Effectuer un rappel post-expositionnel restimule la mémoire immunitaire induite par la vaccination et accélère l'élimination de *Bordetella pertussis* dans les poumons et la trachée : Evidence préclinique

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Thèse préparée sous la direction de la Professeure honoraire Claire-Anne Siegrist

" Effectuer un rappel post-expositionnel restimule la mémoire immunitaire induite par la vaccination et accélère l'élimination de Bordetella pertussis dans les poumons et la trachée: Evidence préclinique"

Thèse
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par

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Introduction de thèse

La coqueluche est une maladie respiratoire hautement contagieuse causée par la bactérie *Bordetella pertussis* (Bp). Bp est un coccobacille aérobique pléomorphe. Elle est très contagieuse et se propage par le biais de gouttelettes respiratoires lors de la toux, ou des contacts étroits avec une personne infectée. La coqueluche peut affecter des personnes de tous les âges, mais elle est particulièrement grave chez les nourrissons de mères non vaccinés et les jeunes enfants. Les symptômes typiques de la coqueluche comprennent des accès de toux violents suivis d'une respiration sifflante ou de bruits de coq étouffés (d'où son nom "coqueluche"), une toux persistante pouvant durer plusieurs semaines (« la toux des 100 jours »), des difficultés respiratoires, des vomissements après les accès de toux et une fatigue générale. Elle est caractérisée par trois stades : phase catarrhale, phase paroxystique et phase de convalescence. Une à trois semaines après la contagion, des symptômes d'allure grippale apparaissent, 1 à 2 semaines après la toux se transforme et les quintes de toux caractéristiques apparaissent et peuvent persister jusqu'à 6 semaines, ensuite survient la phase de convalescence pendant laquelle les symptômes diminuent progressivement. Les nourrissons sont les plus à risque de développer des complications graves et en particulier des apnées centrales, pouvant aller jusqu'à l'arrêt respiratoire et au décès. Le risque de décès est estimé à 1% chez les nourrissons, il diminue à moins de 0.5% chez les enfants de 2 à 11 mois. La coqueluche peut aussi se compliquer par une pneumonie (6% en général, mais 25% chez les nourrissons), une otite moyenne aigue, des convulsions (1% chez les nourrissons) et des lésions cérébrales (5 enfants sur 1'000) qui peuvent laisser des séquelles à vie. Jusqu'à 70% des enfants atteints de coqueluche doivent être hospitalisés. En Suisse, en 2016, 46 enfants ont été hospitalisés pour une coqueluche confirmée, contre 25 en 2015 et 33 en 2014. La prévalence de

la coqueluche est très probablement sous-estimée aux vues de sa présentation aspécifique. (1–3)

En général, Le diagnostic est posé grâce à l'identification de Bp dans les sécrétions nasopharyngées par PCR. La problématique avec le traitement antibiotique vient du fait que le diagnostic est difficile à faire dans la phase catarrhale et que dans la phase paroxystique il n'a quasiment aucun effet sur le déroulement de la maladie. Malheureusement, le traitement antibiotique ne protège pas contre les complications de la coqueluche, cependant il permet de diminuer la contagiosité et donc la transmission de la maladie, et c'est pour cette raison qu'il est indiqué. (4)

Depuis 1996, les vaccins acellulaires contre la coqueluche (aP) ; composés de quelques protéines de surface de la bactérie (2 ou 3) y compris la toxine pertussis (PT) formulés avec du sel d'aluminium ; ont été progressivement introduits dans la plupart des pays développés pour remplacer le vaccin à cellules entières (wP) très efficace mais plus réactogène. (5–13) Cette tendance se poursuit avec un nombre croissant de pays émergents qui adoptent les vaccins aP. Malheureusement, ce changement a entraîné une diminution de l'efficacité du vaccin, car l'immunité induite par les vaccins aP s'atténue rapidement et nécessite donc des rappels fréquents pour maintenir la protection. (14–19) Malgré une couverture vaccinale d'environ 85% dans le monde, Bp est encore responsable d'une morbidité et une mortalité significatives à l'échelle mondiale et sa prévalence est en augmentation dans les pays ou les vaccins aP sont préférés. (20–23) La Figure A illustre le nombre de cas de coqueluche rapportés par Centers for Disease Control and Prevention.

