MINIREVIEW

T-cell immune responses to Bordetella pertussis infection and vaccination

Giorgio Fedele1,*, Antonio Cassone1,2 and Clara Maria Ausiello1

1 Anti-Infectious Immunity Unit, Department of Infectious Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, 00161 Rome, Italy and 2 Center of functional genomics, Polo della genomica, genetica e biologia, University of Perugia, 06132 Perugia, Italy

*Corresponding author: Anti-Infectious Immunity Unit, Department of Infectious Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 299-00161 Rome, Italy. Tel: (+39)-06-4990-3354; Fax: (+39)-06-4990-3168; E-mail: giorgio.fedele@iss.it

One sentence summary: Pertussis is one of the hardest-to-control vaccine preventable disease. We here review current knowledge about the role of T-cell immunity in response to Bordetella pertussis infection and vaccination against pertussis.

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ABSTRACT

The recent immunological investigations, stemming from the studies performed in the nineties within the clinical trials of the acellular pertussis vaccines, have highlighted the important role played by T-cell immunity to pertussis in humans. These studies largely confirmed earlier investigations in the murine respiratory infection models that humoral immunity alone is not sufficient to confer protection against Bordetella pertussis infection and that T-cell immunity is required. Over the last years, knowledge of T-cell immune response to B. pertussis has expanded broadly, taking advantage of the general progress in the understanding of anti-bacterial immunity and of refinements in methods to approach immunological investigations. In particular, experimental models of B. pertussis infection highlighted the cooperative role played by T-helper (Th)1 and Th17 cells for protection. Furthermore, the new baboon experimental model suggested a plausible explanation for the differences observed in the strength and persistence of protective immunity induced by the acellular or whole-cell pertussis vaccines and natural infection in humans, contributing to explain the upsurge of recent pertussis outbreaks. Despite the progress, open questions remain, the answer to them will possibly provide better tools to fight one of the hardest-to-control vaccine preventable disease.

Keywords: pertussis; T-cell immune response; vaccines; infection

INTRODUCTION

Pertussis (whooping cough) remains a widespread, global disease despite the availability of safe and efficacious vaccines. Its causative agent, Bordetella pertussis, is a Gram-negative bacterium endowed with a rather remarkable apparatus of virulence traits allowing invasion and persistence in the respiratory tract, and then triggering systemic events that can ultimately be lethal. Some of the above traits confer to B. pertussis exquisite ability to avoid host eradicating immunity (Locht 1999; Mills 2001; Fedele, Bianco and Ausiello 2013). Initially considered as an exclusive extracellular pathogen, several studies have documented that B. pertussis can also thrive as an immune escape strategy, within cells of the mammalian host, including immune cells critical for mounting immune responses, such as monocytes and dendritic cells (DC) (Ewanowich et al. 1989; Hellwig et al. 1999; Bassinet et al. 2000; Weingart and Weiss 2000; Ishibashi, Relman and Nishikawa 2001; Schaeffer and Weiss 2001; Shumilla et al. 2004; Fedele et al. 2005).

Although the infection is cleared by the immunocompetent host, the immune response raised during the infection is not durable and does not protect against reinfection. Despite
considerable recent progress of our understanding of the mechanisms of immune responses to B. pertussis, no unequivocal immunological correlates of protection against pertussis have been identified. Serum antibody titers are important but not sufficient to represent a correlate of protection (Cherry et al. 1998; Storsaeter et al. 1998). Besides IgG, mucosal IgA antibodies were shown to play a role in anti-B. pertussis mechanisms (Hellwig et al. 2001). In addition, serum levels of virulence-neutralizing antibodies wane rapidly after infection or vaccination as inferred from studies by ourselves and others, some of which performed during clinical trials of acellular pertussis (aP) vaccines or soon after their approval (Cassone et al. 1997; Edwards and Berbers 2014; Plotkin 2014). Coupled with B. pertussis intracellular location, all this suggests that T-cell responses play a role in protection or in disease containment to the respiratory tract.

We here review current knowledge about the role of T-cell immunity in response to B. pertussis infection and vaccination against pertussis, as shown by studies in laboratory animals, human ex vivo cellular models and infected or vaccinated subjects.

