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Regulation of Vaccine Immunity :
from myeloid cell functions to antibody responses

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REGULATION OF VACCINE IMMUNITY

— FROM MYELOID CELL FUNCTIONS TO ANTIBODY RESPONSES

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REGULATION OF VACCINE IMMUNITY

— From myeloid cell functions to antibody responses

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To my parents

献给我的父母

ABSTRACT

The generation of vaccine-induced protection against infections relies on a well-coordinated network of innate immune responses, adaptive cellular responses, and humoral responses. Sufficient stimulation of the innate immune system by vaccination is critical to subsequently induce effective pathogen-specific T cell and B cell responses. The magnitude, characteristics and functions of the T cell and B cell response will further determine the protective effect of the vaccines. This thesis has investigated aspects of both the early innate immune response as well as the adaptive T cell and B cell response after vaccination. Specifically, the first part focuses on the functions of different innate myeloid cell subsets in regulating vaccine responses. The second part focuses on the antibody responses induced by a new live pertussis vaccine compared to the currently used acellular pertussis vaccine (aPV).

Neutrophils are the major circulating myeloid cells. Heterogeneity and plasticity of neutrophils have received much attention during the past years. In **Paper I**, we show that neutrophils can present antigens to antigen-specific memory $CD4^+$ T cells. This is dependent on the upregulation of MHC-II and costimulatory molecules on neutrophils which can occur in the presence of antigens and autologous antigen-specific memory $CD4^+$ T cells. Furthermore, we found that neutrophils isolated from lymph nodes draining vaccine injection sites of rhesus macaques can present vaccine antigens to antigen-specific $CD4^+$ T cells. Neutrophils may therefore play a role in the induction and regulation of vaccine-specific T cell responses through antigen presentation.

There have been efforts trying to understand how vaccines induce immune responses but much less is known about immune suppressive regulation. In **Paper II**, we demonstrate that myeloid-derived suppressor cells (MDSCs), which is a unique population of myeloid cells with suppressive functions particularly on T cells, accumulate transiently after vaccination. We found that monocytic (M)-MDSCs and polymorphonuclear (PMN)-MDSCs are present in rhesus blood and possess inherent suppressive effects on T cells. The frequency of M-MDSCs rapidly and transiently increased in the blood, and these cells infiltrated the injection sites after vaccination. We speculate that MDSCs contribute with an immune-balancing role to prevent excessive immune activation and inflammation upon vaccine exposure.

In **Paper III**, we evaluated the immune responses in humans receiving the live attenuated *Bordetella pertussis* vaccine BPZE1 in a clinical trial. A single intranasal immunization of BPZE1 induced well-detectable plasmablasts, activated circulating T follicular helper cells, vaccine-specific Th1-polarized $CD4^+$ T cells, memory B cells and antibodies. In contrast to the antibodies induced by the currently used aPV, BPZE1-induced antibodies showed substantially broader specificities to several *B. pertussis* antigens, many of which were identified for the first time. The BPZE1-induced antibodies were also more potent in mediating bacterial opsonization to stimulate reactive oxygen species production in neutrophils, which further led to enhanced bactericidal function.

Collectively, these studies help in the understanding of how myeloid cells dictate immune responses and how the quality and specificities of antibody responses can be influenced by different pertussis vaccine platforms. This information will ultimately aid in the development of better future vaccines.

LIST OF SCIENTIFIC PAPERS

I. Maria Vono, **Ang Lin**, Anna Norrby-Teglund, Richard A. Koup, Frank Liang, and Karin Loré.

Neutrophils acquire the capacity for antigen presentation to memory $CD4^+$ T cells in vitro and ex vivo.

Blood. 2017 Apr 6;129(14):1991-2001.

II. **Ang Lin**, Frank Liang, Elizabeth A. Thompson, Maria Vono, Sebastian Ols, Gustaf Lindgren, Kimberly Hassett, Hugh Salter, Giuseppe Ciaramella, and Karin Loré.

Rhesus macaque myeloid-derived suppressor cells demonstrate T cell inhibitory functions and are transiently increased after vaccination.

The Journal of Immunology. 2018 Jan 1;200(1):286-294.

III. **Ang Lin**, Danijela Apostolovic, Maja Jahnmatz, Frank Liang, Sebastian Ols, Teghesti Tecleab, Chenyan Wu, Marianne van Hage, Ken Solovay, Keith Rubin, Camille Locht, Rigmor Thorstensson, Marcel Thalen, and Karin Loré.

The live attenuated *Bordetella pertussis* vaccine BPZE1 induces a Th1-polarized broad antibody response in humans

Manuscript

PUBLICATIONS NOT INCLUDED IN THESIS

I. Luca Cassetta, Espen S. Baekkevold, Sven Brandau, Anna Bujko, Marco A. Cassatella, Anca Dorhoi, Carsten Krieg, **Ang Lin**, Karin Loré, Olivia Marini, Jeffrey W. Pollard, Mikael Roussel, Patrizia Scapini, Viktor Umansky, and Gosse J. Adema. Deciphering myeloid-derived suppressor cells: isolation and markers in humans, mice and non-human primates.

Cancer Immunology, Immunotherapy. 2019 Apr;68(4):687-697.

II. **Ang Lin** and Karin Loré. Granulocytes: new members of the antigen-presenting cell family.

Frontiers in Immunology. 2017 Dec 11;8:1781.

III. Frank Liang, Gustaf Lindgren, **Ang Lin**, Elizabeth A. Thompson, Sebastian Ols, Josefine Röhss, Shinu John, Kimberly Hassett, Olga Yuzhakov, Kapil Bahl, Luis A. Brito, Hugh Salter, Giuseppe Ciaramella, and Karin Loré. Efficient targeting and activation of antigen-presenting cells in vivo after modified mRNA vaccine administration in rhesus macaques.

Molecular Therapy. 2017 Dec 6;25(12):2635-2647.

IV. Gustaf Lindgren, Sebastian Ols, Frank Liang, Elizabeth A. Thompson, **Ang Lin**, Fredrika Hellgren, Kapil Bahl, Shinu John, Olga Yuzhakov, Kimberly J. Hassett, Luis A. Brito, Hugh Salter, Giuseppe Ciaramella, and Karin Loré. Induction of robust B cell responses after influenza mRNA vaccination is accompanied by circulating hemagglutinin-specific $ICOS^{+}PD\text{-}1^{+}CXCR3^{+}$ T follicular helper cells.

Frontiers in Immunology. 2017 Nov 13;8:1539.

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LIST OF ABBREVIATIONS

ACT	Adenylate cyclase toxin
APC	Antigen-presenting cell
APRIL	A proliferation-inducing ligand
aPV	Acellular pertussis vaccine
BAFF	B cell activating factor
BCR	B cell receptor
BM	Bone marrow
BrkA	*Bordetella* resistance to killing A
CFSE	Carboxyfluorescein diacetate succinimidyl ester
ClITA	Class II transactivator
CMP	Common myeloid progenitor
CMV	Cytomegalovirus
CR	Complement receptor
cTfh	Circulating T follicular helper
DC	Dendritic cell
dLN	Draining lymph node
DNT	Dermonecrotic toxin
FHA	Filamentous hemagglutinin
FIM	Fimbriae
G-CSF	Granulocyte colony stimulating factor
GC	Germinal center
GMP	Granulocyte-monocyte progenitor
HA	Hemagglutinin
IC	Immune complex
ICAM-1	Intercellular adhesion molecule 1
ICOS	Inducible T cell co-stimulator
Ig	Immunoglobulin
LDN	Low-density neutrophil
LOX-1	Lectin-type oxidized low-density lipoprotein receptor 1
MHC	Major histocompatibility complex

MAC	Membrane attack complex
MCP-1	Monocyte chemoattractant protein 1
MDSC	Myeloid-derived suppressor cell
MIP-1α	Macrophage inflammatory protein 1 alpha
NDN	Normal-density neutrophil
NET	Neutrophil extracellular trap
NHP	Nonhuman primate
NK	Natural killer
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
PGE_2	Prostaglandin E_2
PRN	Pertactin
PT	Pertussis toxin
ROS	Reactive oxygen species
SHM	Somatic hypermutation
STAT3	Signal transducer and activator of transcription
TAN	Tumor-associated neutrophil
TCR	T cell receptor
TCT	Tracheal cytotoxin
TNF	Tumor necrosis factor
TRM	Tissue-resident memory T
wPV	Whole cell pertussis vaccine

1 INTRODUCTION

The history of immunization can be traced back to the 10th century in ancient China when inoculation was practiced by blowing powdered material from smallpox scabs into the nostrils[1]. In 1796 Edward Jenner inoculated pus from cowpox blisters into a boy and found him to be protected against smallpox[2]. Jenner's attempt established the concept of vaccination and paved the way for vaccine research and development. Nowadays the field of vaccination has become multidisciplinary and covers aspects of immunology, biology, chemistry, pharmaceutics, and material science[3,4]. The past few decades being an active time for vaccine research have witnessed a remarkable development of vaccines, from traditional live-attenuated vaccines, inactivated vaccines, subunit vaccines, to nucleoid acid vaccines (DNA vaccines, mRNA vaccines, and recombinant vector vaccines), accompanied by a rapid development of vaccine delivery systems especially nanoparticle-based platforms[5-7].

Effective vaccines have saved many lives and contributed to the eradication of diseases such as smallpox[8]. Diseases such as polio and yaws are promising targets to be eradicated in the near future[9]. Vaccine-preventable diseases, i.e., diphtheria, measles, tetanus, and hepatitis B, have been greatly controlled under standardized immunization programs[10,11]. However, today we are still facing a lot of challenges in the fight against infectious diseases. Serious infections such as tuberculosis, HIV/AIDS, and malaria cause vast deaths worldwide and no fully effective vaccines are yet available[12-14]. Resurgence and outbreaks of vaccine-preventable diseases such as pertussis have occurred in recent years despite vaccination[15], which indicates that some vaccines that we are using today need to be improved. The limited response to vaccination observed in immunocompromised individuals has indicated the need for better vaccines or more suitable vaccination strategies for specific populations[16]. In addition, vaccine research focusing on non-infectious conditions such as allergies, autoimmune diseases and cancers is emerging and still at its initial stage[17,18]. To tackle these challenges and drive the vaccine field forwards, we need to have a more thorough understanding of how vaccines interact with the immune system and the immune mechanisms dictating vaccine responses.

When evaluating vaccines, it is a common practice to focus on the ultimate outcomes – the magnitude and quality of antibodies, T cell responses, and the resulting protection against infection. Effective protection induced by vaccines is multifactorial and requires an intimate collaboration between specific antibodies, T cells, and in most cases, also innate effector cells[19]. Upon administration into the host, vaccines first trigger the innate immune system[20], which is structured by complements and distinct populations of innate immune cells that are widely distributed throughout the body. Innate immune activation subsequently leads to events such as a local production of inflammatory mediators (cytokines and chemokines), recruitment of cells to the sites of inflammation, activation of complement cascades, and the initiation of antigen presentation process[21]. The adaptive immune system, divided by humoral and cellular immunity, is considered to combat foreign threats more efficiently due to their high degree of diversity and specificity, as well as the memory capacity that enables them to quickly respond upon secondary antigen exposure. Humoral response is mediated by B cells that produce

specific antibodies functioning through neutralization, opsonization and activation of complements[22]. Cellular response is mediated by activated T cells that either differentiate into distinct effector subtypes producing cytokines or exert direct cytotoxic effects[23]. The degree of early innate immune reactions fundamentally determines the level of adaptive immune responses. In return, adaptive immune responses can synergize with innate immune system to mount a more efficient outcome. Therefore, it is important to understand the vaccine-host interactions and how innate and adaptive immune system cooperate to elicit sufficient vaccine-specific responses.

Using human and nonhuman primate (NHP) samples, I have in my Ph.D. thesis studied vaccine-host interactions and their effects on vaccine responses. The focus of this thesis was originally planned to be on two unique populations of innate myeloid cells (neutrophils and MDSCs), but after studying them in humans receiving a novel live attenuated *B. pertussis* vaccine, the work expanded to characterize antibody responses. In the end, the work revolved to analyze how the vaccine-induced antibodies affected neutrophils in their bacterial clearance process. Altogether, this work contributes to a better understanding of the mechanisms regulating pertussis immunity.

2 AIMS OF THESIS

The overall aim of the thesis was to elucidate how innate myeloid cells (neutrophils and MDSCs in particular) influence vaccine responses and how different pertussis vaccines regulate the quality of antibodies which in turn affect the interaction with myeloid cells to clear bacteria. The specific aims of the individual studies were:

Paper I: To study the antigen-presenting function of neutrophils in regulating vaccine-specific T cell responses

Paper II: To characterize MDSCs in rhesus macaques and explore the role of MDSCs in vaccine-induced responses

Paper III: To characterize the immune responses in humans receiving a live attenuated pertussis vaccine compared to the clinically used acellular pertussis vaccine

3 INNATE MYELOID CELLS

Innate myeloid cells being the key components of the innate immune system contain monocytes, macrophages, granulocytes, mast cells, dendritic cells (DCs), thrombocytes, and erythrocytes[24]. These cells are derived from common myeloid progenitors (CMPs), which are in contrast to natural killer (NK) cells, gamma delta T cells and innate lymphoid cells that are rooted in the lymphoid lineage[25]. Upon vaccine administration, myeloid cells are the first to encounter vaccines[26,27], undergo activation process, and initiate the succeeding immunological events to build vaccine-specific responses.

