, as shown in Figs. 2 and 3. To date, 3D structures have not been reported for *B. pertussis* fimbrials (no Protein database ID), therefore we were unable to map these epitopes.

The targeting of these regions by T cell and antibody responses may therefore provide an advantage to the host either by neutralization/inhibition, steric interference, the promotion of opsonization, cytolysis of infected cells and/or the production of cytokines that promote clearance. The 3D location of observed antibody epitopes reflects targeting of the more surface exposed regions of the toxin; whereas the T cell responses appear to be directed at sites more internal and likely accessible to TCRs following class I/II processing. Data are as yet insufficient for AC, PRN and FHA to provide similar mappings.

3.5. Antigenic drift as a cause of vaccine failure

As indicated above, antigenic drift has also been investigated as a potential contributing factor for increased disease incidence. Antigenic drift occurs as a result of genomic variations between circulating *B. pertussis* strains and those incorporated within licensed vaccines arise over time. This sequence variation could affect antibody and cellular recognition and/or memory responses in the host (vaccinated or immune) population. Indeed, vaccine-induced antigenic shifts associated with clinical breakthrough, as well as vaccine-induced pathogen adaptation have been documented [41,42].

While *B. pertussis* is known to be fairly monomorphic [14,17,43], allelic variation has been documented in several well-known virulence genes: *ptx* (PT promoter and A subunit), *fim* (fimbrials 2 and 3), and *prn* (pertactin). Genomic analysis has identified single nucleotide polymorphisms (SNPs), as well as insertions and deletions (in *prn*; though less frequent) leading to the occurrence of multiple allelic forms globally [12,15,17,19,44]. While these studies have suggested that these variations may play a role in adaptation following immunization [15,45–48], the extent to which these variations fall within epitopic regions was not addressed.

Using the data provided in the studies, we examined the relationship between SNPs identified to date and reported antibody and T cell epitope locations. It was found that of the 12 unique SNPs previously identified, 10 (83%) are present within antibody and T cells epitopes, which includes those specificities captured in the IEDB, as well as those within larger regions recognized by certain monoclonal antibodies and T cell clones. Table 8 provides details of the SNPs analyzed and the corresponding epitope(s). However these results are not statistically significant, because the inclusion of the larger regions greatly reduces the resolution of the analysis, and results in the sequences of those antigens being very extensively covered. Taken together, these results suggest that a more stringent definition of the actual epitopes recognized in humans following vaccination is required, to conclusively address this issue.

4. Discussion/conclusions

The resurgence of *B. pertussis* infections has received considerable attention in recent years. Several hypotheses have been formulated in terms of apparent waning of vaccine efficacy. First, it is possible that the breadth of responses induced by aP is significantly less comprehensive than the one induced by wP, and immunity against crucial antigens is not elicited by the aP vaccine (breadth of immunity). Second, it has been hypothesized that the quality of responses, in terms of phenotypes of responding T cells/neutralizing capacity of antibodies, or duration of responses, induced by aP is markedly different from the one induced by wP or natural infection (quality of immunity). Lastly it is also possible that new *B. pertussis* strains might be evolving, and lacking the antigens targeted by the vaccine, or carry mutations in key epitopes (pathogen escape). All three of the above hypotheses can be at least partially addressed by immunological data. Here we present a comprehensive review of immune epitope data related to *B. pertussis* to investigate whether the available data are sufficient to address these issues.

Our review and analysis of the epitope data reveals that the characterization of *B. pertussis* immunity is limited. In terms of the breadth of the responses induced by natural infection versus the different vaccine constructs, epitope data were defined from a relatively small number of *B. pertussis* antigens (6 of >3400). The majority of epitopes were defined for PT, and far fewer were reported for antigens incorporated within currently licensed aP vaccines, and also associated with vaccine-induced immunity and in immunity to natural infection [11,49–51]. Thus a meaningful assessment of how the epitope breadth elicited by natural infection versus aP and wP vaccination could not be undertaken. However, this analysis was able to present interesting insights into structural and functional relationships with respect to

epitope location and to examine the association between epitope location and sites of antigenic diversification (SNP analysis). Taken together these findings underscore the need for a more comprehensive epitope mapping of *B. pertussis* antigens in the context of human disease and active immunization.