T-cell immune responses in the murine respiratory infection model

The respiratory challenge of mice by intranasal or aerosol administration with live bacteria is a widely used model for studying immunity to B. pertussis (Sato et al. 1980). This model presents some caveats, since cough, which is the prototypical symptom of pertussis, is absent and lung pathologic findings are different from those seen in humans. However, the course of infection and many of the pertussis pathognomonic, systemic effects, such as leucocytosis and hypoglycemia, are similar to those observed in infants (Higgs et al. 2012). In addition, the immune mechanisms induced in infected mice overlap those seen in the recently developed baboon model (see below) and in infected humans. As for other pathogens, the availability of inbred wild-type, knockout and transgenic mouse strains, as well as mouse-specific reagents, has made mice attractive and commonly used model organisms.

In this model, following the infectious challenge, B. pertussis cells adhere to the upper respiratory tract of mice and start to multiply. Bacterial burdens in the lungs reach a peak at 7–10 days and then start to decrease and the infection is cleared 5–10 weeks after challenge (Mills et al. 1993; Mahon et al. 1997; Carbonetti et al. 2003; Krimanjeswara, Mann and Harvill 2003; Mann et al. 2005; Andreassen and Carbonetto 2009; Andreassen, Powell and Carbonetti 2009; Ross et al. 2013; Raeven et al. 2014). Time-course analysis of ongoing immunity showed that host response starts right after infection with an acute phase response and infiltration of immune cells in the lungs (Carbonetti et al. 2003; Raeven et al. 2014). At this stage, innate immune response, phagocytosis, antigen processing and antigen presentation are initiated. Immune effectors recruited to the infectious site include macrophages, DC, neutrophils, natural killer cells and γδ T cells, which infiltrate the lungs up to day 7. Instructed by the innate response, the induction and activation of the adaptive immune response occurs from day 10 until day 28 post-infection, marked by infiltration of CD4+ and CD8+ T cells, humoral immune response and antibody-mediated immunity (Mills 2001; Raeven et al. 2014).

The prominent and direct role of T cells in protection of mice against B. pertussis infection was first demonstrated by Mills et al. (1993), who showed that mice able to clear the infection display consistent antigen-specific T-cell responses in the absence of a detectable serum antibody response. More important, it was shown that the adoptive transfer of CD4+ T cells in nude mice unable to clear the infection, resulted in bacterial clearance while injection of serum from protected animals only marginally reduced bacterial loads. T cells induced by infection were predominantly T-helper (Th1) cells that secreted interleukin (IL)-2 and interferon (IFN)-γ (Mills et al. 1993).

The importance of T-cell immunity in clearing the primary infection with B. pertussis was further strengthened by experiments with mutant mice lacking T or B cells, and showing that protective immunity was dependent on CD4+ T cells and B cells (Barbic et al. 1997; Leef et al. 2000). CD4+ T cells provided significant functions other than helping for specific antibody production as protection was demonstrated in the absence of specific IgG, suggesting a direct, non-antibody-mediated role for CD4+ T cells in anti-B. pertussis immunity (Leef et al. 2000).

Further insight into the mechanisms of T-cell protection came from studies using mice defective in individual cytokines and receptors, which confirmed the crucial role for Th1 cells. Indeed, primary infection with B. pertussis was not significantly different in IL-4 KO (IL-4−/−) and wild-type mice, suggesting that IL-4 is not essential for protection. In contrast, infection in IFN-γ−/− and IFN-γ receptor (IFN-γR)−/− mice demonstrated that this cytokine plays a key role both in the clearance of a primary infection and in protection against subsequent challenge (Barbic et al. 1997; Mahon et al. 1997).