3.1 NEUTROPHILS

Neutrophils are the most abundant myeloid cells within the blood circulation in humans[28]. They are derived from granulocyte-monocyte progenitor cells (GMPs) that undergo sequential differentiation stages in the bone marrow (BM) and mature neutrophils egress into circulation when terminally differentiated[29]. At steady state, neutrophils circulate in the blood for a limited period of time (approximately 12h) before being cleared. This physiological process has recently been referred to as "neutrophil aging", which follows a circadian rhythm and plays an important role in maintaining homeostasis[30,31]. When "danger signals", such as infection or tissue damage, appear in peripheral tissues, circulating neutrophils are quickly alerted and usually represent the first cells migrating to the sites of inflammation. This is known as "extravasation" that involves multiple steps coordinated by adhesion molecules and chemokines[28].

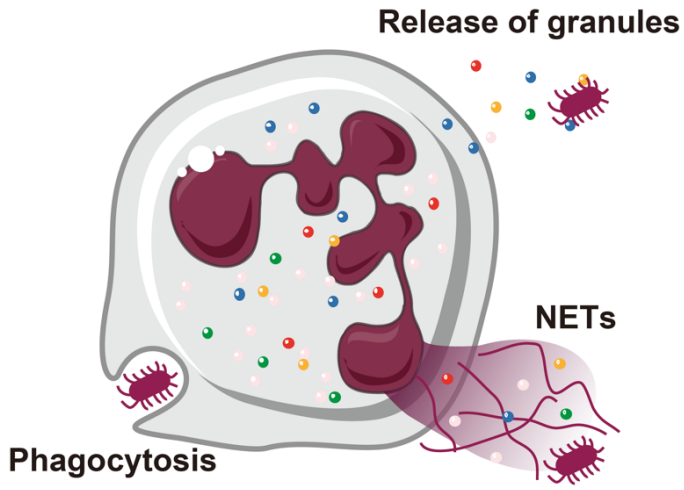


Figure 1. Microbicidal mechanisms of neutrophils.

As one of the major phagocytes, neutrophils are specialized at taking up pathogens, and subsequently eliminate them through oxidative burst or release of destructive antimicrobial proteins stored in granules[32]. In addition, neutrophils can extrude web-like structures containing nuclear chromatin and granule proteins, which are named neutrophil extracellular traps (NETs)[33] that capture extracellular pathogens and degrade them (Figure 1). However, the traditional view that the functions of neutrophils are restricted to the initial phase of the defense against pathogens has been changed[34]. A substantial body of evidence has demonstrated that there is a high diversity of neutrophils with regard to their versatile functions in partaking in both innate and adaptive immune responses[35,36].

3.1.1 Heterogeneity of neutrophils

Neutrophils have long been considered as a homogeneous population of terminally differentiated cells with a short lifespan. This view has been changing in recent years as studies demonstrated the heterogeneity and plasticity of neutrophils, which manifest as differences in phenotypes, maturation or activation status, locations, buoyancy, and importantly the functions[29,37].

Heterogeneous populations of neutrophils have been reported at steady state, as well as under inflammatory or pathological conditions. The aforementioned spontaneous neutrophil aging is a typical example showing the heterogeneity of circulating neutrophils in the absence of inflammation. Once released into the blood circulation, neutrophils undergo phenotypic changes over time, e.g. upregulation of CXCR4, CD11b and downregulation of CXCR2, until they become aged neutrophils and leave the circulation[38]. Although the physiological consequence of neutrophil aging is largely unknown, the altered expression of certain surface molecules related to cell adhesion may affect their trafficking and migration into peripheral sites. In addition to neutrophils at different age, phenotypically different neutrophil subsets exist in the blood under homeostasis. In healthy individuals, a proportion of mature neutrophils express CD177, a glycoprotein that mediates the interaction between neutrophil and endothelial cells[38,39]. In addition, 20-25% circulating neutrophils possess the expression of olfactomedin-4 (OLFM4)[40] and these cells are more prone to produce NETs as compared to their $OLFM4^-$ counterparts[41]. Recent evidence also demonstrated the presence of neutrophils in multiple tissues under normal conditions, which challenged the traditional understanding that tissues are devoid of neutrophils[42]. In healthy individuals, neutrophils are found in the lung vasculatures constituting the "marginated pool"[43]. These cells resemble aged neutrophils and highly express CXCR4, which allows them to be retained in the lung through the interaction with CXCL12 released by pulmonary endothelia cells[44]. In the spleens of healthy humans and mice, a unique population of neutrophils located around marginal zone exhibit B cell helper functions and produce cytokines such as B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) to regulate antibody production[45,46]. Neutrophils have also been found in many other healthy tissues, while the biological relevance is largely unknown[42]. Whether these neutrophils transiently appear in tissues before being cleared or they are *bona fide* cells able to reside long term to exert unique functions are still unknown[47].

Heterogeneity of neutrophils is more commonly seen in pathological conditions[35]. This is largely due to an abnormal granulopoiesis process, during which several neutrophil subsets with different levels of maturity are generated and released to the circulation or peripheral tissues[48]. In the blood, heterogeneity of neutrophils is usually reflected by the difference in buoyancy. During inflammation, a population of neutrophils co-localize with peripheral mononuclear cells (PBMCs) after density gradient centrifugation using Ficoll (1.077g/L)[49]. These neutrophils are referred to as low-density neutrophils (LDNs), in contrast to the normal-density neutrophils (NDNs) that sediment together with erythrocytes. LDNs normally contain immature and mature cells indicated by the different morphology and phenotype (Figure 2). These cells may increase in numbers in the context of infection, cancer, or other disorders and

their frequencies may correlate with disease progression[50,51]. Of note, a unique population of cells, mostly known as PMN-MDSCs, with suppressive functions on T cells may exist in the LDN pool[52]. Much effort has been put on identifying unique markers for PMN-MDSCs. Recent evidence indicated lectin-type oxidized low-density lipoprotein receptor-1 (LOX-1) to be a specific marker for PMN-MDSCs, at least in cancer patients[53]. In contrast with LDNs, NDNs are usually considered as a homogeneous population of mature cells under homeostasis. However, NDNs may represent a heterogeneous population in non-homeostatic conditions. For example, $CD10^+$ NDNs and $CD10^-$ NDNs were reported in the blood of healthy individuals receiving G-CSF treatment for stem cell mobilization, and these two subsets possess inhibitory and stimulatory effects on T cells, respectively[54]. Apart from the cells in the circulation, heterogeneous tissue-infiltrating neutrophils have also been described in pathological conditions. One typical example is tumor-associated neutrophils (TANs)[55,56] that include anti-tumorigenic (N1) and pro-tumorigenic population (N2). However, as the discrimination of N1 and N2 is mainly based on their functional difference, identification of unique surface markers is still needed.

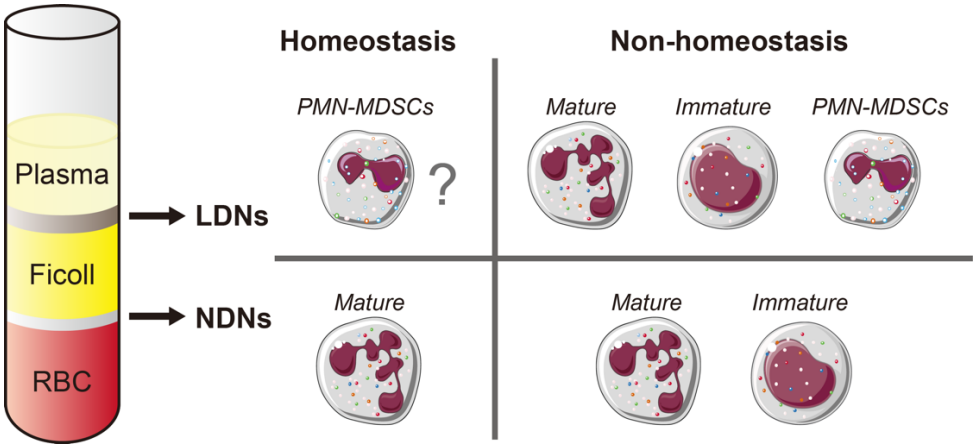


Figure 2. Heterogeneity of neutrophils in human blood.

3.1.2 Neutrophils as antigen-presenting cells (APCs)

The functions of neutrophils in regulating T cell responses through antigen presentation have attracted considerable attention recently[57,58]. Early evidence of the antigen-presenting ability of neutrophils mostly came from in-vitro studies showing that IFN-γ or GM-CSF-stimulated neutrophils could express MHC-II and co-stimulatory molecules (CD80 and CD86), and thereby stimulating T cell proliferation[59,60]. It was also shown that GM-CSF-treated human and murine neutrophils could differentiate into hybrid cells with dual features of dendritic cells (DCs) and neutrophils[61,62], implying a potential antigen-presenting ability of these cells. Similar evidence that neutrophils could express HLA-DR was also observed in patients receiving GM-CSF or IFN-γ treatment[63,64]. In these contexts, cytokine-induced expression of MHC-II on neutrophils is mainly mediated by the activation of class II transactivator (CIITA), a key regulator controlling MHC-II transcription[65].

In addition to the findings that cytokine-treated neutrophils can function as APCs, several recent studies including ours (**Paper I**) showed that T cells could deliver signals to neutrophils

that provided them with antigen-presenting functions[66-68]. We have shown that freshly isolated neutrophils can acquire the expression of HLA-DR, CD80 and CD86 when co-cultured with antigens and autologous antigen-specific memory T cells. This enables them to present the antigens to antigen-specific T cells, thereby stimulating proliferation and cytokine production of T cells[68]. Similarly, mouse studies showed that neutrophils loaded with ovalbumin (OVA) were able to present the antigen to OVA-specific T cells and drive the expansion of effector T cells[67]. Of note, no evidence so far shows that neutrophils can prime naïve T cells, which is a typical feature of classical APCs such as myeloid DCs. Therefore, neutrophils are considered as atypical APCs restrictive to the regulation of memory T cells[69]. While the exact signals delivered from memory T cells to neutrophils still are unclear, possible candidates are the cytokines released by bystander-activated T cells. It is likely that neutrophils are triggered by cytokines released from T cells that are activated by other neighboring APCs (Figure 3). In addition, surface antigen-mediated cell-cell contact between memory T cells and neutrophils may also play a role, for example, the binding of intercellular adhesion molecule I (ICAM-1) on memory T cells to CD11b/CD18 on neutrophils. This initial cell-cell interaction may provide "prime" signals to evoke MHC-II expression by neutrophils[58]. In addition to these, some other mechanisms may exist. A very recent study showed that neutrophils could acquire the function of APC after they phagocytosed antibody-opsonized erythrocytes[70]. In this scenario, fresh neutrophils are able to express MHC-II and costimulatory molecules even in the absence of T cells or cytokine stimuli (Figure 3).

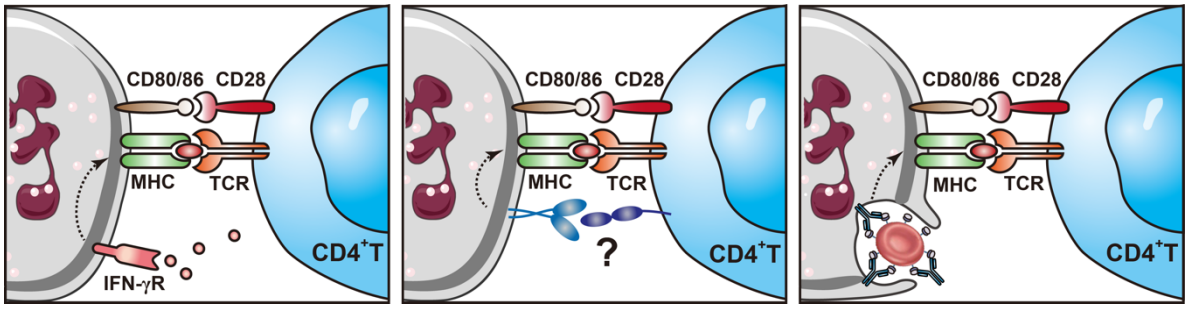


Figure 3. Potential mechanisms involved in the induction of APC feature by neutrophils.

In conclusion, the antigen-presenting function of neutrophils starts to be a well-established phenomenon. However, the physiological or pathological significance of this remains to be understood. Although neutrophils cannot compete with classical APCs in their capacity of antigen presentation on a per cell basis, their abundance may make up for some of the weak ability.

3.2 MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS)

MDSCs are a heterogeneous population of myeloid cells that demonstrate strong suppressive function on T cells[71]. They are considered as activated immature cells that cannot fulfil normal differentiation in the BM due to an aberrant myelopoiesis. MDSCs appear in a variety of conditions associated with cancer, infections, autoimmune diseases or other disorders[72]. For example, they are generated in the BM and spread to periphery in response to tumor-secreted growth factors[73] such as granulocyte colony stimulating factor (G-CSF), IL-6, prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF) (Figure 4). The roles of MDSCs in the pathology of these immune-related diseases are not fully understood and appear to be heterogeneous.

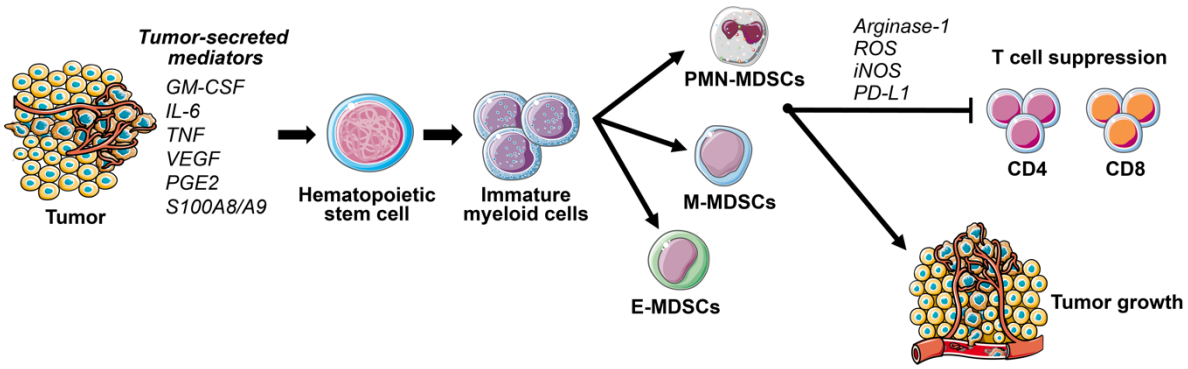


Figure 4. Differentiation and function of MDSC populations observed in cancer patients.