The consensus in the literature is that vaccine-induced protection is largely antibody-mediated [52–56]. And indeed, antibody epitopes represent the majority of those reported herein (>80%), however, relatively few antibody epitopes were associated with human subjects (<30%). Furthermore, our analysis revealed a surprising lack of discontinuous B cell determinants given the overwhelming focus on humoral responses. There were a relatively small number of monoclonal antibodies (all murine) and few reports defining specific isotypes or subtypes other than IgG. Further mapping in this context may provide additional insight for protective responses, as work to date has shown that IgG2a, which facilitates opsonization and complement fixation has been associated with protection [55,57], and IgA isolated from convalescent human sera has been shown to inhibit adherence of bacteria to ciliated epithelial cells [58]. Indeed, both the wP and aP vaccines induce high levels of specific antibodies. However different isotypes predominate resulting in functional differences [55,57,59] and these have not been investigated at this level to any extent.

T cell epitopes represent a mere 16% of the total data. Numerous studies published to date have demonstrated a role for T cells/cell-mediated immunity following immunization and natural infection [11,49,50,60–62]. Despite this, very little of the T cell epitope data were characterized phenotypically. While the majority of reported T cell epitopes were defined for CD4⁺/class II T cells, these represent mostly proliferation data. Few cytokine data were described (minimal IFN γ and IL-2). Studies to date have implicated certain cytokine reactivity in protection thereby making this an area for future focus [33,50,60,63,64]. Similarly, while the wP vaccine has been associated with a Th1/Th17 type response, the aP formulations tend to induce Th2 cytokines [50,65]. Thus far there is little or no characterization of CD8⁺ T cell epitopes, despite some evidence of their role in natural infection and following immunization [49,61]. Lastly, MHC restriction data was limited. Moreover, tetramer reagents, which can provide powerful tools for characterization of T cell responses in disease, and following different vaccination strategies, were absent, thus highlighting another area for future work.

To date, few epitopes were defined in the context of functional assays, and thereby little data are available to ascertain correlates of protection. Thus, at the level of response quality the available data does not provide clear association of epitope reactivity with different antibody type and T cell phenotypes, functional activities or correlates of protection. Similarly, since not all relevant epitopes for aP vaccine components are known, it is not possible to comprehensively assess how they may differ from those epitopes recognize following natural infection. Lastly, in terms of evaluating the possible role of pathogen escape in relation with waning vaccine efficacy, it is apparent from what is presented above that only a small subset of the relevant antibody and T cell epitopes is known. While some evidence is emerging that shows antigenic variants in circulating strains of *B. pertussis* [12,17], whether this is as a result of natural host-pathogen evolution, selective pressure exerted by vaccines

containing few antigens, or a combination thereof, it not yet known. A more comprehensive epitope mapping of critical vaccine-related epitopes is therefore necessary to ascertain whether mutations are occurring within the key epitopes or not.

In conclusion, taking into consideration the overall impact of this pathogen on human health, the characterization of *B. pertussis* at the molecular level is surprisingly minimal. Perhaps one unexpected and positive outcome of this recent resurgence of disease will be renewed efforts to more fully characterize the immunobiology of this important pathogen, including definition of human reactivity at the molecular level.

Assuming that more epitope knowledge will become available in the coming years, it may be possible to use this knowledge to a better vaccine design or understanding of the current aP vaccine's role in the dramatic increase in observed pertussis cases. For example, knowledge of the crucial epitopes induced by natural infection and vaccination would indicate whether they are modified by fixation, and might suggest different inactivation strategies. Epitope definition on a genome-wide basis, as recently shown in the case of TB [66], would allow for a more accurate measure of responses to a wide variety of antigen, and compare responses resulting from natural infection, and vaccination with whole cell and cellular vaccines. Further important issues that await clarification are which epitopes are important for protection and which are non-protective, and where are they located on the toxin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge Drs Mark Sawyer, Rino Rappuoli and Alison Deckhut for their contributions in reviewing this manuscript. This work is supported by the National Institute of Allergy and Infectious Diseases (NIAID), under the Immune Epitope Data-base and Analysis Resource: contract number HHSN272201200010C.

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Fig. 1.

Epitope distribution by protein of prominent hosts. The distributions of epitopes from each of the main *B. pertussis* antigens are shown for each host species in which they were defined. These data were generated using the IEDB Search interface (www.iedb.org) by individual queries to the Epitope Source Molecule Finder for each antigen along with the Host Organism Finder to filter on human, mouse or rabbit data.

Fig. 2.