IL-17-producing Th17 cells, although initially identified as crucial players in autoimmunity and chronic inflammation (Weaver et al. 2007), were subsequently linked to the control of several extracellular bacterial and fungal infections (Ye et al. 2001; Chung et al. 2003; Huang et al. 2004; Khader et al. 2007; Wu et al. 2007). The dual nature of B. pertussis as either intracellular and extracellular pathogen, as well as findings showing production of the Th17-promoting cytokine IL-23 by infected human DC (Fedele et al. 2005), suggested a role for Th17 cells in anti-pertussis immunity. A clear-cut indication for the involvement of Th17 cells in protection from B. pertussis came from the observation that either murine macrophages infected with the closely related pathogen B. bronchiseptica and bone-marrow-derived DC stimulated with whole-cell pertussis (wP) vaccine drove the immune response toward a Th17 phenotype (Higgins et al. 2006; Siciliano, Skinner and Yuk 2006). In the latter study, it was also shown that immunization of mice with wP vaccine induced Th17 cells in vivo (Higgins et al. 2006). Thereafter, several studies confirmed the induction of antigen-specific Th17 cells upon B. pertussis infection in mice as early as 7 days after the infection (Feunou, Bertout and Locht 2010; Ross et al. 2013; Scanlon et al. 2014). The role of Th17 cells in protection was definitely demonstrated by using IL-17A−/− mice which were unable to clear the infection up to 6 weeks after respiratory challenge (Ross et al. 2013). In summary, almost 20 years of studies with the murine respiratory challenge model support the notion of a key role exerted by Th1/Th17 cells in protection from pertussis, while curtailing the contribution of Th2 effectors. Interestingly, CD8+ CTLs, which were disregarded as result of initial studies (Leef et al. 2000), were more recently added to the scenario, as indicated by evidence of CTL infiltration in lungs and formation of filamentous hemagglutinin (FHA)-specific IFN-γ-producing CTLs upon B. pertussis infection (Raeven et al. 2014).

The induction of regulatory T (Treg) cells to counteract potentially eradicating T-cell immunity is a common strategy adopted by bacteria to enhance their own survival in hostile environment (Russell 2015). At the same time, Treg cells can take part into homeostatic processes to prevent a tissue-damaging, Th1/Th17-driven, inflammatory host response. IL-10-secreting T cells and
Foxp3+ Treg cells were found in the lungs during infection of mice with B. pertussis (Coleman et al. 2011). Interestingly, protective immunity in the lung was not affected in IL-10−/− mice nor by CD25− cells depletion in wild-type mice, but it was enhanced in IL-10−/− mice depleted of CD25+ cells, suggesting that both CD25+ Treg cells and IL-10-producing cells induced by B. pertussis infection might play complementary roles in promoting bacterial survival in the lungs.

**Murine respiratory challenge model to study vaccine-induced T-cell immunity**

The mouse respiratory infection model has proven a useful tool for the evaluation of vaccine-induced immunity, since it has been demonstrated that bacterial clearance from lungs following exposure to B. pertussis correlates with vaccine efficacy (Mills et al. 1998; Guiso et al. 1999). Moreover, the model has provided crucial information concerning the type of T-cell immune response induced by different vaccines. Immunization with WP vaccines was found to be associated with the induction of strong Th1 responses, similarly to natural infection, whereas aP vaccines preferentially induces Th2 cells (Mills 2001). Indeed, mice immunized with an aP vaccine had high levels of B. pertussis-specific antibodies and spleen cells secreting IL-5 but not IFN-γ. On the contrary, immunization with a WP vaccine induced CD4+ Th1 cells and serum antibody responses.

Results of murine studies reflect distinct patterns of cellular and humoral immune responses induced by aP and WP, and are largely consistent with the Th1- and Th2-dominated responses observed in human recipients of WP or aP vaccines (see below). Remarkably, the bias towards Th1 or Th2 appears to affect the quality and persistence of the protective immune response. Complete clearance of bacteria usually does not occur before 14 days or more in mice immunized with aP, whereas mice primed by previous infection or by immunization with WP clear the bacteria within 5–7 days from the challenge (Redhead et al. 1993).

This aspect was confirmed when the protective efficacy of WP and aP vaccination was assessed in 2- to 3-week-old mice, chosen as a model resembling infant vaccination in humans. A more rapid bacterial clearance from lungs (day 8) occurred in infant mice immunized with a WP rather than an aP vaccine (Roduit et al. 2002).

Vaccine-induced responses wane with time, in particular antibody responses rapidly decline after immunization, while T-cell responses persist longer (Mahon, Brady and Mills 2000). Experimental studies have demonstrated that protection induced by WP or aP is maintained after waning of circulating antibodies, through the generation of immunological memory. Moreover, it was shown that the Th1/Th2 dichotomy does not impinge on memory induction (Mahon, Brady and Mills 2000).

After discovery of the key role of Th17 cells in immunity to B. pertussis, the murine respiratory infection model has been used to analyze the contribution of Th17 cells in response to pertussis vaccination. In a recent paper, it has been shown that WP vaccines induce a Th1/Th17 response and that IFN-γ largely contributes to WP-mediated protection, with a smaller contribute of Th17 cells. In contrast, aP immunization induces a Th2/Th17 response. In this case, however, IL-17A played an essential role, while IL-4 was unnecessary for bacterial clearance (Ross et al. 2013).