3.2.1 Heterogeneity of MDSCs

The immune regulatory functions of MDSCs have been well documented. However, current understanding of their heterogeneity is still elusive, in particular regarding their phenotypes[74]. MDSCs have intensively been studied in humans and mice (Figure 5). In **Paper II** of this thesis we characterized these cells in NHPs (rhesus macaques)[75]. Human MDSCs do not express the lineage (Lin) markers CD3, CD19 or CD56 but typically express CD11b and CD33, and have low or absent expression of HLA-DR. They can be further divided into three major subsets: monocytic (M)-MDSCs, polymorphonucelar (PMN)-MDSCs and early-stage (E)-MDSCs. Amongst them, M-MDSCs and PMN-MDSCs have been much investigated and they are present in humans, mice and NHPs[76]. E-MDSCs have recently been termed in humans, but this subset has yet to be defined in animal models[77].

In humans, M-MDSCs are normally identified as HLA-$DR^{-/low}CD14^{+}$ cells. CD11b and CD33 are also highly expressed by M-MDSCs and therefore can be used as additional markers to identify them[52]. Being considered as the counterparts of monocytes, M-MDSCs share the morphology and surface markers with monocytes to a large degree, such as CD11b, CD33 and CCR2[75]. Therefore, phenotypic discrimination of M-MDSCs from monocytes is normally determined by the lack of HLA-DR expression. What remains to be investigated is whether M-MDSCs and monocytes are generated in different differentiation pathways or if M-MDSCs are simply a subpopulation of monocytes acquiring suppressive function in diseases. PMN-MDSCs are immature neutrophils present inside LDNs. Identification of PMN-MDSCs

remains to be a challenge due to the lack of unique markers[78]. Human PMN-MDSCs have therefore long been defined as LDNs, which are typically identified as $CD15^{+}CD33^{dim}SSC^{high}$ cells that resemble NDNs except for their lower buoyancy[52]. Recently, LOX-1 was demonstrated to be a unique marker for PMN-MDSCs in patients with non-small cell lung cancer and head and neck cancer[53]. Whether this can be applied to other disease models remains to be validated. To this end, in the MDSC field it has been widely accepted that functional assessment of potential MDSCs following purification based on surface markers is the "gold standard" to identify them.

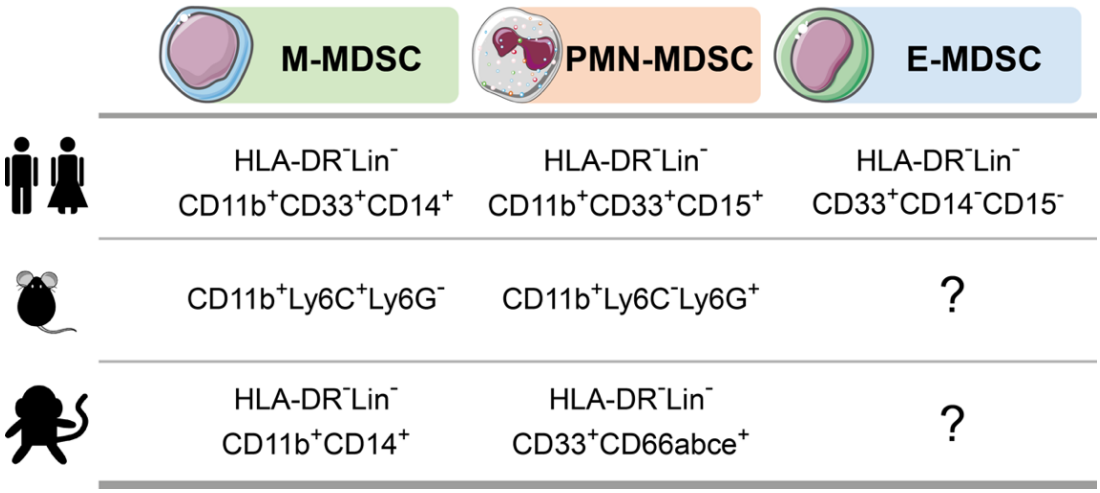


Figure 5. Phenotypes of MDSCs in humans, mice and rhesus macaques.

Apart from the cell morphology and phenotype, some intrinsic differences exist between M-MDSCs and PMN-MDSCs. Compared with PMN-MDSCs, M-MDSCs are more potent at excreting suppressive effects[79]. They possess a longer lifespan and are more tolerable to freeze-thaw procedures[80], which adds the technique feasibility to study them. In addition, M-MDSCs can be generated from monocytes in vitro upon stimulation with cytokines such as GM-CSF and IL-6[81]. M-MDSCs also maintain some degree of plasticity to further differentiate to non-suppressive cells[82]. For example, TLR7/8 agonists can induce human M-MDSCs to differentiate to macrophages[83]. So far, there is no such evidence showing the plasticity of PMN-MDSCs.

3.2.2 MDSCs in diseases and inflammation

The most well-known function of MDSCs is their suppressive effect on T cells. This is mainly mediated by two mechanisms[84] namely secretion of suppressive mediators (e.g. arginase-1, inducible nitric oxide synthase) and expression of co-inhibitory molecules (e.g. PD-L1). Aside from these, MDSCs can cross talk with other immune cells to negatively regulate immune responses. For instance, MDSCs have been shown to promote the expansion of regulatory T cells (Treg)[85], suppress NK cell cytotoxicity as well as inhibit the maturation of DCs[86].

The role of MDSCs appears to be widespread in different diseases. Being first reported in tumor models, MDSCs have intensively been studied in the context of cancer[73]. Elevated levels of MDSCs in the blood, tumor microenvironments, and lymphoid organs have been described in

a variety of malignancies[84]. Their suppressive effects impair anti-tumor T cell responses and were shown to support tumor progression. The level of MDSCs has been proposed to be a predictor of poor prognosis and is associated with poor survival in patients with breast, pancreatic, melanoma and colon cancer[87-90]. Therefore, strategies to block MDSC expansion, disturb inhibitory mechanisms, promote MDSC maturation, as well as prevent their migration to tumor sites have been employed in the development of therapeutic drugs and treatment of cancer[91]. For examples, sunitinib, being used in the treatment of renal cell carcinoma, can block the signal transducer and activator of transcription (STAT3) pathway to inhibit MDSCs expansion[92]. All-trans retinoic acid (ATRA) that can induce the differentiation of MDSCs into non-suppressive DCs is used to treat acute promyelocytic leukemia in clinic[93,94].

In contrast to the wealth of information on MDSCs in cancer, the roles of MDSCs in several other pathological or inflammatory conditions have recently emerged as an intense area of investigation[95]. Much has been studied was the functions of MDSCs during infections[96]. Accumulating evidence demonstrated the expansion of MDSCs under conditions of bacterial, viral, fungal and parasitic infection[97]. However, their respective role remains diverse in different contexts. Taking advantage of the suppressive function, MDSCs can help to counter-balance overwhelming inflammation to prevent tissue damage. However, an excessive expansion of MDSCs may impair the anti-infection immunity of the host, thereby favoring pathogen persistence and inducing chronic infection. MDSCs therefore function as "double-edged sword" in the context of infection[98]. Whether they are beneficial or detrimental for the host depends on the type of infection and stage of diseases.

MDSCs are also associated with several inflammatory conditions. In individuals with obesity, MDSCs are elevated as a consequence of low-grade persistent inflammation. This has been proposed to explain why obese populations showed compromised immune responses to vaccination[99]. Accumulation of MDSCs was also observed in experimental hypertension mice[100]. In this scenario, MDSCs can help alleviate high blood pressure and limit inflammation through the production of ROS. MDSCs also play a critical role in maintaining maternal-fetal tolerance to ensure normal pregnancy[101]. Vaccination-elicited inflammation was also shown to induce MDSCs, which in turn negatively influenced the vaccine efficacy[102]. Consistently, GM-CSF, an adjuvant used in a melanoma cancer vaccine formulation was reported to induce MDSCs and thereby impaired the therapeutic outcome[103]. These studies prompted a relationship between MDSCs and vaccination. A further investigation on this would increase our understanding of any interfering role that MDSCs may have in vaccination.

4 ANTIBODY RESPONSES

While the innate immune system can provide a rapid protection in face of infections, their contribution is usually limited. Adaptive immune responses built on the innate system are therefore needed to confer more effective and specific protection. Moreover, the memory capacity of the adaptive immune system allows it to quickly respond upon successive antigen exposure. In the context of vaccination or infection, the antibody response, being one arm of the adaptive immune system, is of particular importance. Almost all the vaccines to date function through the induction of specific antibodies in the serum or on the mucosal surface to combat infections. It has been well documented that vaccine-elicited antibody responses dominantly determine the ultimate outcome and, in many cases, operate as correlates of protection[104]. For any pathogen, a vaccine that could induce a broad, long-term antibody response with high neutralizing capacity would be able to confer efficient protection.

4.1 DEVELOPMENT OF ANTIBODY RESPONSES

B cell progenitors in the BM go through several successive steps including immunoglobulin (Ig) gene rearrangement, negative selection and finally differentiate into naïve B cells emerging into the periphery. Naïve B cells continuously patrol the blood circulation and the lymphatic system until encountering their cognate antigens in the spleen or LNs, whereupon they continue their maturation and differentiation program, which ultimately leads to the establishment of antibody responses[105,106].

Naïve B cells carry surface IgM and IgD as B cell receptors (BCRs) to sense the pathogenic antigens[107]. Some microbial components can directly stimulate B cells to produce antibodies without the help from T cells[108]. These antigens are named as thymus-independent (TI) antigens that can be divided into two groups: TI-1 antigens, which possess intrinsic B-cell activating capacity such as lipopolysaccharide (LPS), and TI-2 antigen[109], which contain highly repetitive epitopes such as polysaccharide antigens. Antibody responses induced by TI antigens can provide early protection but are usually considered less efficient due to no or limited somatic hypermutation (SHM) and class switching. In contrast, antibody responses generated with the involvement of T-helper cells are endowed with higher diversity and better efficacy[110,111]. In this scenario, B cells function as APCs and can process antigens and present them to T follicular helper (Tfh) cells that have encountered the same cognate antigens during an earlier antigen presentation process mediated by DCs. As a consequence, activated Tfh cells deliver helper signals to B cells to promote cell proliferation, survival, differentiation, as well as the succeeding SHM and class switching[112]. These signals are present in the form of either cytokines such as IL-21 or expression of surface molecules such as CD40L and inducible T-cell co-stimulator (ICOS) that interact with corresponding receptors on B cells[113]. Some of the B cells receiving helper signals will differentiate into short-lived antibody-secreting plasmablasts that constitute an initial phase of the antibody response. The remaining B cells, together with their cognate Tfh cells, will migrate into lymphoid follicles and form the germinal center (GC), where activated B cells undergo affinity maturation and class switching with the involvement of Tfh cells[112]. The GC reaction[114,115] ultimately leads to the induction of long-

lived plasma cells secreting a diverse repertoire of high-affinity antibodies and the generation of a memory B cell pool, which are also the goals of a successful immunization regimen.

4.2 FUNCTIONS OF ANTIBODIES

The whole antibody pool is composed of five antibody isotypes (IgM, IgD, IgG, IgA and IgE), distinguished by their difference in the constant region of the heavy chain[116,117]. Antibodies are distributed selectively throughout the body and exert specific functions in the context of infections. IgM is the first antibody produced and is present in the blood as a pentamer[118]. Soluble IgD is present at a very low level in the blood and its function remains unknown. However, surface IgD on B cells was shown to regulate B cell survival via BCR signaling[119]. IgG is the most abundant antibody isotype in the blood, as well as in the extracellular fluids. Four IgG subclasses exist, namely IgG1, IgG2, IgG3, and IgG4. Each subclass differs in their constant region and therefore possesses different functional activities[120]. IgA molecules exist either as monomers in the blood or as secreted IgA (sIgA) in a dimeric form present at mucosal surface and in secretions such as breast milk and saliva[121]. IgE is rare in the serum, but is closely associated with allergic reactions. IgE mainly works through the ligation with Fc receptors on mast cells and basophils, thus stimulating the cells to release functional mediators[122].