3D Mapping of prominent epitopes for PT S1 catalytic domain. These data were generated using the Immunome Browser feature accessible through the results summary page. Prominence was given to any epitope with RFscore >0.30. The data for each epitope is accessible using the Epitope ID through the Identifier Search (Advanced Search menu). RFscores are calculated as # respondents-square root of respondents/# tested. The square root is a correction factor, approximating one standard deviation for the number of responding donors. This gives a higher score to epitopes studied with larger sample sizes. Bold sequence indicates residues overlapping among epitopes from protection studies shown in Table 6. 3D mapping was accomplished using Homology Mapping (FASTA of antigens from Tohama I) available through the IEDB Epitope Analysis Tools. Blue color indicates residues involved in epitopes. (For interpretation of color in Fig. 2, the reader is referred to the web version of this book).

T cell

Epitope ID	Sequence	Frequency	RFscore
80106	YEVTPTRMLVCGIAAKLGAAASSPD	12/12	0.71
79756	AMTHLSPALADVPPYVLVKTNMNVTS	11/12	0.64
79939	MVVTSVAMKPYEVTPTTRMLVCGIAA	6/12	0.30
79771	CGIAAKLGAAASSPDAHVPFCFGKD	6/12	0.30
79761	ASSPDAHVPFCFGKDLKRPGSSPME	6/12	0.30

Fig. 3.
3D Mapping of prominent epitopes for PT S4 receptor binding domain Refer to previous Fig. 2 legend for details.

B. Pertussis antigens with defined epitope data.

Table 1

Antigen name	T cell	Antibody	MHC binders	Total epitopes	References
Pertussis toxin (PT) [*]	43	147	2	156	27
Adenylate cyclase (AC) [†]	0	1	40	41	2
Pertactin (PRN) [*]	0	38	0	38	4
Filamentous hemagglutinin (FHA) [*]	0	23	0	23	2
Fimbrial 2 (Fim2) [*]	0	18	0	18	2
Fimbrial 3 (Fim3) [*]	0	18	0	18	2

Note: Protein nomenclature is that of the NCBI Protein database; the IEDB links directly to this database for the purpose of providing the source of the epitope sequence. When the accession number or GenBank ID is not provided by the authors, a representative antigen is chosen with 100% homology. Nomenclature is therefore not assigned by the IEDB. The proteins names listed above represent, in some cases, numerous different GenBank IDs.

^{*} A component of the aP vaccine formulations. Three additional *B. pertussis* antigens are present in the IEDB: cyaE [accession 117799], fhaE [accession 462083] and the putative autotransporter [accession 3411270] proteins, however, these represent only MHC binding data not related to disease characterization. Queries were performed using Source Antigen Molecular Finder and the Immune Recognition Context selections provided on the IEDB search interface (www.iedb.org).

[†] 294/254 Negative T cell and MHC binding epitopes reported for this antigen, respectively.

Table 2**a**
Host distribution for T cell epitopes.

	Epitopes	Class I	Class II
All hosts	47	1	35
Human	26	1	14
Mouse	36	0	25

b
Host distribution for antibody epitopes.

	Epitopes	Linear	Discontinuous
All hosts	248	247	1
Human	65	65	0
Mouse	98	97	1
Rabbit	99	99	0
Goat	1	1	0

Queries to enumerate epitopes by host were performed using the Source organism Finder to select “*Bordetella pertussis*,” the Host Organism Finder to select human, mouse, rabbit, etc., the Immune Recognition Context to select ‘B cell Response’ or ‘T cell Response,’ the MHC class pull-down for class I versus class II and the ‘Discontinuous peptide’ radio button under Epitope Structure within the IEDB search interface (www.iedb.org). *Note:* The total number of epitopes reported for each individual host may be greater than the total number of epitopes for all hosts as some peptides (identical molecular structures) are positive in more than one host.

Table 3Assays defining epitopes with *in vitro* correlates of protection.