**The baboon model of pertussis infection**

Recently, baboons showed to be a very reliable animal model to study B. pertussis infection and vaccine-induced protection. In baboons, the disease is very similar to the human illness. When animals are inoculated with B. pertussis, very high numbers of bacteria are recovered from the nasopharyngeal washes from day 2 to day 25 after challenge, and infected animals have leukocytosis. Contrary to mice, baboons develop severe cough that persist for more than 2 weeks. As in humans, there is a correlation between the age of the infected animal and the severity of disease. When young infant baboons were challenged, severe disease was observed, full-grown adult baboons challenged with the same dose exhibited very mild signs of disease or were asymptomatic (Merkel and Halperin 2014). Baboon studies confirmed the important role of T-cell immunity. A major finding of the baboon model is the achievement of a near-sterilizing immunity. Rechallenged baboons did not display boosting of anti-pertussis toxin (PT) serum antibodies until day 5, suggesting that T-cell memory plays a substantial role in the sterilizing immunity (Warfel et al. 2012). Further studies showed mucosal expression of IL-17 and IL-17-related IL-6, IL-23 and IL-1β in infected animals. The induction of IFN-γ and IL-17-secreting cells in convalescent animals 2 years after infection was consistent with the induction of adaptive Th1 and Th17 responses. Remarkably, no Th2-associated cytokines such as IL-4, IL-5 or IL-13 were detected (Warfel and Merkel 2013).

In the baboon model, vaccination with WP induced a more rapid clearance than aP vaccination even though only previously infected animals fully controlled the challenge. All vaccinated or previously infected animals had robust serum antibody responses. A key difference in T-cell immunity was found: WP-vaccinated animals, similarly to infected baboons, had strong B. pertussis-specific Th17 and Th1 cells, whereas aP vaccination induced a Th1/Th2 response, in line with murine studies. Of interest, aP-vaccinated baboons were protected from the disease but not from colonization. Transmission of B. pertussis to unvaccinated contacts occurred in those animals which were unable to clear the infection rapidly. WP vaccination on the contrary induced a rapid clearance of the lungs. It has been hypothesized that the failure of aP vaccination of preventing airways colonization, then transmission, could represent a plausible explanation for the resurgence of pertussis in vaccine high-coverage countries (Warfel, Zimmerman and Merkel 2014).

**Human ex vivo cellular models**

DC play a key role in the orchestration of immune responses, from pathogen recognition to Th cells polarization. In the last decades, an ex vivo cellular model based on the differentiation of DC from freshly isolated monocytes has been established. Such monocyte-derived (MD)DC constitute a powerful experimental tool for the evaluation and analysis of the human immune response (León, López-Bravo and Ardavin 2005). Contrary to animal models of respiratory infection, the MDDC model is host specific, since in nature B. pertussis is an exclusive human pathogen, and has flanked the murine and the non-human pri mate models providing key information on T-cell response induced by B. pertussis and the mechanisms involved, hence supporting and complementing the data obtained from the animal models (Fedele, Bianco and Ausiello 2013).

Although B. pertussis has a low capability to be internalized by and to survive in MDDC, bacterial contact induces immature MDDC to undergo phenotypic maturation and acquire antigen-presenting-cell functions (Fedele et al. 2005). Several studies have shown that bacteria trigger TLR2 and TLR4 signaling mediated by surface receptors including PT and the lipooligosacharide (Wang et al. 2006; Fedele et al. 2008, 2010;...
Nasso et al. 2009). Despite the production of high levels of IL-10 and barely detectable levels of IL-12p70, B. pertussis-experienced MDDC induce Th1-polarized effector cells (Fedele et al. 2005; Spensieri et al. 2006).

These apparently contradictory findings were confirmed later in the baboon model where despite Th1 induction, IL-12p70 was neither detected in vivo after infection nor in PBMC stimulated ex vivo with heat-killed B. pertussis (Warfel, Zimmerman and Merkel 2014). The induction of Th1 effectors in the absence of IL-12p70 was shown to occur through an ERK1/2 dependent mechanism; adenylate cyclase toxin activity and activation of p38 MAPK were essential for MDDC-driven Th17 expansion (Fedele et al. 2010).