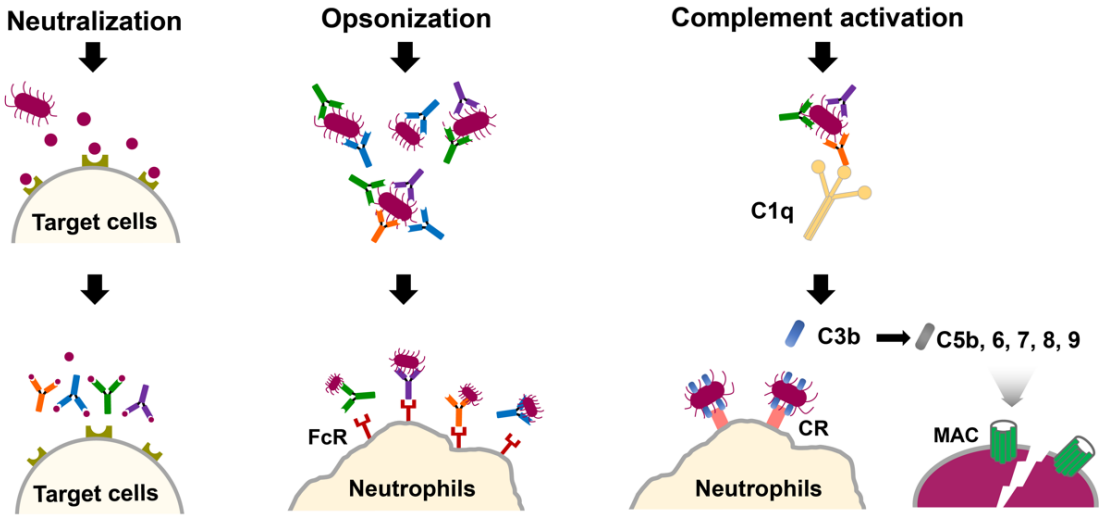


Figure 6. Overview of pathogen elimination mechanisms of antibodies

Antibodies mainly exert their effects through three mechanisms: neutralization, opsonization, and complement activation (Figure 6). Neutralization is mediated by the intrinsic binding capacity of antibodies to the corresponding pathogen epitopes, which leads to the inhibition of the infectivity and pathogenesis of pathogens[22]. Several different ways of neutralization have been described[116]. The most common type of neutralization is mediated by the antibodies that bind to adhesins on pathogens and therefore interfere with their attachment to target cells. In addition, antibodies, especially polymeric IgM and IgA, can bind to pathogens and induce aggregation[123,124]. Some antibodies can immobilize the pathogens and impede their random movement[125]. In some cases, neutralizing antibodies can directly inhibit the growth of pathogens or even kill them by forming pores on the outer membrane upon surface binding[126]. Among the various antibody classes and subclasses, IgG and IgA are strong neutralizing

antibodies due to their high binding affinity (Table I). While IgM has a low neutralizing ability on a per molecule basis, it is compensated by the avidity effect of its pentameric form. In contrast, IgD and IgE have no neutralizing capacity.

Beyond neutralization, the collaboration between antibodies and innate effector cells provides more efficient clearance of infections, especially for pathogens that cannot be neutralized by antibodies[22]. To this end, innate phagocytes such as neutrophils and macrophages are critical for host defense due to their surface expression of Fc receptors that bind to the Fc portion of antibodies[127]. The formation of immune complexes (ICs) as a result of binding between pathogens and antibodies can subsequently initiate a biological process referred to as "opsonization", through which the pathogens are phagocytosed and eliminated by effector cells via Fc receptor ligation. This is of particular importance as some pathogens are naturally resistant to phagocytosis[128], however, upon opsonization by antibodies, they can easily be recognized by effector cells. For example, in **Paper III** we showed that vaccine-induced antibodies can facilitate the clearance of *B. pertussis* by neutrophils through opsonization in vitro. In terms of opsonization, the different antibody isotypes and subclasses differ in their capacity (Table I). IgG1 and IgG3 are the most potent opsonizing antibodies due to their high binding affinity to Fc receptors.

Table I. Antibody isotypes and subclasses differ in their functions

	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	−	++	++	++	++	++	−
Opsonization	+	−	++	+−	++	+−	+−	−
Complement activation	+++	−	++	+	+++	−	+−	−

Antibodies can also function in synergy with the complement system[129]. It is important to note that only the classical complement pathway, being one of the three complement activation cascades, is associated with antibodies. In this scenario, antibodies first bind to pathogens to form ICs and then the ICs bind to the complement component C1 to initiate the classical complement activation, which ultimately generates molecule C3b. After that, C3b molecules are deposited on pathogens and form C3b-pathogen complexes, which can be recognized by complement receptors (CRs) expressed by phagocytes, and as a consequence being phagocytosed and eliminated by the cells. In addition, C3b can bind to C3 convertases to continue the complement cascades and finally generate other complement molecules including C5b, C6, C7, C8, and C9. These molecules assemble together to form the membrane attack complex (MAC), which creates a pore on the outer membrane of pathogens and leads to the lysis and death of organisms. While the antibody-dependent activation of classical complement pathway is not the only way to generate the MAC, this effect can be further promoted by specific antibodies. For example, several studies including ours (**Paper III**) showed that complement killing of *B. pertussis* can be promoted in the presence of vaccine-induced

antibodies. Amongst the different antibody molecules, IgM is the most potent at activating the complement especially when polymeric IgM is formed.

A major focus of investigation is to determine the immune correlates of protection in the case of vaccination or infection[104]. A better understanding of the correlates of protection can help us understand what type of immune response is favorable and therefore provides references for rational vaccine design. Early evidence has indicated that antibody titers can function as correlates of protection for pathogens such as tetanus and diphtheria[130]. However, for many infectious diseases, such as malaria, pertussis, and HIV, there is yet no consensus on definitive and quantitative immune correlates of protection. Therefore, instead of solely relying on the quantity, functional characteristics of antibodies may more likely serve as the candidates associated with protection. For example, recent evidence suggested that levels of complement-fixing antibodies were associated with protection in the case of malaria[131].

5 PERTUSSIS AND PERTUSSIS VACCINES

Pertussis, also known as whooping cough, is a highly contagious respiratory disease caused by gram-negative bacterium *Bordetella pertussis*[132]. During the pre-vaccine era, pertussis was one of the major causes of infant morbidity and mortality. Nowadays, pertussis has been largely controlled thanks to the wide use of vaccines. That being said, it still remains to be one of the major vaccine-preventable diseases, which affects infants and young children frequently[15].

5.1 HISTORY OF PERTUSSIS VACCINATION

The pertussis vaccine is usually combined with the vaccines against tetanus and diphtheria. These together form a combinatorial vaccine named DTP, which usually includes aluminum as the adjuvant. The first generation of the pertussis vaccine, whole cell pertussis vaccine (wPV), was developed in the 1930s and contained the entire formalin-killed bacterium[133]. During the 1940s in the United States (US), wPV was introduced and used in babies and young children with a high coverage as a routine immunization (Figure 7). In Europe, routine vaccination with wPV started in the late 1950s[134]. Since the wPV was globally used, pertussis became largely in control and the incidence almost dropped by 99%[135]. However, it was later noticed that wPV tended to induce adverse reactions, both locally and systemically. Especially, there was a possible association between the use of wPV and the occurrence of chronic neurological damages and encephalopathies, which raised a major concern in the public[135]. Because of the high reactogenicity, the use of wPV was reduced or even discontinued in some countries[135]. One example is that in Sweden the pertussis vaccination program was suspended from 1979 to 1996. This had resulted in two outbreaks of pertussis in 1983 and in 1985 in Sweden[136]. In countries with reduced coverage of wPV, there was also a sharp increase in the incidence of disease[137].

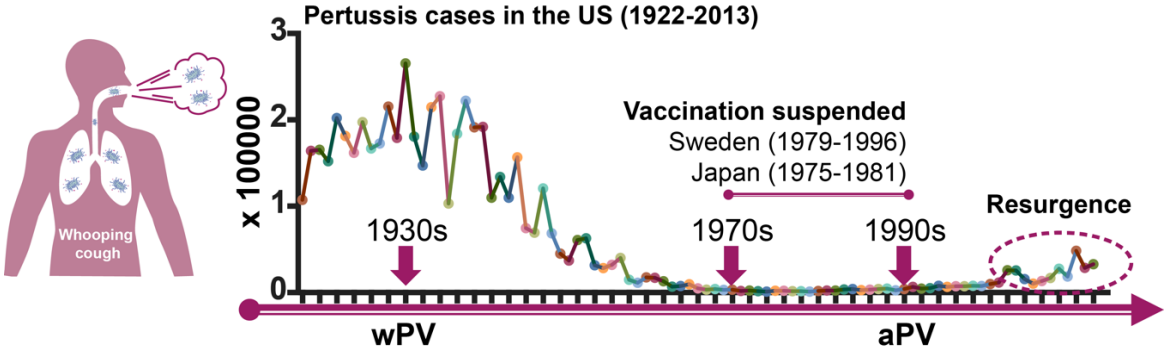


Figure 7. Overview of pertussis vaccination history and reported pertussis cases in the US

To tackle these problems, the next generation of pertussis vaccine, an acellular pertussis vaccine (aPV) was developed during the 1980-1990s[133]. aPV contains one to five inactivated *B. pertussis* immunogens in different combinations, accompanied by aluminum as an adjuvant. The most commonly used aPV formulation is a 5-component vaccine including pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), fimbriae 2 and fimbriae 3 (FIM2/3). aPV has now taken the place of wPV in the majority of industrialized countries. However, in less developed areas wPV is still being used because of the lower cost[138]. During the past

decades, an extensive course of immunization with aPV has provided a good level of protection and to a large extent controlled the disease[139]. However, it was noted that the incidence of pertussis has been growing during the last ten years even in industrialized countries with sustained vaccination history[133,140]. Some countries have even experienced outbreaks of pertussis occurring within school-age children that represent a highly-vaccinated population[133]. Further studies demonstrated that aPV-immunized individuals were more susceptible to *B. pertussis* infection compared to wPV-immunized people[141]. This has raised new concerns about the prevention of pertussis, especially the effectiveness of the current aPV[142,143]. Therefore, we are still facing the problem to develop more effective pertussis vaccines, which requires a more comprehensive understanding of pertussis immunity.

Recently, a live attenuated *B. pertussis* vaccine, BPZE1, was developed and has been tested in several animal studies[144-146], as well as in two human clinical trials (Jahnmatz, et al. In preparation)[147]. This live vaccine has been shown to elicit effective mucosal and systemic immune responses, long-lasting protection, and blocking of nasal colonization upon infection in animal models. In human clinical trials, BPZE1 showed excellent safety profiles and promising immunogenicity. In **Paper III**, we performed multiple analyses to in-depth characterize the immune responses in humans generated to BPZE1.

5.2 PERTUSSIS IMMUNITY

So far, considerable progress has been made to understand how the immune system responds to *B. pertussis* infection. Three major steps are involved in the pathogenesis and the induction of host immunity (Figure 8): (*a*). Attachment of *B. pertussis* to the lung epithelium and formation of colonies, (*b*). Release of toxins that cause tissue damages, inflammation and immune cell infiltration, (*c*). Induction of systemic adaptive immune responses. Substantial evidence has demonstrated that effective protection against *B. pertussis* is multifactorial and requires both specific antibodies and T cell responses, in synergy with innate effector cells[148].

5.2.1 Innate immunity to *B. pertussis*

B. pertussis, as a respiratory pathogen, invades the host by attaching to the lung epithelial cells through adhesins such as FHA and PRN, which allows them to further grow and colonize the epithelium. This is usually accompanied by the release of virulence factors, such as dermonecrotic toxin (DNT) and tracheal cytotoxin (TCT), which cause local tissue damage and generate inflammation[149,150]. Following *B. pertussis* infection, the innate immune system is first alerted[151]. Complement molecules present on mucosal surface of upper respiratory compartments usually provide the initial phase of defense. However, *B. pertussis* has developed a range of strategies to evade complement killing[152]. One typical mechanism is the production of BrkA (*Bordetella* resistance to killing A) that inhibits the deposition of complement molecules on bacterial surface[153].

Following the reaction of complement system, local production of inflammatory molecules and infiltration of immune cells to the sites of infection are the succeeding innate immune events. In mice and baboons, *B. pertussis* infection was shown to induce an early production of

inflammatory cytokines (IL-6, IL-8 and IL-1β) and chemokines (monocyte chemoattractant protein-1, MCP-1 and macrophage inflammatory protein 1 alpha, MIP-1α) in the nasopharynx[154,155]. Infection of naïve mice with *B. pertussis* resulted in an early infiltration of DCs and macrophages into the lung, followed by an influx of neutrophils and NK cells[148]. DCs, being professional APCs, can recognize pathogen-associated molecular pattern (PAMPs) molecules on *B. pertussis*, take up the bacteria, undergo cell activation/maturation, and finally activate cognate T cells via antigen-presentation. A unique subset of DCs ($CD11c^{+}CD8\alpha^{+}$ DCs) are highly involved in the generation of Th1 cell response against *B. pertussis*[156]. In addition, DCs primed by ACT were able to induce pertussis-specific Th17 response through the activation of inflammasome and IL-1β production[157]. Macrophages, being potent phagocytes with inherent bactericidal ability, also play a role in clearing *B. pertussis*. Their killing activity can be further augmented by IFN-γ and IL-17[158,159]. However, ACT was shown to induce the apoptosis of macrophages[160,161]. Some evidence also showed that *B. pertussis* can evade the killing by macrophages and replicate in macrophages by residing in non-acidic compartments within the cells, suggesting that macrophages may serve as intracellular haven for *B. pertussis*[162].