Response type	Epitope	Assay	PMID
mAb E251, mouse	PT S2 (134–147) TATRLLSSTNSRLC	CHO cell clustering	1718872
pAb, rabbit	PT S3 (37–51) PKALFTQQGGAYGRC	CHO cell clustering	1383153
pAb, rabbit	PT S3 (29–51) VAPGIVPPKALFTQQGGAYGRC	CHO cell clustering	1383153
pAb, rabbit	PT S3 (46–69) GAYGRCPNGTRALTVAELRGNAEL	CHO cell clustering	1383153
pAb, rabbit	PT S3 (65–92) GNAELQTYLRQITPGWSIYGLYDGYTLG	CHO cell clustering	1383153
pAb, rabbit	PT S3 (88–115) GTYLGQAYGGIIKDAPPGAGFIYRETFC	CHO cell clustering	1383153
pAb, rabbit	PT S3 (115–136) CITTIYKTGPAAADHYYSKVTA	CHO cell clustering	1383153
pAb, rabbit	PT S2 (105–125) GAFDLKTTFCIMTTRNTGQPA	CHO cell clustering	1383153
pAb, rabbit	PT S3 (131–155) YSKVTATBLLASTNSRLCAVFVRDG	CHO cell clustering	1383153
pAb, rabbit	PT S3 (151–182) FVRDQSQVIGACASPYEGRYRDLYDALRRLLY	CHO cell clustering	1383153
pAb, rabbit	PT S3 (177–204) LRRLLYMIYMSGLAVRVHVSKEEQYYDY	CHO cell clustering	1383153
pAb, rabbit	PT S3 (106–136) AGFIYRETFCITTIYKTGPAAADHYYSKVTA	CHO cell clustering	1383153
pAb, rabbit	PT S1 (1–18) DDPPATVYRYDSRPPEDV	CHO cell clustering	2017195
pAb, rabbit	PT S1 (121–138) GALATYQSEYLAHRRIPP	CHO cell clustering	2017195
pAb, rabbit	PT S1 (201–235) CMARQAESSEAMAAWSEAGEAMVLVYYESIAYSF	CHO cell clustering	2017195
pAb, rabbit	PT S1 (1–15) DDPPATVYRYDSRPP	CHO cell clustering	2017195
pAb, rabbit	PT S1 (124–138) ATYQSEYLAHRRIPP	CHO cell clustering	2017195
pAb, rabbit	PT S3 (29–40) VAPGIVPPKAL	HA assay	1706321
pAb, rabbit	PT S3 (40–51) LFTQQGGAYGRC	HA assay	1706321
pAb, rabbit	PT S3 (42–57) TQQGGAYGRCPNGTRA	HA assay	1706321
pAb, rabbit	PT S3 (64–79) RGNAELQTYLRQITPG	HA assay	1706321
pAb, mouse	PTS1 (1–17) DDPPATVYRYDSRPPED	CHO cell clustering	2480389
pAb, mouse	PT S1 (70–82) EAVEAERAGRGTG	CHO cell clustering	2480389
pAb, mouse	PT S1 (189–199) GTLVRMAPVIG	CHO cell clustering	2480389
mAbs 2/1:9F, 2/2:5D, 5/1:4C	FHA (2001–2015) RGHTLESAEGRKIFG	HA assay	9180182
T cell clones *	PT S1 (212–235) MAAWSEAGEAMVLVYYESIAYSF	Cytotoxicity, RIA IFN γ	1716614
T cell clones *	PT S1 (30–41) DNVLDHLTGRC	Cytotoxicity, RIA IFN γ	1716614
T cell clones *	PT S1 (27–39) GNNDNVLDHLTGRC	Cytotoxicity	1716614
T cell clones *	PT S1 (180–194) SRRSVASIVGTLVRM	RIA IFN γ	1716614

The query was performed using the Advanced Search interface on the IEDB home page (B cell and T cell Searches done separately) to select 'Bordetella pertussis' from the Epitope Organism Finder and then: all "neutralization/inhibition of antigen activity" assays from the Assay Finder for antibody responses and "cytotoxicity" and "cytokine release IFN γ " for all T cell responses; only positive data were considered.

* T cell clones were generated from the peripheral blood of a donor who had suffered from *B. pertussis* as a child and who had exhibited significant humoral and cellular responses and was therefore considered immune; Polyclonal sera (pAb); Hemagglutination assay (HA assays).

Table 4

Epitopes reported to be protective in vivo.

Response	Immunogen	Immunization/challenge	Assessment	% Protected	PMID
Ab/B cell	PT S1 (1–17) DDPPATVYRYDSRPPED*	BALB/c, 4–5 weeks, 2 doses 5µg i.p. at days 1 and 10. Challenged: lethal shock syndrome; 100 ng of PT given i.v. on days 0 and 2	Protection correlated with the ability of these peptides to elicit high anti-PT titers	54	2480389
	PT1 S1 (53–64) TSSSRRYTEVYL*			50	
	PT S1 (70–82) EAVEAERAGRTG*			65	
	PT S1 (99–112) GAASSYFEYVDITYG*			80	
	PT S1 (189–199) GTLVRMAPVIG*			50	

* Also recognized by sera from two infants vaccinated with pertussis vaccine. This IEDB data can be quickly accessed using the PMID in the 'Identifier search' located on the Advanced Search pull-down menu on the IEDB home page or by pasting the epitope sequence into the linear peptide field (exact match) under Epitope Structure (www.iedb.org). The query was performed using the Advanced Search interface on the IEDB home page (B cell and T cell Searches done separately) to select '*Bordetella pertussis*' from the Epitope Organism Finder and all "challenge assays" from the Assay Finder; only positive data were considered.