Further studies with the MDDC model showed that B. pertussis-treated MDDC induce not only Th1 effectors but also Th17 cells, mixed with Th1 and simultaneously producing IFN-γ and IL-17 (Fedele et al. 2010).

By using the MDDC model, Dirix et al. pointed out important properties of FHA. These authors reported that FHA induces the upregulation of maturation and costimulatory surface molecules, as well as the secretion of IL-10, IL-12p40, IL-12p70, IL-23 and IL-6. The production of IL-10 by MDDC disappeared when stimulating cells with an 80-kDa N-terminal moiety of FHA, shown to induce strong protection against a B. pertussis challenge in mice, while the induction of other cytokines was maintained. The authors conclude that the immune-modulatory functions of FHA can be physically separated from its antigenic properties, by using a truncated molecule (Dirix et al. 2014).

Recently, the MDDC model was used to analyze the effect of BPZE1, a live-attenuated B. pertussis vaccine candidate that has successfully passed a phase 1 vaccine trial (Locht and Mielcarek 2014). BPZE1, similar to wild-type B. pertussis, is able to induce human MDDC to secrete a broad spectrum of proinflammatory and regulatory cytokines. However, at variance with its parental wild-type counterpart BPSM, BPZE1-pulsed MDDCs very efficiently migrate in vitro in response to the lymphatic chemokine CCL21, due to the inactivation of PT enzymatic activity. BPZE1-pulsed MDDC drove a mixed Th1/Th17 polarization. Interestingly, both BPZE1 and BPSM also induced Treg cells acting through cell-to-cell contact rather than production of soluble factors (Fedele et al. 2011). Follow-up studies have shown that BPZE1-treated MDDC drive the expansion of unconventional Treg cells which convert extracellular nucleotides ATP and NAD+ into immunosuppressive adenosine, mediated by the activity of two specific ectoenzyme networks which are upregulated in naïve T cells after coculture with B. pertussis-committed MDDC (Fedele et al. 2015).

T-cell immune response in humans

Seminal studies on the relevance of T-cell response for pertussis protection in humans were performed by De Magistris and Rappuoli during the introduction of a new aP vaccine based on a genetically detoxified (gd) PT. Their studies used in vitro generated specific human clones to identify the immunogenic moiety of aP vaccines components such as gdPT, pertactin (FRN) or FHA, determining the epitope map recognized by human T-cell clones (De Magistris et al. 1988, 1989). Phase I clinical trial of aP vaccines demonstrated that all vaccines acquired cellular immunity against the three antigens (Podda et al. 1991).

Pertussis-specific T-cell responses in infected humans

Studies with B. pertussis-infected human subjects have shown the induction of T-cell-specific proliferation to B. pertussis whole cells and to PT, PRN and FHA antigens. Furthermore, the induction of a Th1-type responses after infection with B. pertussis was demonstrated from studies performed by several groups (Ausiello et al. 1998, 2000; He et al. 1998; Mascart et al. 2003; Dirix et al. 2009; Smits et al. 2013). Protection conferred by Th1-polarized responses was demonstrated in studies performed during the 1990s efficacy trials of aP vaccines (Zepp et al. 1996; Ryan et al. 1997; He et al. 1998; Ausiello et al. 2000) or soon after (Mascart et al. 2003).

The involvement of Th17 in protection, initially derived from animal studies (Ross et al. 2013) and human preclinical models (Fedele, Bianco and Ausiello 2013) and then reinforced by experimental evidence in the baboons model (Warfel, Zimmerman and Merkel 2014), needs more specific studies in humans. Few, non-conclusive data are available, in vaccinated and B. pertussis exposed subjects (Schure et al. 2012a,b).

Studies requiring further confirmation indicated that CD4+ T cells responding at the single epitope level to B. pertussis may comprise Th1 and Th2 components, suggesting that responding cells are not fixed to one lineage (Han et al. 2013).

The same research group found that pertussis epitopes identified in B. pertussis exposed individuals in the context of HLA-DR molecules derived from two envelope proteins and from two cytotoxic proteins. These epitopes were associated with secretion of functional Th1 (TNF-α and IFN-γ) and Th2 (IL-5 and IL-13) cytokines in the absence of IL-10 and IL-17. Surprisingly, no epitopes were detectable from known virulence factors, components of the current aP vaccines, such as PT, PRN or FHA (Stenger et al. 2014).