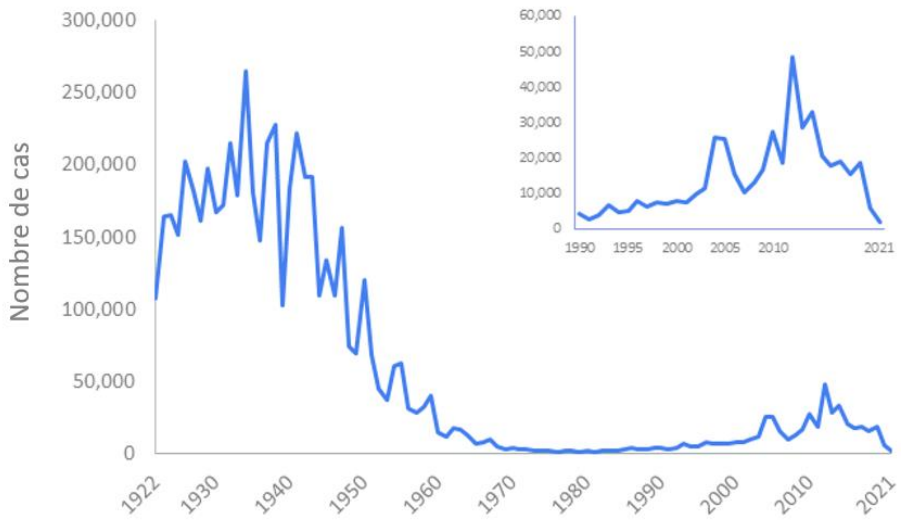

Figure A. Cas de coqueluche rapportés par Centers for Disease Control and Prevention (CDC, Etats-Unis) entre 1922 et 2021

Ni l'infection naturelle, ni la vaccination ne protègent à long terme contre la coqueluche. 9 personnes sur 10 sont protégées des formes graves et 1 personne sur 7 de toutes les forme grâce à la vaccination. La vaccination des femmes enceintes pendant la grossesse protège plus de 90% des nourrissons de toutes les formes de coqueluche confondues. (24) Ainsi, la plupart des pays industrialisés ont recommandé la mise en place de doses de rappel aP au-delà de la petite enfance, mais leur impact est insuffisant et des mesures supplémentaires sont nécessaires, notamment lors des épidémies cycliques de coqueluche. Une observation déconcertante est que la coqueluche peut survenir chez des individus entièrement vaccinés, malgré une période d'incubation longue (de 1 à 3 semaines) qui devrait être suffisante pour restimuler les cellules de mémoire et interrompre la prolifération bactérienne. (25) De plus, les vaccins aP ont été conçus pour limiter la coqueluche sévère chez les nourrissons et ne préviennent pas la colonisation, ne permettant ainsi pas de freiner la transmission/le portage.

Par conséquent, les individus vaccinés participent aux chaînes de contamination, ce qui empêche le contrôle de la maladie. (26)

L'étude des réponses immunitaires suite à une infection à Bp a été le mieux réalisée chez les primates non humains. (27–29) Cependant, les anticorps persistent longtemps après la vaccination chez les primates non humains - comme chez les souris - contrairement aux humains, ce qui empêche de réaliser une analyse approfondie de la réactivation de la mémoire immunitaire en l'absence de persistance des anticorps. (29,30) Pour pallier ce problème, nous avons précédemment développé un modèle murin de transfert adoptif, permettant l'élimination des anticorps circulants et reproduisant ainsi mieux la situation humaine. (30) La Figure B illustre le modèle murin. Un premier groupe de souris sont vaccinées, puis 42 jours après, leurs rates sont extraites afin de récupérer les splénocytes contenant les cellules B mémoires et de les transférer dans des souris receveuses. Les souris receveuses sont ensuite infectées par la coqueluche et la réponse immunitaire ainsi que la charge bactérienne est analysée. (Figure BA) Ainsi, les anticorps circulants spécifiques présents en quantité significative dans les souris donneuses (Figure BB), seront éliminées. Le nombre de splénocytes à transférer suffisant pour que les cellules B mémoires soient réactivées de manière satisfaisante a été optimisé. En effet, le transfert de $25x10^6$ splénocytes de souris vaccinées contre la coqueluche permet de fournir une quantité de cellules B mémoires suffisante et d'obtenir des titres d'anticorps spécifiques à PT significatifs 14 jours après l'infection. (Figure BC)