B

B cell epitopes

Natural infection

T cell epitopes

Natural infection

Overall antigen coverage for human antibody response data.

Immunization

These data were generated by performing a query using the Epitope Source Organism Finder to select 'Bordetella pertussis' and then the Host Organism Finder to select 'Human'. All T cell and B cell Response data were then downloaded in Excel format (full) and combined into one workbook. The data were then parsed by filtering on columns '1st In Vivo Process Type' for 'Occurrence of infectious disease versus 'Administration in vivo', and then all 1st Immunogen fields to assess the type of immunogen. The total number of assays and epitopes was tallied following removal of redundant data (Advanced filter, unique records only).

Table 6

MHC restrictions defined.

<i>Epitope restriction defined in T cell assays</i>		
Mouse class II	H-2-IA ^d	1
Human class II	HLA-DQ	1
	HLA-DR	2
	HLA-DR1	4
<i>Epitope restriction defined in MHC binding assays</i>		
Mouse class I	H-2-D ^b	4
	H-2-K ^b	36
Human class I	HLA-A*02:01	1
Human class II	DR1	1
	DRB1*01:01	1
	DRB1*11:01	1

These data were generated by performing a query using the Epitope Source Organism Finder to select '*Bordetella pertussis*.' All T cell Response and MHC binding assay data were then downloaded in Excel format (full) and combined into one workbook. The data were then parsed by filtering on the column 'MHC Allele Name.' The total number of assays and epitopes was tallied following removal of redundant data (Advanced filter, unique records only).

Table 7

Structure-function.

Epitope ID	Antigen	Epitope Sequence	Position	Resp Freq	RFscore	Response type	Structure	Function	Putative activity	Reference
7850	PT S1	DDPPATVYRYDSRPPED	35–51	10/11	0.62 (0.29)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523, 2902632
1416	PT S1	AFVSTSSRRRYTEVY	83–97	5/5	0.55 (0.45)	B	A subunit	CD	Toxin secretion	10678938
9573	PT S1	DNVLDHLTGRSCQ	64–76	4/4	0.50 (0.50)	T	A subunit	CD	NAD glycohydrolase activity/catalytic	8119996, 21740523
66442	PT S1	TSSRRRYTEVYL	87–98	6/10	0.36 (0.24)	T (B)	A subunit	CD	Toxin secretion	10678938
7851	PT S1	DDPPATVYRYDSRPPEDV	35–52	2/2	0.29 (0.71)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
72154	PT S1	VYRYDSRPPEDV	41–52	2/2	0.29 (0.71)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
22756	PT S1	GTLVRMAPVIG	223–233	5/10	0.28 (0.22)	T (B)	A subunit	CD	ADP-ribosyltransferase activity	8168972
9571	PT S1	DNVLDHLTGRSC	64–75	5/10	0.28 (0.22)	T (B)	A subunit	CD	NAD glycohydrolase activity/catalytic	8119996, 21740523
7850	PT S1	DDPPATVYRYDSRPPED	35–51	3/5	0.25 (0.35)	T	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
3053	PT S1	AMAAWSERAGEA	245–256	NA	0.00 (1.00)	B	A subunit	CD	Catalytic/cleavage site	15691377
78112	PT S1	CMARQAESSEAMAAWSERAGEAMVLVYYESIAYSF	235–269	NA	0.00 (1.00)	B	A subunit	CD	Catalytic/cleavage site	15691377
78113	PT S1	CQVGSSNSAFVSTSSRRRYTEVYL	75–98	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity	8119996, 21740523
7849	PT S1	DDPPATVYRYD	35–45	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
78115	PT S1	DDPPATVYRYDSRPP	35–49	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
94492	PT S1	DSRPPEDVFQNGFTAWG	45–61	0/1	0.00 (0.00)	T	A subunit	CD	NAD binding	
10594	PT S1	DVFQNGFTAWGNND	51–64	NA	0.00 (1.00)	B	A subunit	CD	NAD binding	8119996, 21740523
78159	PT S1	GALATYQSEYLAHRRIPP	155–172	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/catalytic	8119996, 21740523