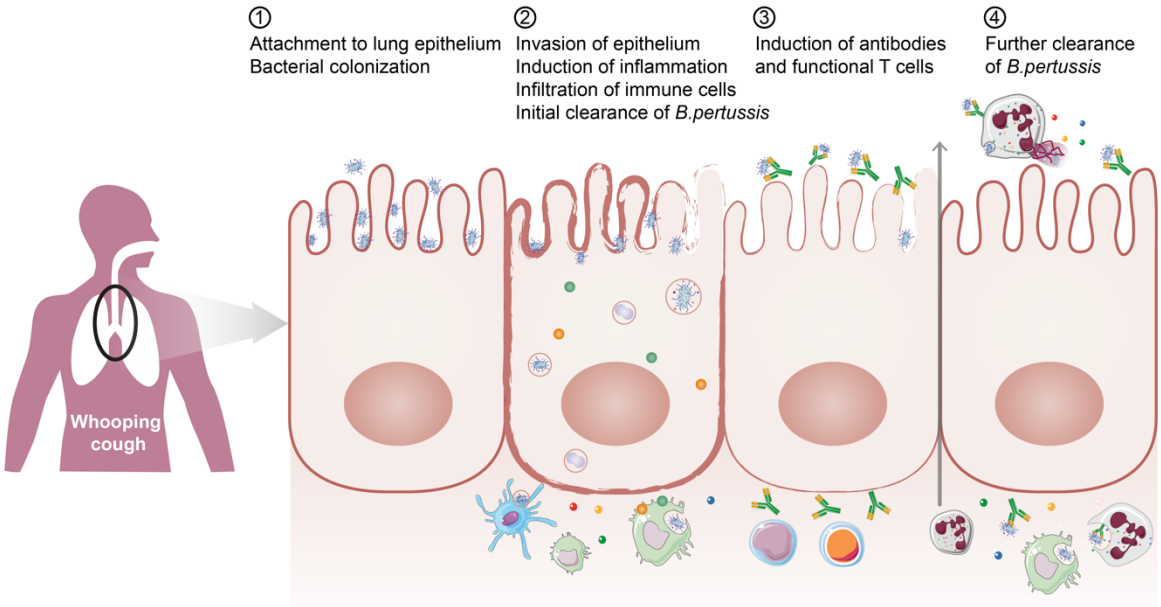


Figure 8. Pathogenesis of B. pertussis and the induction of host immunity

In naïve mice, neutrophils are not the first wave of cells migrating to the local sites following *B. pertussis* infection[163]. This is mainly attributed by the toxins released by *B. pertussis* that inhibit chemotaxis, for example PT and ACT[164,165]. In addition, ACT can inhibit phagocytosis and oxidative burst of neutrophils, thus impairing their killing functions, which was proposed to be one major strategy of immune evasion by *B. pertussis*[166]. This explains why neutrophils are not helpful in clearing *B. pertussis* at early stage following infection in naïve mice. However, neutrophils are the key players to clear infection in convalescent mice, which is caused by the presence of pre-existing antibodies that can neutralize the immune-inhibitory toxins and opsonize bacteria to form ICs that facilitate phagocytosis[163]. Although some evidence showed that *B. pertussis* can also survive within subcellular structures in

neutrophils[167], it is unlikely that neutrophils serve as intracellular reservoirs for *B. pertussis* due to their relatively short lifespan. In addition, in-vitro evidence demonstrated that fresh neutrophils possess killing capacity of *B. pertussis,* which can be further promoted upon stimulation with cytokines that activate neutrophils[168,169].

So far, limited information is available regarding the innate immune responses in vivo following intramuscular (i.m.) administration with wPV or aPV. However, it is possible that injection of wPV or aPV into the muscle sites can induce local inflammation and immune cell infiltration, which are typical innate immune events observed in other similar contexts[26,170]. wPV contains the whole organism that includes a wide range of molecules with inherent immune-stimulatory functions. Even the limited antigens included in aPV formulation are able to activate innate immune system. For example, PT and ACT can activate human monocyte-derived DCs in vitro[171]. In contrast to wPV and aPV, BPZE1 as live attenuated bacteria administrated via intranasal route is supposed to elicit similar innate immune signatures as by natural infection. Of note, BPZE1 produces the inactive form of PT that does not have inhibitory effect on chemotaxis of neutrophils. This leads to that intranasal administration of BPZE1 into naïve mice can induce influx of neutrophils to the lung at early time points[172], which is not observed in naïve mice challenged with virulent *B. pertussis* as mentioned.

5.2.2 Antibody responses to *B. pertussis*

Following *B. pertussis* infection, antibodies slowly develop and usually reach the peak after 4 weeks. Early studies showed that passive transfer of serum from convalescent mice conferred a level of protection in sublethally irradiated mice[173]. Similarly, passive transfer of serum from wPV- or aPV-immunized mice conferred effective protection in naïve mice upon virulent challenge[174]. Further studies demonstrated that antibodies specific to PT and PRN mainly contributed to the protection[174]. The protective function of *B. pertussis*-specific antibodies was also described in humans where infected children receiving anti-*B. pertussis* Ig treatment had demonstrated a less duration of symptom[175].

The duration of the protective response against pertussis after infection or vaccination has been estimated[176]. It is believed that neither natural infection nor vaccination is able to elicit lifelong protection. The estimated duration of protection conferred by infection ranges from 7 to 20 years. wPV-induced protection was speculated to persist for 4-14 years, which is longer than that induced by aPV (3-10 years). It is therefore believed that waning immunity induced by aPV is the major cause of pertussis resurgence[139]. However, studies showed that by 2 years following infection or vaccination with wPV or aPV, titers of pertussis-specific antibodies are barely detectable, whereas the protective activity remains[176]. This suggests that it is not only the antibody titer that should be taken into consideration when evaluating protective responses, the quality and functionality of antibodies may be equally important. This also raises another question about the immune correlates of protection against pertussis that is yet to be defined. Although early clinical data suggested that the anti-PT, PRN, FIM IgG level was indicative of protection[177], there is still no consensus on this and no reliable threshold has been established[130,178]. Since *B. pertussis* has a complex antigen composition and a large proportion

of antigens overlap with other pathogens, it is likely that other immunogenic antigens associated with protection exist. To this end, I have in my studies (**Paper III**) identified novel *B. pertussis* immunogens that may potentially be related to protection.

Functions of antibodies are mediated by the aforementioned mechanisms and are closely related to the antibody isotypes and subclasses (Table I). IgG being the most abundant antibody has been well investigated in both humans and animal models in the context of infection or pertussis vaccination. Infected mice or wPV-immunized mice showed a higher level of IgG2, IgG3 and lower level of IgG1 against *B. pertussis* antigens when compared to aPV-immunized mice[179]. In humans, IgG1 was elevated in both wPV- and aPV-immunized individuals. IgG4 was only induced in subjects receiving repeated aPV immunization, whereas the induction of IgG3 was only seen in subjects primed with wPV[180]. Altogether, this suggests that a Th1- and Th2-polarized antibody response is generated by wPV and aPV, respectively (Figure 9). The four IgG subclasses differ in their ability in opsonization and complement activation, which may contribute to the different efficiency of wPV and aPV. In **Paper III**, we dissected the antibody responses induced by BPZE1 compared to that induced by aPV. BPZE1- and aPV-immunized individuals developed Th1 and Th2 type antibodies, respectively. We found that this further led to that BPZE1-induced antibodies were more potent to stimulate reactive oxygen species (ROS) production in neutrophils and enhance bactericidal function.

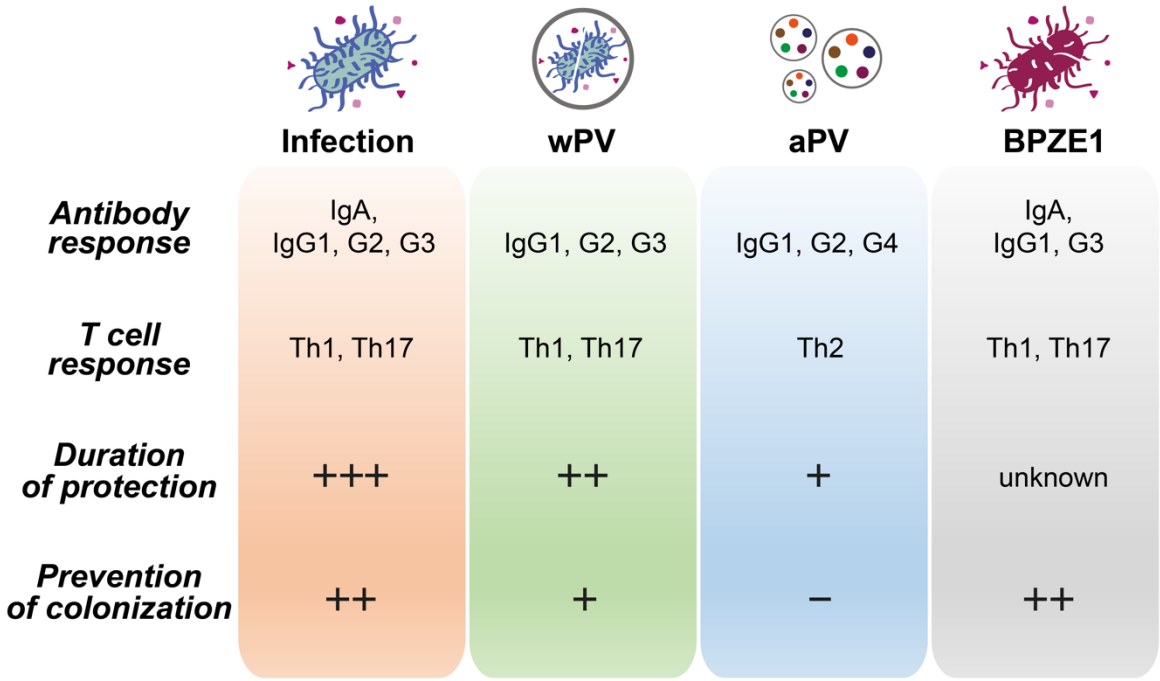


Figure 9. Types and quality of immune responses induced by B. pertussis infection or immunization with different types of pertussis vaccines

Apart from IgG, IgA is also important to control *B. pertussis* infection, especially the secretory IgA (sIgA) residing at mucosal sites. It has been shown that both wPV and aPV are not able to elicit serum IgA in naïve mice, whereas natural infection does so[179]. The findings that wPV- or aPV-immunized individuals could develop an IgA response are largely attributed to their earlier natural exposure[181]. However, it is important to note that the route of vaccine

administration may influence the generation of IgA response. An intranasal administration of wPV was shown to induce both IgA in serum and sIgA in nasal fluid[182]. A recent study showed that even aPV, when given via intranasal route, could elicit protective sIgA response in mice[183]. These provide new vaccination strategies that could be utilized to improve vaccine efficacy.

5.2.3 T cell responses to *B. pertussis*

While an antibody response is critical for combating *B. pertussis* infection, it may not be sufficient to provide a fully protective response. It has been shown that effective protection against pertussis requires both specific antibodies and $CD4^+$ T cells[184].

Substantial evidence has implied the importance of cellular T cells in pertussis immunity. Of note, the distinctly polarized T cell subtypes influence the quality of protection differently (Figure 9). It has been well-evidenced that Th1 and Th17 cells appear to be more effective at conferring protection than Th2 cells[185]. Natural infection or wPV immunization was shown to induce Th1 and/or Th17 $CD4^+$ T cells, but no or limited Th2 cells[186,187]. Animal studies showed that IFN-$\gamma^{-/-}$ or IL-$17A^{-/-}$ mice were not able to clear *B. pertussis* infection, which proved the importance of Th1/Th17 cells in this scenario[186,188]. In line with this, T cells isolated from infants diagnosed with pertussis or at convalescent stage secreted IFN-γ and IL-2, but no or very limited level of IL-4 and IL-5[189]. In addition to the systemic T cell responses, emerging studies have focused on tissue-resident memory T (T_{RM}) cells in the context of pertussis. In *B. pertussis*-infected mice, a population of IL-17 and IFN-γ-producing CD4 T_{RM} cells were induced in the lung and these cells quickly expanded upon secondary infection[190]. Importantly, passive transfer of isolated CD4 T_{RM} cells conferred a level of protection in the recipient mice. Similar results were described in wPV-immunized mice that showed a development of IL-17 and IFN-γ-producing CD4 T_{RM} cell response within the respiratory compartment[191]. In contrast to natural infection or wPV immunization, aPV immunization mainly induces a Th2-biased T cell response. However, it has been shown that IL-$4^{-/-}$ mice and wide type mice could clear *B. pertussis* infection at a similar rate upon virulent challenge, suggesting that Th2 cells are not essential to control pertussis[186]. Moreover, aPV immunization was not able to generate CD4 T_{RM} cells in the lung as that by wPV immunization or natural infection[191].

T cell responses elicited by the live vaccine BPZE1 have also been studied. It has been shown that BPZE1 could induce Th1 and Th17 cellular responses in mice similar to natural infection. IL-17 and IFN-γ-producing CD4 T_{RM} cells also appeared at nasal sites upon BPZE1 immunization[192]. In **Paper III**, we detected a Th1 $CD4^+$ T cell response in the blood induced by BPZE1[193]. While Th17 cells were not detectable in the circulation, they may be elicited but reside in the airway mucosa.

Finally, although early studies indicated that $CD8^+$ T cells are not needed for the protection against pertussis[184]. Recent evidence proposed a potential role of cytotoxic $CD8^+$ T cells, especially due to the intracellular infection properties of *B. pertussis*[162]. IFN-γ-producing $CD8^+$ T cells were shown to be induced in *B. pertussis*-infected individuals[194]. A booster with aPV was able to generate pertussis-memory $CD8^+$ T cells[195]. Anyhow, IFN-γ secreted from

activated $CD8^+$ T cells may help control the infection by activating innate effector cells such as neutrophils and macrophages.

6 MATERIALS AND METHODS

Detailed information is available in the results and discussion session, and in the papers.

6.1 IMMUNIZATIONS

All animal studies in this thesis were approved by the Animal Care and Use Committees of the Vaccine Research Center, National Institutes of Health (**Paper I**), or the Stockholm Local Ethical Committee on Animal Experiments (**Paper II**). Approval for human studies in **Paper III** was granted by the Swedish Medical Product Agency (MPA) and the Stockholm Local Ethical Committee.