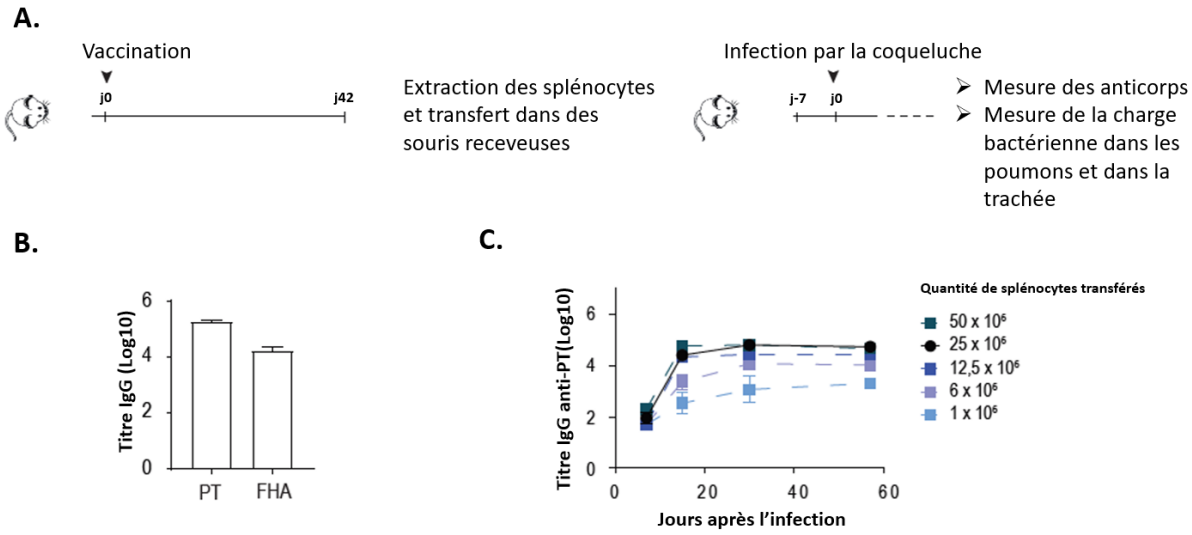

Figure B. Un modèle murin pour étudier la réponse immunitaire à la coqueluche aussi fidèlement que possible, en retirant les anticorps circulants.

(A) Schéma du modèle de transfert adoptif. Un premier groupe de souris est vacciné, 42 jours après les rates sont extraites et les splénocytes sont isolés pour être transférés dans des souris receveuses. Les souris receveuses sont infectés par la coqueluche 7 jours après le transfert. Les anticorps et la charge bactérienne dans les poumons et la trachée sont ensuite analysées à différents points après l'infection. (B) Les IgG spécifiques pour PT et FHA sont déterminées par ELISA avant le transfert. (C) Les splénocytes (10^6 à 50x 10^6) de souris vaccinées sont transférés dans des souris receveuses et la production des anticorps spécifiques à PT par les cellules B mémoires est quantifiée par ELISA à 7, 14, 28 et 56 jours après l'infection par Bp. Les données représentent la moyenne +/- SEM de 2 expériences différentes incluant 8 souris par groupe.

Pour la réalisation de cette thèse, nous avons utilisé ce modèle murin adapté pour évaluer si un rappel vaccinal réalisé avec un vaccin aP rapidement après une exposition à Bp (« vaccin post-expositionnel ») pourrait réactiver rapidement la mémoire immunitaire induite par les vaccinations précédentes et accélérer l'élimination bactérienne à la fois dans les poumons et la trachée. Les résultats des expériences sont détaillés dans la publication en anglais ci-après, soumise à Frontiers in Immunology.

Titre et page de garde de l'article en anglais

Post-exposure booster vaccination recalls vaccine-induced memory and accelerates *Bordetella pertussis* clearance in the lungs and trachea: preclinical evidence

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Abstract

Despite vaccine prevention, the prevalence of pertussis is increasing in countries where acellular vaccines (aP) are preferred. The efficacy of the current aP vaccines demonstrated in clinical trials is hampered by its transient nature. In addition, aP vaccines do not prevent asymptomatic carriage in the upper airways, facilitating transmission even by asymptomatic vaccinated individuals. In humans, antibodies induced by the aP vaccine wane rapidly, suggesting limited induction of long-lived plasma cells. This contrasts with non-human primates and murine models in which vaccine-induced antibody titers persist, in correlation with the persistence of bone marrow plasma cells. To study B cell responses following *Bordetella pertussis* infection, we have developed an adoptive murine transfer model in which memory cells induced by aP vaccination, are transferred to naïve recipients prior to intranasal challenge with *Bordetella pertussis* (Bp). This allows characterizing recall responses in the absence of serum antibodies, best mimicking the human situation. Using this model, we found that post-exposure boosting with aP vaccines accelerates Bp clearance in the lungs and the trachea, mediated by efficient B cell memory recall. If applicable to humans, post-exposure vaccination could reduce bacterial carriage and transmission, supporting the United Kingdom's empiric recommendations for post-exposure vaccination in susceptible populations.

Introduction

Despite pediatric vaccination coverage of roughly 85% globally, *Bordetella pertussis* (Bp) is still causing significant morbidity and mortality worldwide, and its prevalence is increasing in countries where acellular pertussis (aP) vaccines are preferred. (20–23) Since 1996, aP vaccines were progressively introduced in most developed countries to replace the very efficient but more reactogenic whole cell vaccine (wP). (5–13) This trend is continuing as a growing number of less privileged countries adopt aP vaccines. Unfortunately, this switch led to a decrease in vaccine efficacy, as immunity induced by aP vaccines wanes rapidly and thus frequent boosters are required to maintain protection. (14–19) .

Most industrialized countries have recommended the implementation of aP booster doses beyond infancy and childhood, but their impact is insufficient and additional measures are needed especially during the cyclic outbreaks of Bp. One puzzling observation is that pertussis occurs in fully vaccinated individuals, despite a long (1 to 3 weeks) incubation period, which should be sufficient to recall memory cells and interrupt bacterial proliferation. (25) Moreover, aP vaccines were designed to limit severe infant pertussis and do not prevent colonization and therefore cannot hamper transmission/carriage. (27,31) Therefore, vaccinated individuals participate to contamination chains, precluding disease control. (26)

The study of immune responses following pertussis challenge has been best achieved in non-human-primates (NHPs). (27–29) They are the only available animal models to allow studying pertussis transmission; however, to our knowledge no study showed that pertussis antibodies wane in NHPs as rapidly as in humans. Antibody persist long after vaccination in mice, in contrast to humans preventing to perform a deep analysis of immune memory reactivation in

the absence of antibody persistence. (32) To overcome this issue, we have previously developed an adoptive transfer murine model, enabling the elimination of circulating antibodies, and thus better mimicking the human situation.(30)

Here, we used this adapted murine model to assess whether - and under which conditions - a post-exposure aP vaccine booster could recall vaccine-induced memory and accelerate bacterial clearance both in the lungs and the trachea.