Epitope ID	Antigen	Epitope Sequence	Position	Resp Freq	RFscore	Response type	Structure	Function	Putative activity	Reference
21476	PT S1	GNNDNVLDHLTGR	61–73	NA	0.00 (0.50)	T	A subunit	CD	NAD glycohydrolase activity/catalytic	8119996, 21740523
22516	PT S1	GSSNSAFVSTSSRR	78–92	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity	8119996, 21740523
78205	PT S1	MAAWSERAGEAMVLVYYESIAYSF	246–269	NA	0.00 (1.00)	B	A subunit	CD	Catalytic/cleavage site	15691377
94607	PT S1	MARQAESSE	236–244	0/1	0.00 (0.00)	T	A subunit	CD	Cleavage site	15691377
78213	PT S1	MVLVYYESIAYSF	257–269	NA	0.00 (1.00)	B	A subunit	CD	Subunit B binding domain	8168972
78223	PT S1	PATVYRYDSRPPEDV	38–52	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
48751	PT S1	PPATVYRYDSRPPE	37–50	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
55389	PT S1	RQAESSEMAAWSERAGEA	238–256	NA	0.00 (1.00)	B	A subunit	CD	Catalytic/cleavage site	15691377
56519	PT S1	RYDSRPPEDVF	43–53	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
61920	PT S1	STSSRRYTEVY	86–97	NA	0.00 (1.00)	B	A subunit	CD	Toxin secretion	10678938
94726	PT S1	TVYRYDSRPPED	40–51	0/1	0.00 (0.00)	T	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740523
72153	PT S1	VYRYDSRP	41–48	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity site/NAD binding	8119996, 21740524
75548	PT S1	YQSEYLAHRR	160–169	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/catalytic	8119996, 21740525
75686	PT S1	YRYDSRPP	42–49	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740526
12527	PT S1	YRYDSRPPEDV	42–52	NA	0.00 (1.00)	B	A subunit	CD	NAD glycohydrolase activity/NAD binding	8119996, 21740527
188005	PT S1	Y8, R9, Y10, D11, S12, R13, E16, R39, R79, T81, H83, Y148, N150, T153	discn	NA	NA	B	A subunit	CD	Binding of mAb 1B7 keep A-B linked	19899804
18572	PT S2	GAFDLKTTFCIMTTRNTGQPA	105–125	2/5	0.12 (0.28)	B (T)	B subunit	RBD	CRD	1353482
39107	PT S2	LRGSGDLQEYLHVTR	62–77	NA	0.00 (1.00)	B	B subunit	RBD	CRD	1729677
66072	PT S2	TRNTGQPATDHYYSNV ^a	118–133	NA	0.00 (1.00)	B	B subunit	RBD	CBD	21740523, 1353482

Epitope ID	Antigen	Epitope Sequence	Position	Resp Freq	RFscore	Response type	Structure	Function	Putative activity	Reference
1508	PT S3	AGHYRETFCTITTYKTGQPAADHYYSKVTA ^a	106–136	2/5	0.12 (0.28)	B (T)	B subunit	RBD	CBD	21740523
6459	PT S3	CTTTYKTGQPAADHYYSKVTA ^a	115–136	NA	0.00 (1.00)	T, B	B subunit	RBD	CBD	21740523
80772	PT S3	CPNGTRALTV	51–60	0/1	0.00 (0.00)	B	B subunit	RBD	CRD	1353482
18843	PT S3	GAYGRCPNGTRALTV AELRGNAEL	46–69	NA	0.00 (1.00)	T, B	B subunit	RBD	CRD	1729677
21374	PT S3	GNAELQTYLRQITPGWSIYGLYDGTYL	65–92	NA	0.00 (1.00)	T, B	B subunit	RBD	CRD	1729677
82843	PT S3	GQPAADHYYSKVT	123–135	NA	0.00 (1.00)	B	B subunit	RBD	CBD	21740523
87884	PT S3	RGNAELQTYLRQITPG	64–79	NA	0.00 (1.00)	B	B subunit	RBD	CRD	1729677
79761	PT S4	ASSPDAHVPFCFGKDLKRPSSPME	83–107	6/12	0.30 (0.20)	T (B)	B subunit	RBD	ATP binding site	8500874
79771	PT S4	CGIAAKLGAASSPDAHVPFCFGKD	73–97	6/12	0.30 (0.20)	T (B)	B subunit	RBD	ATP binding site	8500874
79769	PT S4	CFGKDLKRPSSPMEVMLRAVFMQQ	93–117	4/12	0.17 (0.17)	T (B)	B subunit	RBD	ATP binding site	8500874
79768	PT S4	CFGKDLKRPSS	93–104	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
6225	PT S4	CFGKDLKRPSSPMEV	93–108	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79760	PT S4	ASSPDAHVPFCF	83–94	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79773	PT S4	DAHVPFCFGKDL	87–98	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79781	PT S4	DLKRPSSPMEV	97–108	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79839	PT S4	GKDLKRPSSPM	95–106	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79840	PT S4	GKDLKRPSSPME	95–107	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79854	PT S4	HVPFCFGKDLKR	89–100	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
79960	PT S4	PFCFGKDLKRP	91–102	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874