The acellular versus whole-cell vaccines

T-cell immune responses to pertussis vaccine antigens were assessed during the safety and efficacy trials of aP vaccines conducted in Sweden and Italy in the 1990s (Greco et al. 1996; Gustafsson et al. 1996). These investigations documented for the first time the generation of a robust T-cell immunity in infants and very young children recipient of the pertussis vaccines. In addition, it was shown that T-cell immunity persisted far after the decline of antibodies to vaccine constituents and could be boosted by natural infection (Ausiello et al. 1997, 1999; Cassone et al. 1997; Meyer et al. 2007; Ryan et al. 1997).

The results of T-cell studies in humans were in substantial agreement with those obtained in mouse models. Protection conferred by wP vaccine was much closer to that which follows natural infection. Both infection and vaccinations with wP vaccine were able to induce a Th1-polarized responses whereas the protection conferred by aP vaccines was characterized by a predominant Th2 response, but could change from a mixed Th2/Th1 to a robust Th1 profile following a natural booster (Zepp et al. 1996; Ausiello et al. 1997, 1999; He et al. 1998; Ryan et al. 1998; Edwards and Berbers 2014). The data supported the notion that protection from pertussis is not lifelong and that a high flexibility of T CD4 cells in shifting Th cytokines profile exists (Han et al. 2013).

Due to waning immunity conferred by the aP vaccine, an issue deserves great attention is when to administer the aP booster to assure an efficient protection. Several studies have pointed out the importance of booster immunizations in enhancing T-cell responses to pertussis antigens. Tran Minh et al. (1999) and Edelman et al. (2004) in a follow-up study evaluated pertussis-specific T-cell responses in adolescents. At 1 month and 3 years after the boost, the response levels were higher than the pre-booster immunization levels. Rieber et al. (Rieber et al. 2008) measured, in adolescents, humoral and T-cell...
pertussis-specific immune response with different pre-vaccination schedules after a five-component Tdap booster vaccination. T-cell parameters to PT, FHA, PRN and fimbriae increased after booster immunization.

More recently, a more complicated picture emerged. We demonstrated that 5 years after primary vaccination with two aP vaccines a positive response, evaluated in terms of proliferation and IFN-γ positive CD4+ T cells, was present in 36.8% of vaccines. PT-specific proliferation was higher in children tested before than after the preschool booster dose (Palazzo et al. submitted). Similar results were obtained when responses to the FHA antigen were analyzed (Palazzo et al. submitted). Vermeulen et al. (2013) reported that the aP booster administered to preterm infants between 13 and 16 months had no major effect on antigen-induced cytokine production but it allowed maintaining significant immune responses in the same infants tested before and after the booster dose. Schure et al. (2012b) showed that in children primed with aP vaccine an increase in cytokine production was missed after boost vaccination, in contrast to wP-vaccinated children. The same research group (Schure et al. 2012a) reported that in children at 9 years of age, T-cell responses did not increase after a second aP vaccine booster boost. These authors suggested that the enhancement of T-cell immunity during the 5 year following the booster at 4 years of age is probably caused by natural boosting, due to the high circulation of B. pertussis (Schure et al. 2012b). These conclusions resemble those obtained by our group in an earlier study in which it was shown that vaccination-induced T-cell response could wane by 4 years of age and can be naturally boosted by symptomless B. pertussis infection (Ausiello et al. 1999). This might explain, at least in part, the persistence of protection against pertussis in aP vaccine recipients despite a substantial waning of both Ab and T responses induced by the primary immunization.

It is possible that the different results reported above are due to differences in study design. Earlier studies were planned as to sample the same subject before and after the booster at an optimal time to detect a B- and T-cell recall response (Tran Minh et al. 1999; Edelman et al. 2004; Rieber et al. 2008). In more recent studies, subjects in the non-boosted and the boosted groups were different (Schure et al. 2012a,b; Palazzo et al. submitted) and in one case the time after the booster was extremely variable (Palazzo et al. submitted).

It is nevertheless probable that the duration of immunity of aP vaccines introduced in the mid-1990s of the last century was overestimated due, in part, to an asymptomatic natural booster in countries with high B. pertussis circulation. Nowadays, B. pertussis circulation due to herd immunity in high-income countries with high vaccine coverage is reduced and can no longer boost T-cell response. This could in part explain pertussis resurgence (see below).