Material and methods

Mice

Adult female BALB/cByJ mice were purchased from Charles River (L'Arbresle, France), kept under specific pathogen free conditions and used in all experiments. Mice were used at 6-8 weeks of age. All animal experiments were carried out in accordance with Swiss and European guidelines and approved by the Geneva Veterinary Office.

Immunizations

Mice were immunized intra-muscularly (i.m.) on days 0 and 21 (priming) and/or on days 1 or 3 post-Bp exposure (boosting) with 1/5th of a human dose (50 µl in both hind legs) of DTaP-IPV (Infanrix®, GlaxoSmithKline). Some mice were boosted on day 1 post-Bp exposition with 50 µl in both hind legs of Tdap (Boostrix®, GlaxoSmithKline, 1/5th of the human dose). Passive immunization was performed on day 5 after Bp exposure by administering intra-peritoneally (i.p.) 200 µl of hyperimmune sera (collected on day 41 after two DTaP-IPV doses given in naïve mice at days 0 and 21).

Adoptive transfer

Spleens were harvested 42 days after DTaP-IPV priming and boosting. Single cell suspensions were obtained by mechanical disruption and processed for red blood cell lysis. $25x10^6$ splenocytes (experimentally defined as optimizing the recall of immune memory, unpublished data) were resuspended in 100 µl of saline solution and transferred intravenously (i.v.) by retro-orbital injection into naïve mice.

Bordetella pertussis challenge

Streptomycin-resistant *Bordetella pertussis* 18323 (U.S. Food and Drug Administration) were grown on Bordet-Gengou agar (Difco) supplemented with 1% glycerol, 10% defibrinated sheep blood (Chemie Brunschwig AG) and 100 µg/ml streptomycin. $1x10^6$ colony-forming units (CFU) were instilled intranasally in a volume of 20 µL into mice anesthetized by i.p. injection of Ketasol® (100 mg/kg; Graeub) and Rompun® (10 mg/kg; Bayer). Mice were sacrificed 2-3 hours after infection for quantification of the initial numbers of viable Bp CFUs in the lungs and trachea, and at different time-points post challenge for determination of bacterial colonization. Lungs and trachea homogenates were plated onto Bordet-Gengou agar plates supplemented with 1% glycerol, 10% defibrinated sheep blood (Chemie Brunschwig AG) and 100 µg/ml streptomycin; and the number of CFUs was counted after 4 days of incubation at 37°C.

Antibodies quantification

Bp antigen-specific antibody titers were determined by ELISAs using 96-well plates (Nunc MaxiSorp™; ThermoFischer Scientific) coated with pertussis toxin (PT, WHO International Standard *Bordetella Pertussis* Toxin 2nd IS, NIBSC code: 15/126) (1 µg/ml) or filamentous hemagglutinin (FHA, The Native Antigen Company) (1 µg/ml). Wells were incubated with 2-fold serial dilutions of individual or pooled mouse prior to incubation with secondary horseradish

peroxidase (HRP) conjugated anti-mouse IgG (Invitrogen). The optical density of each well was measured at 405 nm and the data analyzed with SoftMax Pro software. IgG titers were expressed as Log10 in reference to a pool of hyperimmune sera harvested from vaccinated mice to quantify antibody levels with precision and minimal inter-plate variation. (33) Titers under 10^2 are not detected by the assay.

Statistical analysis

Values are expressed as mean ± SEM. Statistical analysis were performed using one-way ANOVA followed by a Tukey multiple comparison test. All analyses were done using the GraphPrism software.

Results

Post-exposure boosting is required for rapid and strong antibody recall

We first compared antibody responses to Bp challenge in three experimental groups: 1) naïve control mice; 2) immune mice, i.e. recipient mice of splenocytes from immunized mice; and 3) in immune mice which were also boosted post-Bp exposure.

In naïve control mice, serum antibodies to PT (Figure 1A) only started to increase 3 weeks (day 21) after Bp challenge. In immune mice, PT and FHA antibodies were undetectable on the day of Bp challenge, confirming the absence of serum antibodies following the adoptive transfer of immune splenocytes. These antibodies increased significantly faster after Bp challenge in immune compared to control mice. It nevertheless still required 2 weeks (day 14) for PT- and FHA-specific-IgG to reach statistically significant (10^3) and 3 weeks (day 21) to reach high (10^{4-5}) ELISA titers. This pattern is similar to the 3-weeks delay required for the serological diagnosis of pertussis in humans. (34) In contrast, boosting immune mice with DTaP-IPV one day after

challenge rapidly recalled both PT- and FHA-specific-IgG antibodies, which reached significantly higher levels on days 7, 10 and 14 in boosted compared to non-boosted immunized mice (Figure 1).