Epitope ID	Antigen	Epitope Sequence	Position	Resp Freq	RFscore	Response type	Structure	Function	Putative activity	Reference
80031	PT S4	SPDAHVPFCFGK	85–96	NA	0.00 (1.00)	B	B subunit	RBD	ATP binding site	8500874
26839	fim2	IKLKDCP	83–89	12/12	0.71 (0.29)	B	pili	Attach	HBD	9573115
21713	fim2	GPNH TKV	52–58	12/12	0.71 (0.29)	B	pili	Attach	HBD	9573115
70651	fim2	VQTGGTSRTVTMYLAS	170–186	12/12	0.71 (0.29)	B	pili	Attach	HBD	9573115
34243	fim2	KVVQLPKISKALKANG	57–73	12/12	0.71 (0.29)	B	pili	Attach	HBD	9573115
73808	fim2	YFEPGPT	99–105	8/12	0.43 (0.24)	B	pili	Attach	HBD	9573115
78434	fim2	KNGDVEASAITTYVGFVVYP	190–210	NA	0.00 (1.00)	B	pili	Attach	HBD	9573115
78392	fim2	GDLRAYKMYATNPQTQLSN	111–130	NA	0.00 (1.00)	B	pili	Attach	HBD	9573115
34244	fim3	KVVQLPKISKALRNDG	53–69	12/12	0.71 (0.29)	B	pili	Attach	HBD	9573115
4856	fim3	ASYVKKPKEDVD	178–189	11/12	0.64 (0.28)	B	pili	Attach	HBD	9573115
31932	fim3	KLKECPQ	80–86	7/12	0.36 (0.22)	B	pili	Attach	HBD	9573115
78437	fim3	KVTNGSKSYTLRYLASVVK	164–182	NA	0.00 (1.00)	B	pili	Attach	HBD	9573115
78490	fim3	QALGALKLYFEPGTTNYDTGDLIAYKQTYNASGN	86–120	NA	0.00 (1.00)	B	pili	Attach	HBD	9573115
78375	fim3	EPGITTNYDT	96–105	NA	0.00 (1.00)	B	pili	Attach	HBD	9573115
49037	PRN	PQPGQPQPQPQPQPEAPAPQPPAG	579–603	3/37	0.03 (0.05)	B	OMP	Attach	PQP motif ⁵	17597264
19684	PRN	GGAVPGGAVPGGAVPGGFGPGGFGP	266–290	2/37	0.02 (0.04)	B	OMP	Attach	GCXXP motif ^d	8609998
98699	PRN	APKAPQPGQPQPQ	574–588	NA	0.00 (1.00)	B	OMP	Attach	PQP motif ^{ee}	17597264
3757	PRN	APQPGQPQPQPQPEAPAPQ	578–599	NA	0.00 (1.00)	B	OMP	Attach	PQP motif ^e	17597264
98741	PRN	EAPAPQPPAGRELSA	594–608	NA	0.00 (1.00)	B	OMP	Attach	PQP motif ^e	17597264

Epitope ID	Antigen	Epitope Sequence	Position	Resp Freq	RFscore	Response type	Structure	Function	Putative activity	Reference
98788	PRN	GDAPAGGAVP	261–270	NA	0.00 (1.00)	B	OMP	Attach	GGXXP motif ^d	8609998
98798	PRN	GGFGPGGFGP	281–290	NA	0.00 (1.00)	B	OMP	Attach	GGXXP motif ^d	8609998
98799	PRN	GGFGPVLGDW	286–295	NA	0.00 (1.00)	B	OMP	Attach	GGXXP motif ^d	8609998
98940	PRN	PQPGPQPQPQPQP	579–593	NA	0.00 (1.00)	B	OMP	Attach	PQP motif ^e	17597264
98687	PRN	AKAPPAPKPAPQPGP	569–583	NA	0.00 (1.00)	B	OMP	Attach	PQP motif ^e	17597264
98698	PRN	APKPAPQPGP	574–583	NA	0.00 (1.00)	B	OMP	Attach	PQP motif ^e	17597264
187658	FHA	GPIVVEAGELVSHAGG	1229–1244	NA	NA	B	HA	Attach	CRD ^b	8514379
22921	AC	GVATKGLGVHAKSSDWG	54–70	NA	0.00 (1.00)	B	A subunit	CD	Membrane insertion ^c	2224147, 11348687

OMP, outer membrane protein; CD, catalytic domain; RBD, receptor-binding domain; attach, attachment; CRD, carbohydrate recognition domain, HBD, heparin-binding domain; The number in parentheses is the standard deviation; discn, discontinuous; NA, not available

^aYYSN or YYSK, the K and N considered mitogenic sites.^s

^b constitutes a CRD critical for bacterial adherence.