In **Paper I**, Indian rhesus macaques were immunized with 100μg of recombinant HIV-1 envelop glycoprotein (Env) (TV1 strain, Novartis) in adjuvant via i.m. injection at 0, 4, 12 and 24 weeks. After one and half years, the animals received a boost immunization with Alexa Fluor 680 labeled Env (AF680-Env, 100μg) in one arm and PBS in the contralateral arm as control. In **Paper II**, Chinese rhesus macaques were i.m. immunized with a vaccine (50μg) composed of mRNA encoding for the hemagglutinin (HA) of H10N8 influenza A virus formulated in a lipid nanoparticle (LNP) at 0 and 4 weeks. For vaccine tracking experiments, the animals received vaccination with an Atto-655 labeled LNP/mCitrine mRNA vaccine (50μg) in one arm and PBS in the contralateral arm as control. In **Paper III**, the human clinical trials were registered in ClinicalTrial.gov, NCT02453048 (BPZE1 vaccine study) and NCT00870350 (Tetanus, diphtheria, and pertussis, Tdap5 vaccine study). In BPZE1 vaccine study, healthy adults (18-32 years of age) received a single intranasal administration with 10^9 colony forming units (CFU) of BPZE1 in a volume of 0.4 mL per nostril. In Tdap5 vaccine study, adolescents (14-15 years of age) with a vaccination history of three primary immunization of DTaP5 (at 3, 5, and 12 months of age) plus a boost immunization of Tdap5 (at 5.5 years of age) received another booster dose of Tdap5. DTaP5 and Tdap5 vaccines contain the same components. Pertussis vaccine is composed of 5 protein antigens including FHA, PT, PRN, FIM2, and FIM3. Tdap5 has a reduced dose of the diphtheria and pertussis vaccine antigens compared to DTaP5.

6.2 SAMPLING AND CELL ISOLATION

In **Papers I-II**, muscle tissues of the injection site and injection site draining LNs were collected and processed to obtain single cell suspension as previously described[26,196]. In **Papers I-III**, human and rhesus PBMCs were isolated from fresh blood using Ficoll (GE Healthcare) gradient centrifugation. Human monocytes and neutrophils were isolated from fresh peripheral blood using RosetteSep™ Human Monocyte Enrichment Cocktail (STEMCELL Technology) and Polymorphophrep solution (Axis-Shield), respectively. For cell sorting experiments, different cell populations were purified using fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS).

6.3 FLOW CYTOMETRIC ANALYSIS

Cells were washed and stained with LIVE/DEAD Fixable Blue Dead Cell Stain Kit (ThermoFisher Scientific) prior to incubation with human FcR blocking reagent and antibody cocktails for 20 min at room temperature (RT) in the dark. Intracellular staining of cytokines was performed using Fixation/Permeabilization Kit (BD Bioscience) according to the manual. Flow cytometric analysis was carried out on LSRFortessa cell analyzer (BD Biosciences). Data was analyzed using FlowJo V.10.1 (Tree Star).

6.4 ANTIGEN PRESENTATION ASSAY

Different subsets of APCs were loaded with antigens for 1h and then co-cultured with carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled autologous purified $CD4^+$ T cells at a ratio of 1: 10. After 5 days of culture, proliferation of $CD4^+$ T cells was measured by flow cytometry.

6.5 ANTIBODY ELISA ASSAY

96-well half-area plates (Greiner bio-one, Germany) were coated with antigens or bacterial lysates overnight at 4°C. Serum samples were diluted in PBS-T containing 0.2% BSA prior to incubation at RT. Total IgG was evaluated using HRP-conjugated mouse anti-human IgG. IgA and IgG subclasses were evaluated using mouse anti-human IgA, IgG1, IgG2, IgG3, IgG4 and further analyzed with HRP-conjugated sheep anti-human IgG (Jackson). TMB Substrate (Biolegend) was used for development and the absorbance was read at 450nm (minus 550nm for wavelength correction).

6.6 MEMORY B CELL ELISPOT

In **Paper III**, antigen-specific memory B cells were evaluated by ELISpot. MAIPSWU 96-well plates (Millipore) were coated with vaccine antigens. PBMCs were stimulated for 4 days to expand memory B cells and then added to the respective wells for 24h culture, followed by sequential incubation with biotinylated goat anti-human IgG or IgA and streptavidin-conjugated alkaline phosphatase. Spots were developed with BCIP/NBT substrate (Mabtech) and counted using an AID ELISpot Reader (Autoimmun Diagnostika). Results were depicted as spot-forming cells (SFC) per million stimulated cells.

6.7 ANTIBODY OPSONIZATION ASSAY

10^7 CFU of live BPZE1 were incubated with pooled serum from vaccinees at 37°C for 25min, followed by co-culture with fresh human neutrophils ($1x10^6$ cells). After 1h of incubation, cells were evaluated by flow cytometric analysis to evaluate the activation and maturation. Production of reactive oxygen species was measured using cellular ROS Assay Kit (Abcam) according to the manual.

6.8 2-DIMENSIONAL IMMUNOBLOTTING

Isoelectric focusing (IEF) was performed to separate protein of bacterial lysates by the difference in isoelectric point using an Ettan IPGphor 3 IEF system (GE Healthcare). After IEF, 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to further separate the proteins by molecular weight. After SDS-PAGE, proteins were transferred to polyvinylidene difluoride (PVDF) membrane in a Trans-Blot Turbo Transfer System (Bio-Rad). The membrane was blocked in 5% skim milk overnight at 4°C, followed by sequential incubation with pooled serum. For IgG detection, membrane was incubated with HRP-conjugated mouse anti-human IgG. For IgA detection, membrane was incubated with mouse anti-human IgA followed by incubation with HRP-conjugated sheep anti-mouse IgG. After all incubations, the signals were visualized using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare) under ChemiDoc MP Imaging System (Bio-Rad).

6.9 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

Protein spots were excised manually from CBB G250-stained acrylamide gel and placed in Eppendorf tubes for in-gel digestion. Following sequential washing, reduction, alkylation and digestion, the tryptic peptides were acidified and further cleaned up on C-18 Stage Tips (Thermo Scientific). The reconstituted peptides were first separated on a 50 cm long EASY-spray column (Thermo Scientific) connected to an Ultimate-3000 nano-LC system (Thermo Scientific). Mass spectra were acquired on a Fusion Orbitrap mass spectrometer (Thermo Scientific) for full mass, followed by data-dependent HCD fragmentations from most intense precursor ions. The tandem mass spectra were acquired and analyzed using the Mascot search engine v.2.5.1 (Matrix Science Ltd., UK) and searched against *B. pertussis* protein database from UniProt (6,516 entries). Initial search results were filtered with 5% FDR using Percolator to recalculate Mascot scores.

7 RESULTS AND DISCUSSION

7.1 NEUTROPHILS PRESENT VACCINE ANTIGENS TO COGNATE MEMORY T CELLS (PAPER I)

Several early studies demonstrated that neutrophils could acquire the antigen-presenting function upon cytokine stimulation in vitro[58,59]. These findings were overlooked for a long time mainly because of the assumed short lifespan of neutrophils. However, emerging evidence has shown that neutrophils can survive much longer than originally thought. Their half-life was estimated to range from 13 to 19 hours in vivo under homeostasis and to be even longer in non-homeostatic conditions[197,198]. Apart from that, several studies including ours have shown that upon infection or vaccine injection, neutrophils are the first and most abundant cells that migrate to the sites of infection/injection, take up the antigens, and transport them to secondary lymphoid organs such as LNs[26,170,199]. Based on that, we speculated that neutrophils may play a role in regulating T cell responses if they could act as APCs. The first study (**Paper I**) in my Ph.D. work therefore revisited this topic and found evidence to support that neutrophils can function as APCs in vitro and ex vivo.

First, we assessed the antigen-presenting function of human neutrophils in a side-by-side comparison with several other APC subsets. We established a flow cytometry-based approach to isolate distinct DC subsets, monocytes, neutrophils, and $CD4^+$ T cells with high purity from healthy cytomegalovirus (CMV)- or influenza virus- seropositive individuals. The sorted APC subsets and neutrophils were pulsed with CMV pp65 antigens or influenza HA antigens, followed by co-culture with autologous CFSE-labeled $CD4^+$ T cells for 5 days. We found that BDCA-1^+ myeloid DCs (MDCs) being classical potent APCs demonstrated superior capacity to present the antigens and stimulate antigen-specific T cell proliferation. Two subsets of monocytes ($CD14^+$ monocytes and $CD14^-CD16^+$ monocytes) showed moderate to high antigen-presenting ability. Neutrophils and plasmacytoid DCs (PDCs) demonstrated low but persistent capacity to present the antigens and induce antigen-specific T cell proliferation (Figure 10A).

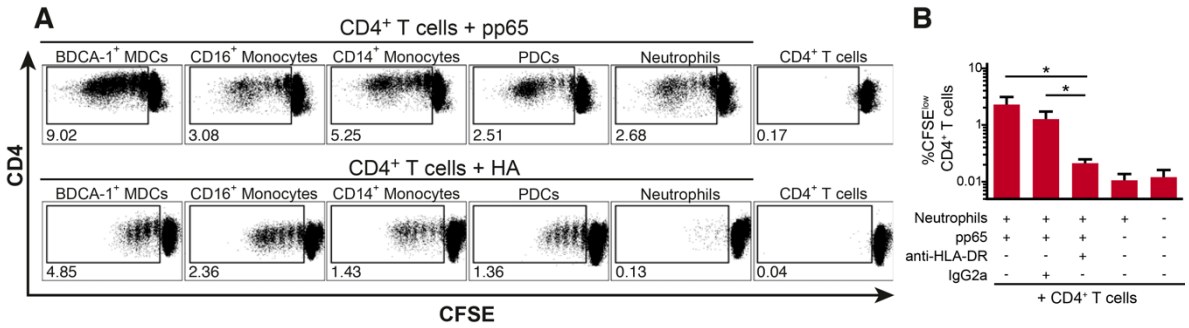


Figure 10. (A). Antigen presentation by distinct APC subsets and neutrophils. (B). Antigen presentation by neutrophils is dependent on HLA-DR.

Engagement between the MHC-II-peptide complex on APCs and T cell receptor (TCR) on T cells is indispensable for the induction of antigen presentation[200]. The observed antigen presentation by neutrophils was also mediated by HLA-DR on neutrophils as the presence of

HLA-DR neutralizing antibodies dramatically reduced T cell proliferation (Figure 10B). However, we and others have shown that fresh neutrophils are devoid of HLA-DR on the cell surface[201], which we found was further supported by their inability to elicit a mixed lymphocyte reaction (MLR) when co-cultured with allogeneic T cells (Figure 11A).

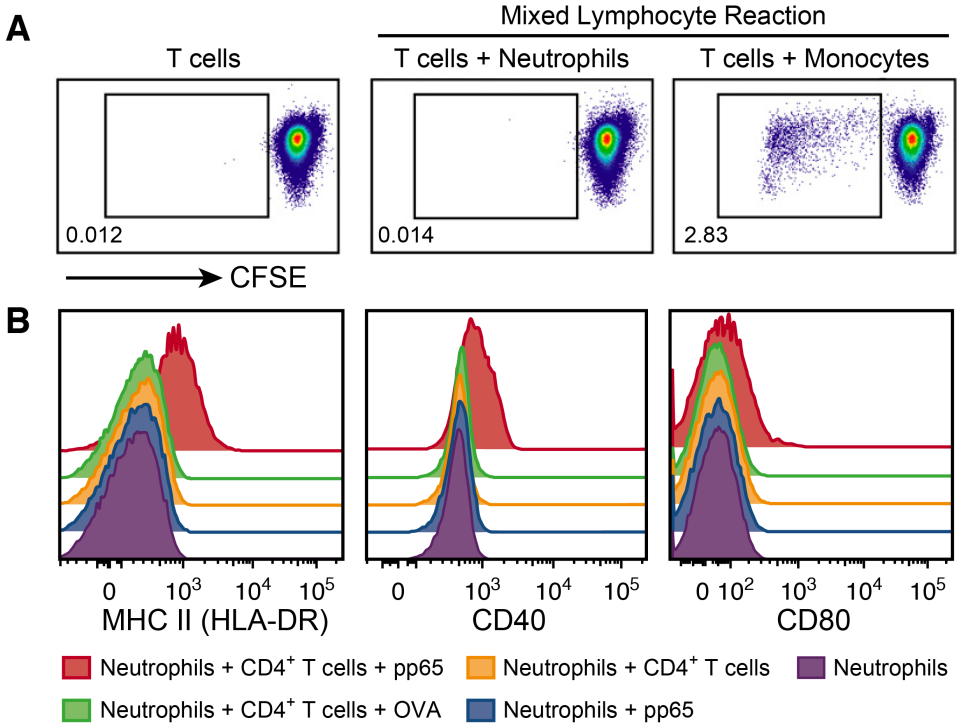


Figure 11. (A). Neutrophils cannot drive allogeneic T cell proliferation in a MLR system. (B). Neutrophils acquire the expression of HLA-DR, CD40 and CD80 when co-cultured with autologous CD4$^+$ memory T cells and cognate antigens.

This raised the question of how neutrophils acquired the expression of HLA-DR to suffice them as APC. Using cells from CMV-seropositive individuals, we found that neutrophils expressed a marked level of surface HLA-DR and costimulatory molecules (CD40 and CD80) when they were incubated with autologous CD4$^+$ T cells and cognate pp65 antigens (Figure 11B). Of note, expression of these APC machinery on neutrophils required the presence of both antigens and antigen-specific memory T cells. These molecules were not induced on neutrophils that had been cultured with T cells or antigens alone, or with T cells in the presence of irrelevant antigens such as ovalbumin (OVA). Neither were they induced when using cells sorted from CMV-seronegative donors.

To further evaluate the antigen-presenting function of neutrophils ex vivo, we utilized rhesus macaques that had received previous immunizations with the HIV-1 vaccine candidate Env glycoprotein and developed Env-specific memory T cell responses. We re-immunized the animals with AF680-Env and isolated the AF680-Env$^+$ neutrophils and MDCs from vaccine draining LNs (dLNs) at 24h after injection (Figure 12A). As control, neutrophils and MDCs were isolated from PBS dLNs after receiving PBS injection in the contralateral arm. After a 5-day co-culture of autologous CD4$^+$ T cells and AF680-Env$^+$ neutrophils, we found that neutrophils that had taken up the vaccine antigens were able to induce robust proliferation of CD4$^+$ T cells through antigen presentation (Figure 12B). As expected, AF680-Env$^+$ neutrophils

showed a much higher expression of HLA-DR than neutrophils sorted from PBS dLNs (Figure 12C).