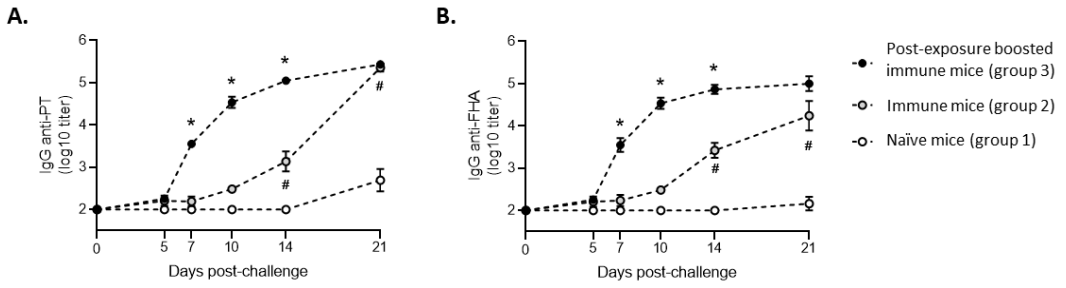

Figure 1. Post-exposure boosting enables a rapid and strong antibody recall.

Splenocytes of DTaP-IPV-vaccinated mice were transferred into naïve recipient mice (group 2 and 3). All mice were challenged with Bp a week later. In group 3, a post-exposure DTaP-IPV boost was administered one day after challenge. PT-specific IgG (A) and FHA-specific IgG (B) antibody titers were determined by ELISA on sera collected on the day of challenge (day 0) and at days 5, 7, 10, 14 and 21 post challenge. Data represents mean +/- SEM from 2 different experiments, including between 4 to 9 mice per group depending on the time point. * $p<0.0001$ comparing group 2 and 3, i.e. with and without post-exposure boosting; ♯ $p<0.0001$ comparing group 1 and 2, i.e. in previously immunized versus naïve mice.

Post-exposure boosting accelerates *Bordetella pertussis* clearance in the lungs and trachea

We next wanted to assess whether the faster and stronger increase of Bp-specific antibodies observed in mice boosted with DTaP-IPV one day after Bp exposure impacted lung and tracheal bacterial loads. In our model, Bp actively replicated in naïve mice or recipients of immune splenocytes, reaching higher numbers of CFU in the lungs (Figure 2A) and trachea (Figure 2B) on day 5 than day 0, and slowly declining during the following 2 weeks. Giving a post-exposure DTaP-IPV booster significantly accelerated bacterial clearance in both the lungs and trachea,

which were cleared of Bp 2 weeks post challenge as illustrated by the significantly lower numbers of CFUs in lungs and trachea on day 7, 10 and 14 (Figure 2).

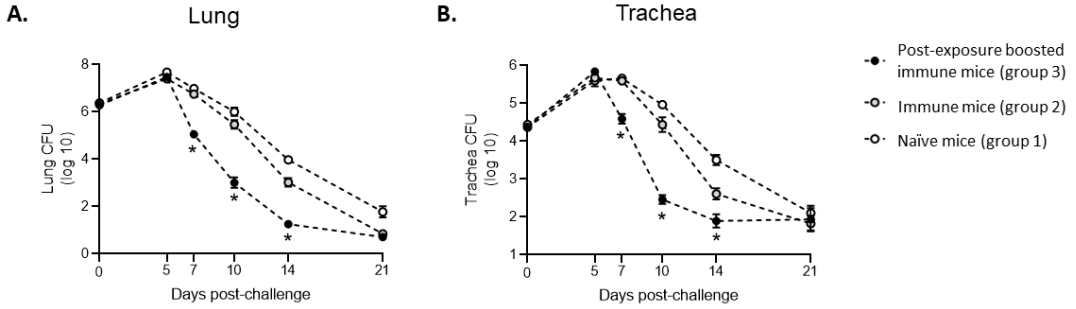

Figure 2. Post-exposure boosting accelerates bacterial clearance both in the lungs and the trachea.

Splenocytes of DTaP-IPV-vaccinated mice were transferred into naïve recipient mice which were challenged with Bp a week later (group 2 and 3). DTaP-IPV boosting was administered one day after challenge in mice of group 3. CFUs were counted in the lungs (A) and the trachea (B) at 3 hours and at days 5, 7, 10, 14 and 21 post Bp challenge, and are displayed on a logarithmic scale. Data represents mean +/- SEM from 5 different experiments, and between 7 to 24 mice per group depending on the time point. * $p<0.01$ comparing group 2 and 3, i.e. with and without post-exposure aP boosting.

We next wanted to see whether a later post-exposure boosting would remain effective: faster lung and tracheal bacterial clearance was still obtained when aP boosting was performed on day 3 after exposure (Figure S1). Boosting on day 3 compared to day 1 after Bp exposure only transiently delayed clearance, with a significant difference only observed on day 7 after challenge: on days 10, 14 and 21, a day 3 post exposure booster was as efficient as boosting on day 1.