^c crucial for membrane insertion and translocation of the catalytic domain of CyaA.

^d mediates interaction with epithelial cells.

^e immune evasion; highlighted residues = functional sites.

Table 8
Polymorphisms identified in *B. pertussis* strains and corresponding reported human epitopes.

Antigen variant	Sequence(s)	Epitope ID	Epitope sequence	Response details	PMID
Fim2-1	TSRTV	70651	VQTGGTSTRIVTMRYLAS (141–157)	Recognized by 12/12 adult WC patients in UK; 4/4 wP vaccinated children; + IgG and IgA response in adults	8731026
Fim2-2	K				
Fim3-1	PQALG	50304	QALGALK (64–70)	Recognized by 12/12 adult WC patients in UK; 3/4 wP vaccinated children; + IgG and IgA response in adults	8731026
Fim3-2	E				
Fim3-3	E				
Fim3-1	SATKA	60914	SSATK (105–109)	Recognized by 11/12 adult WC patients in UK; 4/4 wP vaccinated children; + IgG and IgA response in adults	8731026
Fim3-2	T				
Fim3-3	A				
PTxA1	VLDHL	9568	DNVLDHLTGR (30–39)	T cell clone RR215 proliferation WC; HLA-DR1	7693460
PTxA4	E	9571	DNVLDHLTGRSC (64–75)	Ex vivo T cell proliferation 5/9 WC; HLA-DQ	2460578
PTxA5	E	9573	DNVLDHLTGRSCQ (64–76)	T cell clone proliferation 3/3 subjects vaccination with genetically detoxified PT mutant PT9 K/129G; 1/1 ex vivo response WC patient; HLA-DR1	1313575
		21476	GNNDNVLDHLTGR (61–73)		2469760
PTxA1	EYSNA	20455	GITGETTTTEYSNARYV (185–201)	T cell clones S232, T215, T226 proliferation WC patient; HLA-DR1	2450153
PTxA5	P			Recognized by 5/5 JNH-7 (PT only) vaccinated infants and 5/5 infants vaccinated with wP; IgG	
PTxA1	LVRAPVIGAC	22756	GTLVRMAPVIG (223–233)	Ex vivo T cell proliferation 4/9 adults immunized with wP; Serum Ab of infant immunized with aP	2460578
PTxA2	M				
PTxA4	MV	65995	TRANPNPYTSRRSVASIVGTLVRM (205–228)	T cell clones S105, S201, T209 and T217 from WC proliferated; HLA-DR1	
PTxA5	MM				2469760
PTxA8	M				
Pm1-13	Repeat region	19684	GGAVPGBA VPGGAVPVGFGFGFGP (266–290)	2/37 subjects vaccinated or WC; IgG	17926203
	Variations:	98796	GGAVP (266–270)	Recognized by sera from children vaccinated (aP) or exposed	15213111
	GGAVP				
	GGFGP				
	GGGVP				
flaB	Q831	NA	Region aa390–874 (HBD)	Neutralizing scFv; reduces colonization in respir tract	17724067*

Antigen variant	Sequence(s)	Epitope ID	Epitope sequence	Response details	PMID
cya	V892	NA	Region aa888–1006	Part of region recognized by mAbs 2B12 and 4H2	10225859 *
prn	L532	NA	None reported	NA	21070624 *
ptxB	G44	NA	None reported	NA	21070624 *

Antigen variant data sourced from [12] [PMID: 23406868], [19] [PMID:9453625] and [17] [21070624]. The sequence identified as part of the polymorphism is shown in bold in the epitope. WC = subject who had whooping cough. PTx = pertussis toxin; fim = fimbrial; prn = pertactin. Epitope ID and PMID are searchable parameters on the IEDB search interface.

* not on IEDB webpage. Cya = adenylate cyclase; fhaB = filamentous hemagglutinin; ptx = pertussis toxin; prn = pertactin; fim = fimbrial.