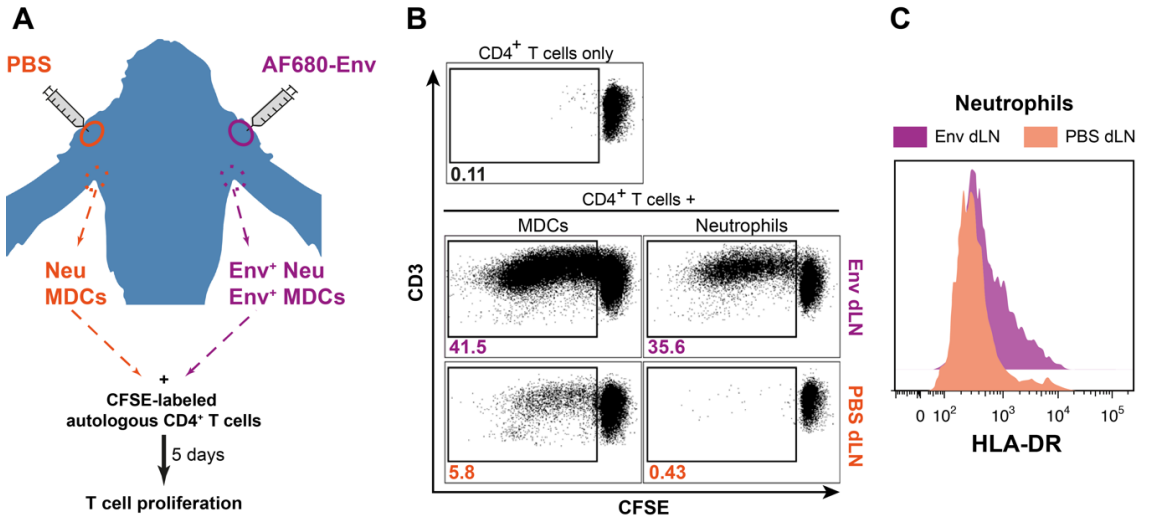


Figure 12. (A). Experiment design for the evaluation of antigen presentation ex vivo. (B). Env$^+$ neutrophils and MDCs could present antigens to antigen-specific T cells. (C). Env$^+$ neutrophils showed a higher level of HLA-DR than neutrophils sorted from PBS dLN.

Collectively, this study demonstrated that neutrophils can acquire antigen-presenting function and can be thought of as atypical APCs. However, the physiological significance remains elusive. In the context of vaccination, as neutrophils are the predominant cells transporting vaccine antigens to LNs they may be able to play a considerable role to act as APCs to activate memory T cells. Although their antigen-presenting capacity cannot be compared with that of classical APCs, their abundance may compensate for some of their weakness. However, the considerable uptake of administrated vaccine antigens by neutrophils may lead to a shortage of antigens for other classical APCs, thus resulting in less efficient generation of T cell responses[202]. Nevertheless, another possibility is that even if neutrophils internalize a large proportion of the administrated antigens and fail to present them timely, these antigens may be re-captured in the form of apoptotic neutrophils in the end by adjacent APCs. Therefore, the role that neutrophils play in regulating vaccine-specific T cell responses is intriguing and merits further investigation.

7.2 RHESUS MDSCS ARE INDUCED BY VACCINE ADMINISTRATION (PAPER II)

Heterogeneity of neutrophils has recently been described in many diseases[35]. In addition to the immune-stimulatory functions such as antigen presentation that we studied in **Paper I**, some neutrophils, for example the subpopulation known as PMN-MDSCs[203], exhibit immune-suppressive functions. In the vaccine field, much focus so far has been put on understanding how vaccines stimulate immune response, but little is known about the negative immune regulators. Information on whether MDSCs change in response to prophylactic vaccination and whether they can suppress the stimulation of vaccine responses is extremely limited. Therefore, in **Paper II**, using NHP as experimental model, we studied these suppressive PMN-MDSCs as well as M-MDSCs with the aim to understand their role in the context of vaccination.

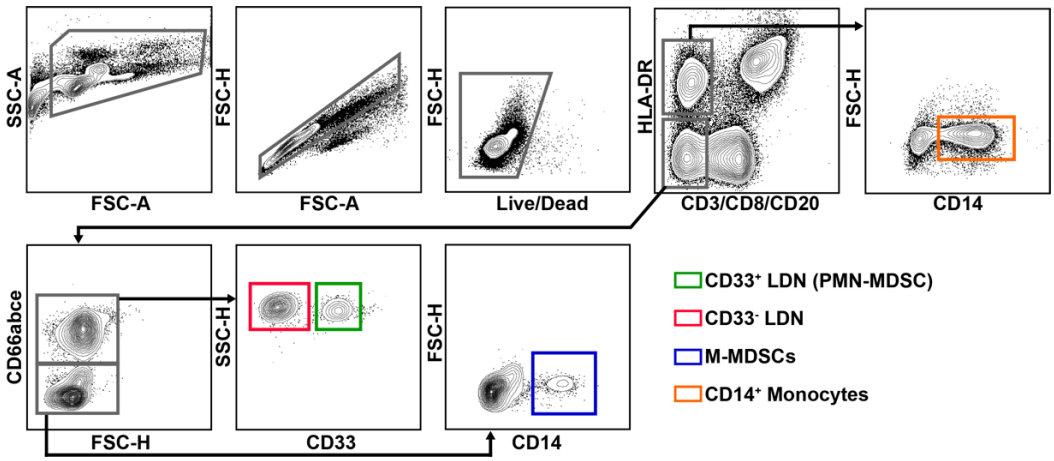


Figure 13. Phenotypic identification of MDSCs in rhesus PBMCs by flow cytometry.

In contrast to the MDSCs in humans and mice[76], the knowledge of MDSCs in NHPs is very limited. A few studies on MDSCs in rhesus macaques have been published, but the information on the phenotypes of these cells is variable and even conflicting between studies[102,204,205]. Therefore, the first aim of this study was to establish a method to identify rhesus MDSCs to ensure an accurate monitoring of these cells. We established a staining panel of antibodies for the phenotyping of rhesus MDSCs using flow cytometry, and further developed a strategy to functionally characterize them based on their suppressive functions. We found that M-MDSCs and PMN-MDSCs exist in rhesus blood at steady state, which can be identified as HLA-DR$^-$Lin$^-$CD14$^+$CD11b$^+$ cells and HLA-DR$^-$CD66abce$^+$CD33$^+$ LDNs, respectively (Figure 13). Importantly, both M-MDSCs and PMN-MDSCs exerted strong inhibitory activities on T cell proliferation in vitro, which confirmed their suppressive function (Figure 14).

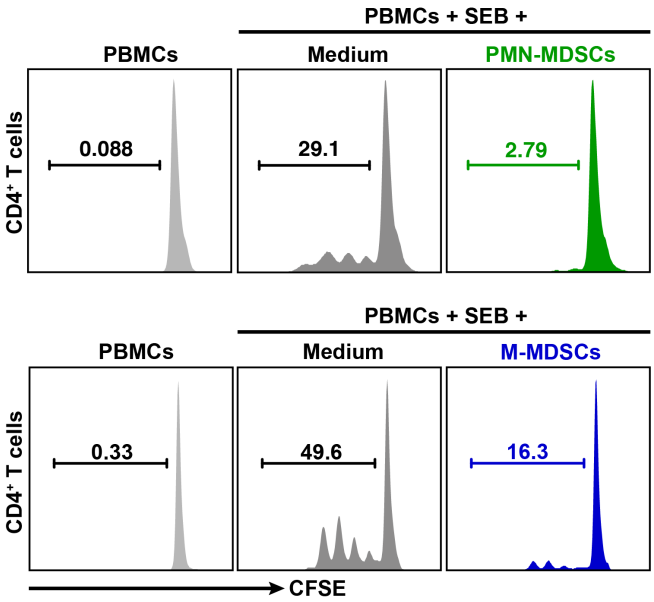


Figure 14. PMN-MDSCs and M-MDSCs suppress T cell proliferation. SEB: staphylococcal enterotoxin B.

To monitor the MDSCs at early time points after vaccinations, we utilized animals that had received an mRNA vaccine encoding for the influenza HA antigen of an H10 strain and developed well-detectable H10-specific T cell and antibody responses. Blood samples from these animals were used to analyze the frequencies of MDSCs. Before termination, the animals also received i.m. injections of the vaccine or PBS in separate arms. This gave us the opportunity to analyze MDSCs in multiple tissue compartments. Prior to and at 24h after injection, the frequencies of MDSCs at different compartments were measured, including blood, injection site (muscle tissue), as well as injection site dLNs (Figure 15). We found that M-MDSCs transiently increased in the blood and vaccine injection site at 24h post vaccine administration, accompanied by an increase of

non-suppressive CD14^{+} monocytes as we reported earlier[170]. We also detected a clear infiltration of monocytes into the vaccine-dLNs, but not for M-MDSCs. Both PMN-MDSCs and the counterpart non-suppressive CD33^{-} LDNs showed a trend of increase in the blood. A clear infiltration of SSC^{high}CD66abce^{+} neutrophils in vaccine injection site and vaccine dLNs was also observed. But due to the limited information about the phenotype of tissue PMN-MDSCs, we could not further distinguish them from other neutrophil subsets. Further, through transcriptomics analysis of MDSC-related genes in the injection site biopsies, we found that a wide range of genes involved in the expansion, functions, and migration of MDSCs were expressed at a much higher level in vaccine injection site than the control PBS injection site.

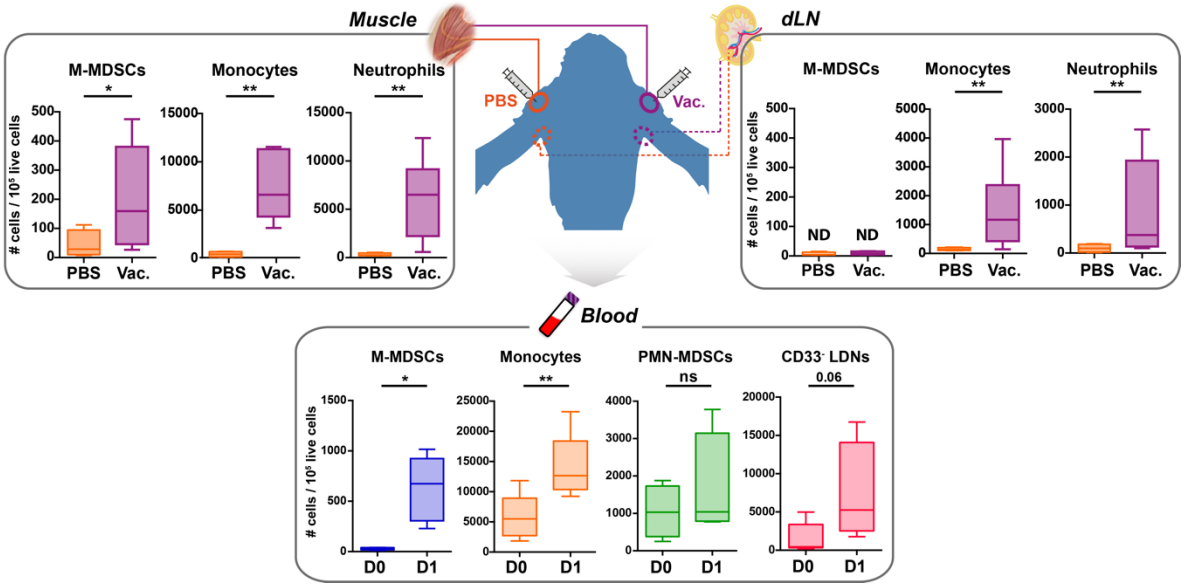


Figure 15. Frequencies of MDSCs, CD14^{+} monocytes, and neutrophils subsets in the blood, injection sites, and the corresponding dLNs at day 1 post injection.

While we showed that MDSCs were induced after vaccination, these cells only increased transiently at an early time point (day 1) and had returned to pre-vaccination level after 7 days. Importantly, despite the finding of an increase of MDSCs, the generation of adaptive vaccine responses was successful and showed well-detectable specific T cell and antibody responses, which suggest that any suppressive capacity these cells exert is not detrimental to the induction of vaccine responses. Vaccine administration usually generates a robust level of local or systemic inflammation that favors the induction of immune responses. However, this type of inflammation needs to resolve timely to avoid excessive inflammation, otherwise leading to a high reactogenicity or even autoimmune reactions. We therefore speculate that the early generation of MDSCs is a reaction of immune system to counteract the inflammatory reactions and therefore plays an important role in the context of vaccine administration.

7.3 A LIVE *B. PERTUSSIS* VACCINE BPZE1 ELICITES TH1 TYPE BROAD ANTIBODY RESPONSES IN HUMANS (PAPER III)

As mentioned earlier, control and prevention of pertussis remain to be an important public health issue due to the rise of pertussis cases during the past years[15]. Although the currently used aPV confers protection against disease, the rapid waning of aPV-induced immunity and inability to block nasal colonization and thereby reduce transmission compared to wPV are believed to cause the resurgence of pertussis[206,207]. There is therefore a critical need for a better pertussis vaccine with high efficacy.