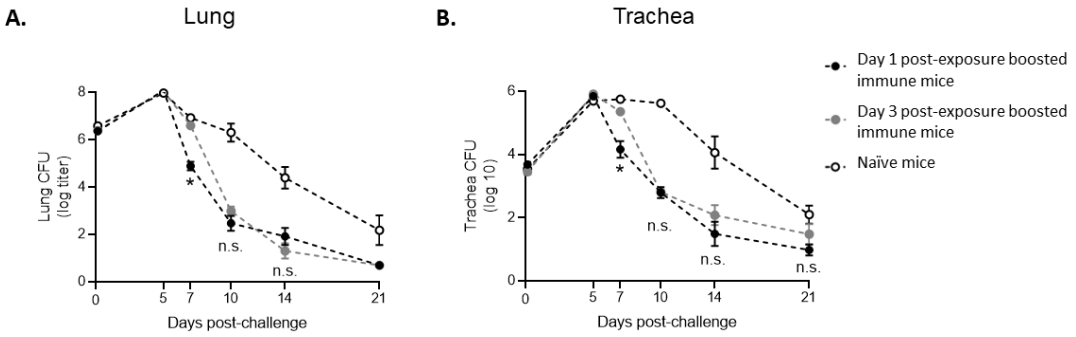

Figure S1. Postponing post-exposure boosting to 3 days post-challenge also accelerates bacterial clearance.

Splenocytes of DTaP-IPV vaccinated mice were transferred into naïve recipient mice which were challenged with Bp a week later. The group "naïve mice" did not receive any transferred splenocytes, but was challenged with Bp at the same time as the other groups. Post-exposure booster immunization with DTaP-IPV was given i.m. on day 1 or 3 after Bp challenge. CFUs were counted in the lungs (A) and the trachea (B) at 3 hours and on days 5, 7, 10, 14 and 21 post challenge, and are displayed on a logarithmic scale. Data represents mean +/- SEM from 5, mice per group. * $p<0.01$, n. s.: $p> 0.05$ comparing post-exposure booster vaccination given 1 versus 3 days post Bp challenge.

Last, we assessed the influence of the dose of vaccine antigens in the recall of Bp immunity: post-exposure boosting with Tdap (one day after Bp exposure), which contains 3 times less PT and FHA than DTaP-IPV, was similarly effective on Bp clearance, both in lungs and trachea (Figure S2).

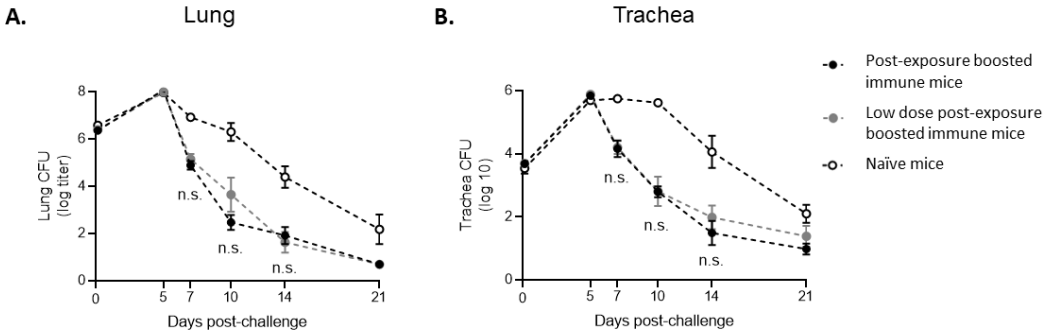

Figure S2. Post-exposure boosting with 3 times less pertussis antigens accelerates bacterial clearance as efficiently.

Splenocytes of DTaP-IPV-vaccinated mice were transferred into naïve recipient mice which were challenged with Bp a week later. The group "naïve mice" did not receive any transferred splenocytes, but was challenged with Bp at the same time as the other groups. Post-exposure boosting was performed with Tdap (3 times less pertussis antigens, "low dose") or DTaP-IPV given i.m. one day after challenge. CFUs were counted in the lungs (A) and the

trachea (B) at 3 hours and on days 5, 7, 10, 14 and 21 post challenge, and are displayed on a logarithmic scale. Data represents mean +/- SEM, 5 mice per group. n. s.: p> 0.05 comparing post-exposure vaccination given with DTaP-IPV versus Tdap ("low dose").

Post-exposure active and passive immunization similarly reduce bacterial clearance

The correlation between a faster/stronger increase of antibodies elicited by post-exposure boosting with a faster bacterial clearance in both the trachea and the lungs suggests that antibodies are the main effectors – without however excluding the requirement of T cell help. This was confirmed by the post-exposure i.p. injection of immune sera from DTaP-IPV vaccinated mice into recipient mice of immune splenocytes instead of one day after Bp challenge with the active immunization. To best mimic the time needed for memory B cells to differentiate into antibody-secreting cells, passive immunization was provided on day 5 after Bp challenge. We observed that passive immunization accelerated bacterial clearance in lungs and trachea as efficiently as a post-exposure DTaP-IPV booster (Figure 3A and B). Thus, providing PT/FHA-specific antibodies was sufficient to accelerate bacterial clearance. The kinetics of PT/FHA-specific antibodies is similar with both passive and active post-exposure booster (Figure S3).