T MEMORY CELLS INDUCTION AFTER wP OR aP VACCINATION

In the search of new parameters to assess the level and duration of protection after vaccination or infection, pertussis-specific T memory cell populations were assessed in humans. A classification is largely agreed upon to identify T memory subsets based on the expression of cell surface markers. CCR7+CD45RA− T cells are T central memory (cm) which have the capacity to proliferate. CCR7−CD45RA− are T effector memory (em) that differentiate, in response to antigenic stimulation, to CCR7−CD45RA+ T effector (e), which are the most differentiated T-cells. CCR7+CD45RA+ T are naive or stem cell memory (n/scm) cells, this last one including a recently described memory subset characterized for self-renewal, multipotent ability and increased proliferative capacities (Appay et al. 2008; Gattinoni et al. 2011; Wyndham-Thomasset et al. 2014).

Data by Sharma and Pichichero (2012), Smits et al. (2013) and Palazzo et al. (submitted) showed that pertussis-specific T-cell responses in infants after aP primary vaccination were mainly restricted to Tcm and Tem subsets. PT antigen stimulation expanded Tem and reduced the levels of Tn/Tscm in the CD4+ subset.

The induction of CD8+ T-cell response during B. pertussis infection was initially reported by Mascart et al. (2003) and it was analyzed in more detail by Dirix V et al. (2012). In CD8+ cells, an expansion of Te was observed leading to assume that pertussis-specific CD8+ T memory cells contribute to protection against pertussis (Rieber et al. 2011; Dirix et al. 2012; de Rond et al. 2015).

However, a vaccine boost had no specific effect on the frequency of memory subsets expansion (Schure et al. 2012a; Smits et al. 2013; Palazzo et al. submitted). Hence, a correlation between the percentage of the different T memory subsets and duration of protection from pertussis appears to be still elusive.

Recently, it has been shown that distinct end-differentiation stages of pertussis-epitope-specific CD4+ T cells correlate with particular cytokine secretion after clinical infection. PBMC from pertussis cases with relatively more Tcm secreted Th1 cytokines while subjects with relatively more Tem secreted Th2 cytokines in addition to Th1 ones (Han et al. 2015).

DURATION OF VACCINE PROTECTION AND PERTUSSIS RESURGENCE

Several potential factors, possibly cooperatively acting, have been identified to explain pertussis resurgence in countries with high vaccination coverage. These include genetic changes in circulating B. pertussis, increased recognition and reporting of pertussis by the application of new, more sensitive, laboratory diagnostic tests (Cherry 2012a). Recent epidemiological studies suggest that the resurgence of pertussis is due to a more rapid waning of aP vaccine than of wP vaccine-induced immunity (Klein et al. 2012; Koepke et al. 2014).

A key aspect to take into account when studying immunity in human populations is the estimation of the length of protection that follows from healing infection or vaccination. This is often problematic because reliable and representative data are of difficult achievement. Several studies tried to define the duration of protection induced by pertussis infection, estimated to fall in such a wide range as 2–30 years (Wendelboe et al. 2005; Wearing and Rohani 2009; Hallander, Nilsson and Gustafsson 2011; Acosta et al. 2015). This substantial uncertainty is due to a combination of differences in study methodology and pertussis epidemiology in different countries. Another confounding factor in the determination of the duration of immunity after pertussis infection is the asymptomatic natural booster. This phenomenon could be particularly relevant in countries with high circulation of B. pertussis and low vaccine coverage, while being less important in countries where the vaccine coverage is higher and, probably, B. pertussis circulation is lower. Additional important point that renders more difficult to quantify the exact duration of immunity in pertussis is the lack of definition of the correlates of protection.

Teenagers who received DTaP vaccines in childhood were more protected during a pertussis outbreak than were those who
received DTaP vaccines (Klein et al. 2013). The study by Smits et al. (2013) in a preadolescent cohort supports this interpretation, indicating that infant vaccination with wP induces longer lasting T-cell immunity than vaccination with aP vaccines. Indeed, even if the time from the last booster vaccine was significantly longer in wP compared to aP-vaccinated children, the T-cell proliferative capacity in response to antigenic stimulation was comparable, and more children had a detectable cytokine response after wP compared to aP vaccination. These data were not universally confirmed. As reported by Schure et al. (2012b, 2013), the induction of T-cell responses measured by pertussis antigen-specific cytokine production improved after shifting from wP to aP priming. The discrepancy of these results may rely on several differences in the study design including the use of probably non-optimal Dutch wP vaccine used in the study by Shure et al. This despite, there is now a rather large consensus for a more rapid waning of protective immunity in aP than in wP vaccine recipients (Plotkins 2013; Edwards and Berbers 2014; Acosta et al. 2015).