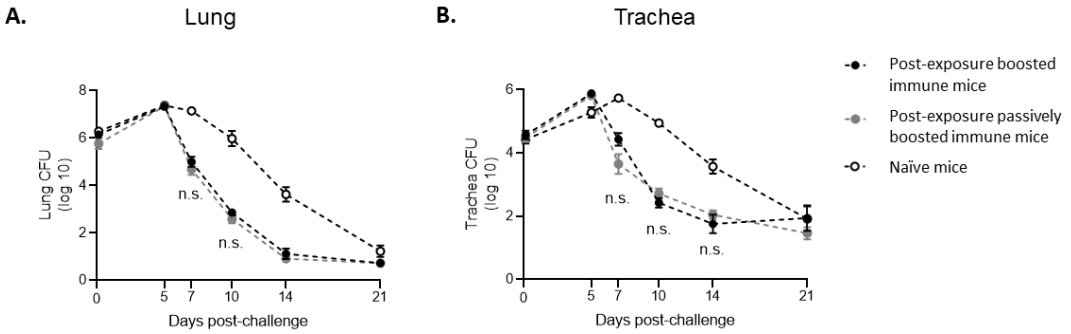

Figure 3. Post-exposure active and passive immunizations exert similar influences on bacterial clearance.

Splenocytes of DTaP-IPV-vaccinated mice were transferred into naïve recipient mice which were challenged a week later with Bp. The group "naïve mice" did not receive any transferred splenocytes, but was challenged with Bp at the same time as the other groups. One group received a post-exposure DTaP-IPV booster i.m. one day after challenge while the second group received immune sera i.p. 5 days after challenge. CFUs were counted in the lungs (A) and the trachea (B) at 3 hours and days 5, 7, 10, 14 and 21 post Bp challenge, and are displayed on a logarithmic scale. Data represents mean +/- SEM from 2 different experiments, 10 mice per group. n. s.: p> 0.05 comparing post-exposure active and passive immunization.

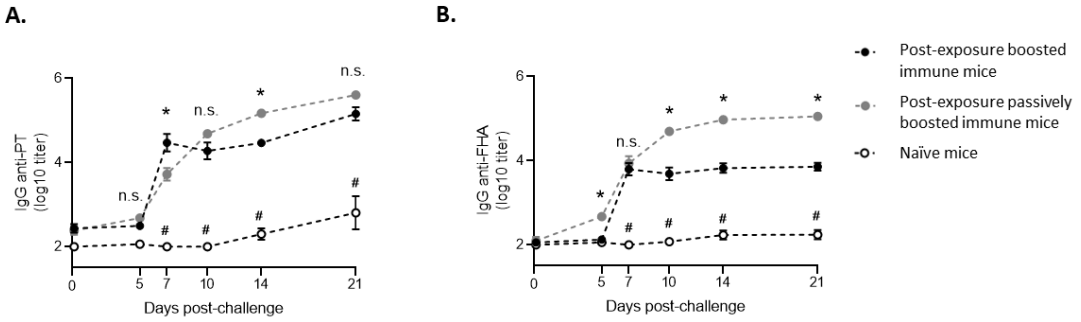

Figure S3. Post-exposure active and passive immunizations induce PT/FHA-specific antibodies with a similar kinetic

Splenocytes of DTaP-IPV-vaccinated mice were transferred into naïve recipient mice which were challenged a week later with Bp. The group "naïve mice" did not receive any transferred splenocytes, but was challenged with Bp at the same time as the other groups. One group received a post-exposure DTaP-IPV booster i.m. one day after challenge while the second group received immune sera i.p. 5 days after challenge. PT-specific IgG (A) and FHA-specific IgG (B) antibody titers were determined by ELISA on sera collected on the day of challenge (day 0) and at days 5, 7, 10, 14 and 21 post challenge. Data represents mean +/- SEM from 2 different experiments, including between 5 to 10 mice per group depending on the time point. * p<0.01, n.s. p> 0.05 comparing post-exposure active and passive immunization; ♯ p<0.0001 comparing the naïve group to the other 2 groups.

Discussion

The current aP vaccination schedule is not fully satisfactory: its transient efficacy fails to prevent carriage and transmissibility and thus does not efficiently reduce disease prevalence and contamination of high-risk neonates or infants too young to be vaccinated. Using a specifically developed murine model (30), we demonstrate the slow and weak reactivation of vaccine-induced immune memory following Bp exposure compared to its prompt and strong activation by aP boosting.

Infection with Bp is very slow at recalling vaccine-induced memory B cells and triggering antibody responses in humans (35). We show that this lack of immune reactivation is similar in our adoptive murine model, and that it is associated with prolonged bacterial loads in the lungs and prolonged carriage in the trachea (Figures 1 and 2). This slow and low reactivation of vaccine-induced immune memory upon Bp infection could result from a combination of factors. First, the incubation period of Bp is long delaying memory B cell reactivation (36) compared to an aP booster that recalls memory in only 4 to 7 days. Second, memory B cell reactivation requires a sufficient amount of antigen to trigger plasma cell differentiation. Since Bp is a strictly mucosal pathogen, without bacteremia (except in severely immune-compromised individuals) (37) we postulate that there is limited antigen dissemination to the draining lymph nodes where memory B cells essentially reside.Last, Bp exerts local immunomodulatory effects which limit the activation and migration of (antigen-loaded) dendritic cells to the draining lymph nodes. (38,39) Thus, the rapid reactivation of pertussis immunity does not occur at time of Bp exposure (32) , resulting in recurrent infection and symptoms. (40)

In contrast to Bp infection, boosting with an aP vaccine even a few days after Bp exposure induce a faster and stronger increase of anti-PT and anti-FHA IgG, reflecting a more effective reactivation of vaccine-induced memory B cells. This post-exposure boosting accelerates Bp clearance with similar patterns and kinetics as the post-exposure administration of immune serum (passive immunization). This strongly suggests a predominant role of memory B cell reactivation without excluding a contribution of T cells (additional to their B cell helper effect). Indeed, although correlates of protection are not well defined for pertussis (41), a critical role has been attributed to antibodies and to mucosal $CD4^+$ Th1/Th17 cells in long lasting-protection and prevention of asymptomatic carriage (42,43). Th2 prone BALB/c mice were used exclusively

as our model, and not C57BL/6 mice with more Th1 skewed responses. Whether this would change the impact of post-exposure boosting - and which strain is more relevant to the human situation - remains unknown.

Importantly, post-exposure boosting does not only increase antibody responses but significantly reduces bacterial load in the lungs and in the trachea. To which extend this would reduce transmissibility cannot be studied in murine models, since mice remain asymptomatic. Interrupting transmission is however key in disease control strategies against pertussis, a highly transmittable infection that spreads from case to close contact via respiratory droplets (26). To our knowledge, none of the currently licensed aP vaccines have been reported to prevent Bp carriage.

Currently Bp infection is classically treated with antibiotics given during the catarrhal phase, which may not affect the course of symptoms but reduce the spread of infection to others. After paroxysms are established, antibiotics usually do not change significantly the course of illness, and infected patients are carriers and transmit the disease for approximately 3 weeks. (4) A post-exposure approach as proposed here might be a more efficient strategy both in terms of antibiotic sparing and in terms of epidemic control. Our data suggests that a booster dose of the currently available DTaP- or Tdap-containing vaccines administered early after Bp exposure may accelerate bacterial clearance and rapidly reduces carriage in the trachea. Public health guidelines in United Kingdom's already consider post-exposure vaccination in susceptible populations to reduce ongoing transmission. (44) Our work provides preclinical evidence to support this approach.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest

Author Contributions

MB, FA, PHL and CAS designed the study. MB and PF performed the experiments. MB, FA, PHL, and CAS analyzed and/or interpreted the results. MB, FA and CAS wrote the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Discussion de thèse

Cette thèse démontre, dans un modèle murin adapté, qu'un rappel vaccinal post-expositionnel contre la coqueluche permet de restimuler efficacement la réponse mémoire induite par la vaccination initiale et ainsi de diminuer rapidement la charge bactérienne aussi bien dans les poumons que dans les voies aériennes supérieures. Ces résultats soutiennent la politique de santé publique déjà mise en place empiriquement au Royaume-Uni, qui préconise un rappel post-expositionnel pour casser les chaines de transmissions de la coqueluche.

Ces résultats mettent en avant le fait que l'infection par Bp ne permet pas de rappeler rapidement la mémoire B et prend bien plus longtemps qu'un rappel vaccinal à initier une réponse anticorps spécifiques en quantité suffisante. D'après nos expériences la protection contre la coqueluche se ferait principalement via les anticorps. En effet en administrant du sérum hyper immun la clairance bactérienne dans les poumons et la trachée suit la même cinétique qu'après un rappel vaccinal. La dose antigénique contenu dans le vaccin à donner après l'exposition à la coqueluche peut être moindre. En effet, dans notre modèle, les vaccins dTpa ont la même efficacité que les vaccins DTPa, au contenu antigénique plus élevé.

Actuellement, l'infection à Bp est classiquement traitée par des antibiotiques administrés pendant la phase catarrhale, ce qui peut ne pas affecter l'évolution des symptômes mais réduire la propagation de l'infection à d'autres personnes s'ils sont donnés précocement. Après l'établissement des paroxysmes, les antibiotiques ne modifient généralement pas de manière significative le cours de la maladie, et les patients infectés transmettent la maladie pendant environ 3 semaines. Une approche post-expositionnelle, telle que celle proposée ici, pourrait

être une stratégie plus efficace à la fois en termes d'économie d'antibiotiques et de contrôle épidémique

Cette mesure de rappel post-expositionnel basée sur des vaccins déjà sur le marché, si elle s'avère aussi efficace chez l'humain que dans notre modèle murin, pourrait être mise en place rapidement et à moindre coût. Ainsi cette thèse apporte une alternative concrète pouvant être testée en clinique pour briser les chaines de transmissions de la coqueluche avec les vaccins déjà à disposition.

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