CONCLUSIONS

The immunogenicity studies performed during the clinical trials of aP vaccines have shed light on the important role played by T-cell immunity to pertussis (Zepp et al. 1996; Ausiello et al. 1997, 1999, 2000; Cassone et al. 1997; Ryan et al. 1997, 1998; He et al. 1998). The establishment of a reliable murine system, and more recently of a primate model, has been instrumental in the definition of part of the mechanisms through which T cells are activated in the context of B. pertussis infection and on the nature of T-cell responses. Mechanistic studies performed ex vivo in human cellular model gave crucial information on the ability of B. pertussis and its virulence factors to modulate host’s T-cell responses (Fedele, Bianco and Ausiello 2013).

Despite some understanding of the role played by T cells in protection against pertussis, several questions remain open. The induction of Treg cells was early described as an escape mechanism whereby the bacterium avoids immune recognition (Coleman et al. 2011); however, the notion that an infectious pathogen is capable of generating immune suppression may be considered as an homeostatic mechanism preventing a response that may be detrimental. This latter possibility has gained importance in the light of human studies showing the ability of wild-type and live-attenuated B. pertussis to induce T cells with regulatory functions (Fedele et al. 2011, 2015). This also applies to the reported role of Th17 cells at the respiratory level, which requires adequate immune control (Way, Chen and Kolls 2013).

Differences in the type of T-cell immunity induced by aP vaccines as compared to natural infection/wP vaccination have been suggested to be involved in the rapid decline of aP vaccine-mediated protection, both in terms of humoral and T-cell immune response, and pertussis resurgence. However, we cannot assume that there is any link between the Th2 response and the protection induced by aP vaccine (Ross et al. 2013). Mascart et al. (2007) reported that the aP vaccines not only induce predominant Th2 response to pertussis antigens but also induce a general Th2 shift of the cellular immune responses.

Furthermore, there is little knowledge of the immunological mechanisms which are specifically stimulated by asymptomatic natural exposure to low doses of B. pertussis. After all, the exact nature of the correlates of protection from pertussis remains still elusive. Studies on vaccine-induced T-cell responses have suggested a key role of natural booster infection for persistence of protection, despite declining of humoral response (Ausiello et al. 1997; Schure et al. 2012a); nowadays, it is possible to hypothesize that reduced circulation of bacteria led to reduced natural booster effect in highly vaccinated populations.

Novel vaccines against pertussis are advocated by some, but whether we really need to replace the current vaccines or simply reshape the use of the current ones is debated (Cherry 2012b; Ausiello and Cassone 2014). In-depth knowledge of T-cell immunity to B. pertussis may clearly help answer this important question. Although there is a consensus that new generation vaccines should be able to skew the immune response towards a mixed Th1/Th17 profile, the way to reach this goal is not clear, as is unclear how to avoid inflammation-associated tissue damage in case of a particularly strong Th1/Th17 bias.

Here, further studies on the regulation of T-cell response to B. pertussis are needed. Several suggestions have been made, mostly focused on the use of novel adjuvants, such as TLR ligands inducing DC activation. Another possibility is to adopt a live-attenuated mucosal vaccine as a first primary vaccine dose or as adult booster. Recently, BPZE1 has successfully passed a Phase1 trial. Pre-clinical studies have shown its ability to induce a Th1/Th17 response both in vivo and ex vivo in the human MDDC model, equally important is the induction of regulatory T cells (Feunou, Bertout and Locht 2010; Li et al. 2010; Fedele et al. 2011), which appears mandatory in order to elude immune-mediated tissue damage which is associated with Th17 responses in the lung.

While waiting for new vaccines and/or adjuvants able to lengthen the duration of protection against the disease and effectively fight the transmission of the disease, more efficacious vaccination strategies are required. Vaccination during pregnancy is currently adopted in various countries to protect the most vulnerable to B. pertussis infection, i.e. the neonates and infants. Improving the knowledge of vaccine-induced anti-B. pertussis immunity in pregnant women appears to be a due corollary of this strategy in view of the distinct regulatory aspects of T-cell immunity during pregnancy.

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