To this end, a live attenuated BPZE1 candidate pertussis vaccine has been developed over several years[144]. This BPZE1 strain was developed through three genetic modifications with the aim to remove DNT, inactivate PT and markedly reduce the production of TCT. In animal models, BPZE1 demonstrated excellent efficacy evidenced by the generation of mucosal and systemic immune responses, long-lasting protection, and the prevention of nasal colonization upon virulent challenge[145,192]. Clinical phase I trials in humans were initiated in Sweden as Sweden had suspended pertussis vaccination for several years (1979-1996) and therefore human study subjects who were vaccination naïve were available. BPZE1 was provided by ILiad Biotechnologies, USA and the clinical studies were led by the Public Health Agency of Sweden. In humans, BPZE1 was found to show good safety and dose-escalating studies showed promising immunogenicity and colonization in the nasal cavity[147]. We were contacted to assist in the work to further dissect the immune responses induced by BPZE1 in humans, which expanded into a large project where we performed multiple analyses to evaluate a wide range of immune responses to BPZE1, with particular focus on the antibody profiles (Figure 16). In addition, since BPZE1 is an anti-bacterial vaccine, it offered us an opportunity to further study how antibodies cooperate with myeloid effector cells such as neutrophils to clear the infection. We also compared the magnitude and quality of antibodies in the BPZE1 vaccinees with that induced by the standard aPV immunization, which helped us to obtain mechanistic insights into immune functions important for pertussis protection and control.

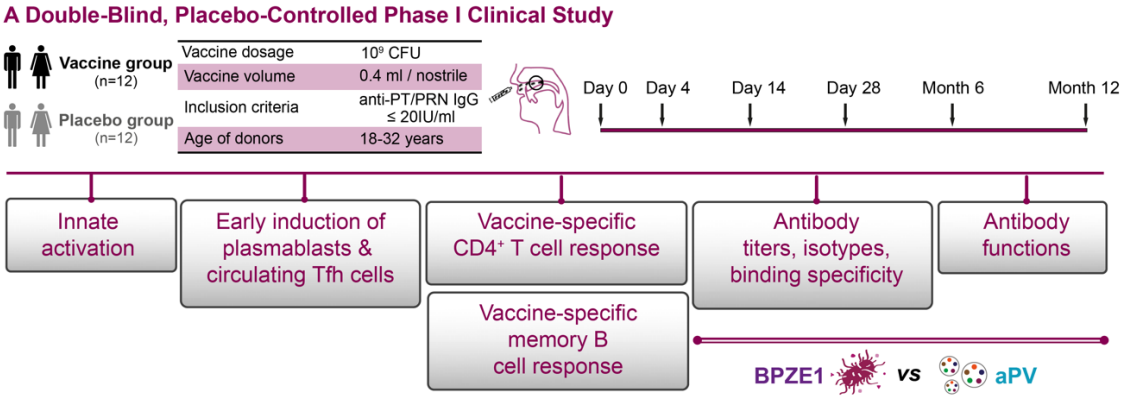


Figure 16. Study design of the evaluation of BPZE1 responses in humans.

We found that BPZE1 administration induced an early transient increase of plasmablasts and activated Th1-type circulating T follicular helper (cTfh1) cells in the blood (Figure 17A), which indicated that an antibody response was generated rapidly following BPZE1 immunization. In addition, the expansion of activated cTfh1 cells in the BPZE1 vaccinees suggested that BPZE1

may preferentially induce a Th1-polarized T cell response. We therefore performed an intracellular cytokine recall assay to evaluate the subtype of BPZE1-specific T cells. At 28 days after vaccination, a dominant Th1-biased $CD4^+$ T cell response was detected in the BPZE1 vaccinees, evidenced by an increase of TNF- or IFN-γ-producing BPZE1-specific T cells. No or very limited numbers of BPZE1-specific Th2 (IL-13) or Th17 (IL-17A) cells were detected (Figure 17B).

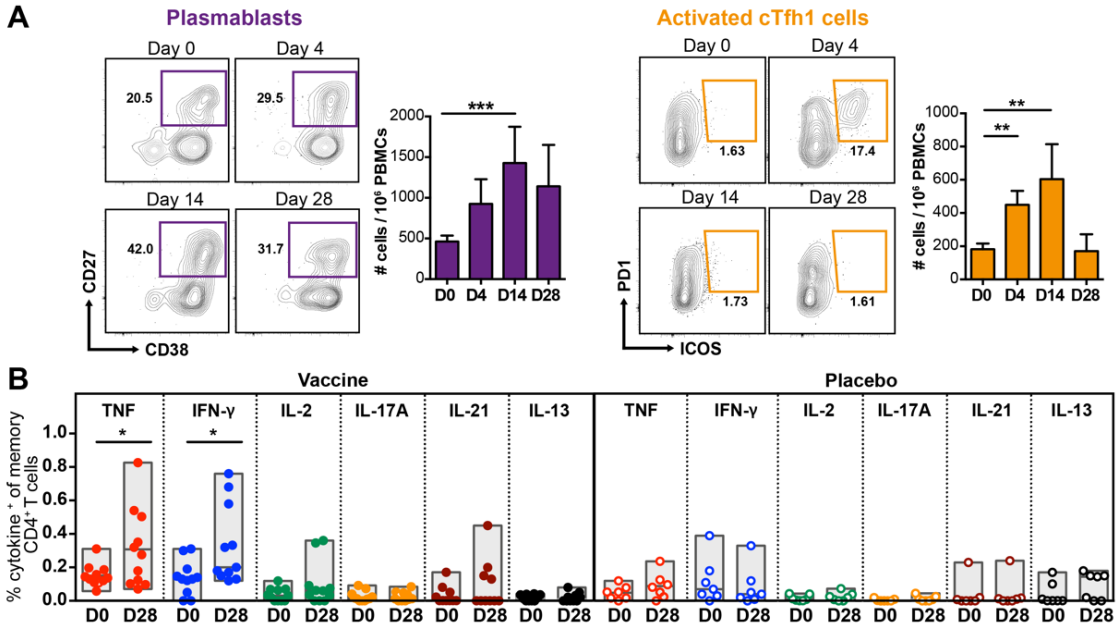


Figure 17. BPZE1 vaccination induces an early increase of (A) plasmablasts and activated cTfh1 cells at day 4-14, and (B) BPZE1-specific Th1-biased $CD4^+$ T cells at day 28.

The polarization towards a Th1 response by BPZE1 immunization was further evaluated by characterizing the antibody responses. We measured the titers of antibodies including different Ig isotypes and subclasses, and benchmarked the responses in the BPZE1 vaccinees to the responses induced by aPV immunization. At 28 days after immunization, BPZE1 vaccinees showed significantly increased total IgG and IgA titers against BPZE1 lysates. The increase of total IgG was exclusively contributed by IgG1 and IgG3 subclasses indicating a Th1-biased phenotype. No increase of IgG2 was observed while IgG4 was not detectable. When evaluating the antibody responses at 28 days after the 5th vaccination of aPV, we found that aPV induced all four IgG subclasses. The IgG2 and IgG4 responses exclusively detected in the aPV group indicated a more Th2-skewed response compared to the BPZE1 group.

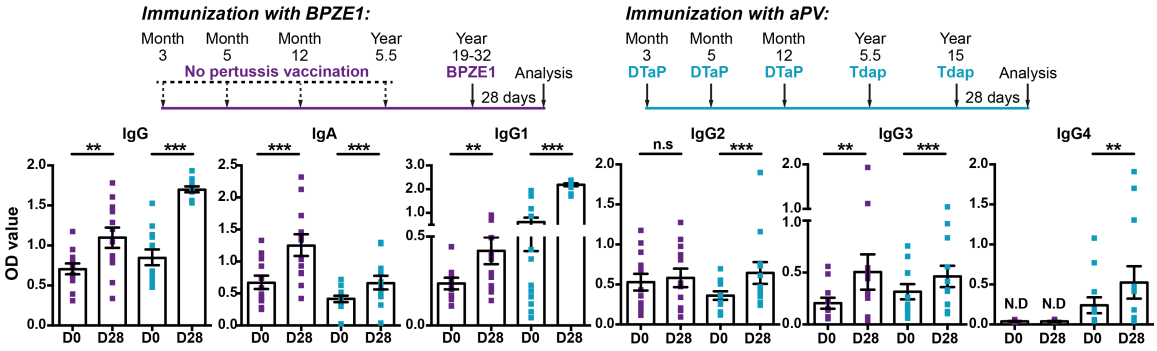
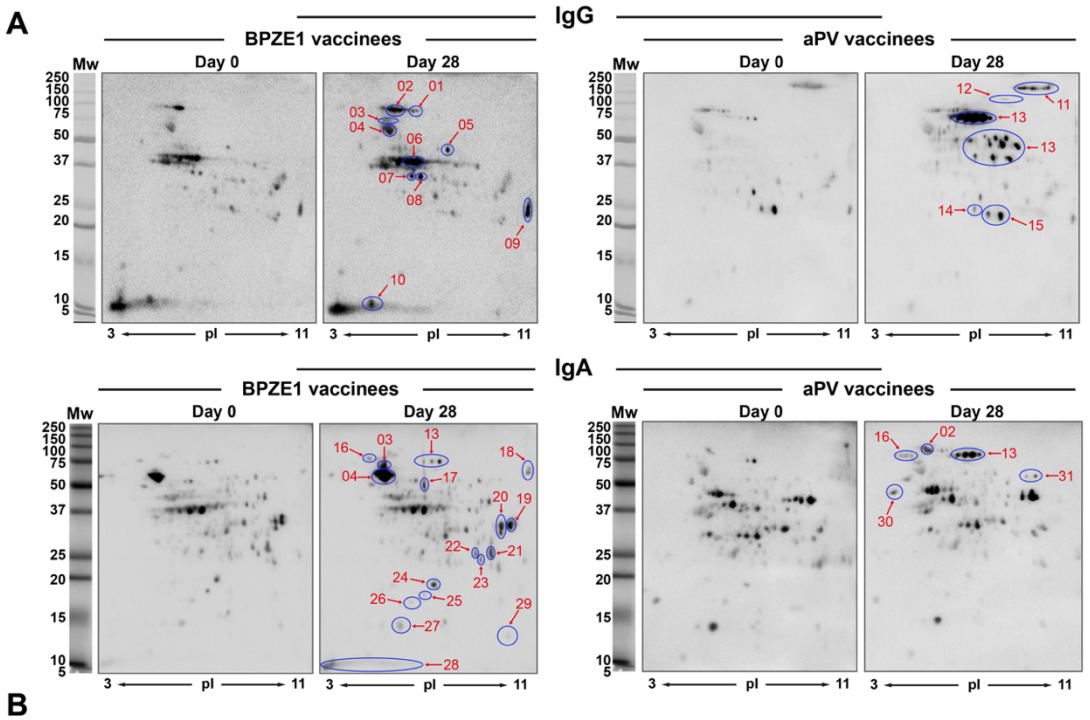


Figure 18. Administration of BPZE1 and aPV induces different types of antibodies in humans

Next, we combined 2D immunoblotting with LC-MS to characterize binding specificities of the antibodies in BPZE1 and aPV vaccinees. This technique also enabled the identification of novel immunogenic antigens. As expected, BPZE1 containing the full spectrum of antigens was shown to induce a much wider repertoire of antibodies with multiple binding specificities compared to the aPV (Figure 19A). Both BPZE1-induced IgG and IgA were found to target multiple distinct antigens. In contrast, aPV-elicited IgG was directed primarily against the vaccine components. aPV-elicited IgA was directed against a few antigens such as PRN, BrkA, DnaK and Enolase, the latter three of which are not included in the vaccine formulation. This is likely the result of natural exposure to *B. pertussis* or other pathogens exhibiting the same antigens since aPV is unable to induce IgA responses in naive individuals[179,181]. In addition to PT, FHA, and PRN included in aPV, multiple immunogenic antigens were identified in this study (Figure 19B). Several of them have previously been shown to be immunogenic in either animal or human studies[179,208-210].



Label	UniProt ID.	Protein/Gene name
01	Q7VZU0	PNPase
02	Q45340	BrkA autotransporter
03	Q7VZG0	RpsA
04	P48210	60 kDa GroEL
05	P14283	Pertactin autotransporter
06	Q7VU66 Q7VYN1 Q7VVC9	TrpD LivJ Putative ABC trasport soluble-binding protein
07	Q7VZB0	CbpA
08	Q7VUV7	Putative exported soluble-binding protein
09	Q7W0S2	RplA

Label	UniProt ID.	Protein/Gene name
10	Q7W0E4 Q7VW32	GcvH ACP: acyl carrier protein
11	P12255	FHAb
12	Q7VVJ2	FHAs
13	P14283	Pertactin autotransporter
14	P04977	PT S1
15	P04978 P04979	PT S2 PT S3
16	Q7VVY2	Chaperone protein DnaK
17	Q7VUE7	WbpO
18	P12255	FHAb
19	Q7VWW8	Putative exported protein

Label	UniProt ID.	Protein/Gene name
20	Q7VXB3	Putative exported protein
21/22	P40415	PPIase
23	Q7VYH5	Putative amino acid ABC transporter
24	P37369 Q7W0K2	SODb DsbA
25	Q7W0S4	NusG
26	Q7VX98	PpiB
27	P0A339	GroES
28		LPS
29	P0A3R5	PT S4
30	Q7VW79	Enolase
31	Q7VSR6	Putative extracellular solute-binding protein

Figure 19. Immunoprofiling of antibody responses in BPZE1 and aPV vaccinees and identification of novel immunogenic antigens.

As described in Figure 6, antibodies work through three major mechanisms. Since the bacterial clearance is largely mediated by phagocytic cells such as neutrophils, we assessed whether the antibodies induced by BPZE1 and aPV have different abilities to opsonize bacteria and mediate

killing by neutrophils. We found that neutrophils exposed to live BPZE1 opsonized by BPZE1-induced antibodies showed a much higher level of ROS, the molecules heavily involved in bacteria killing, as well as increased expression of cell activation markers CD11b and CD66. Bacterial opsonization by aPV-induced antibodies also led to the activation of neutrophils, indicated by elevated expression of CD11b and CD66. However, ROS production was not increased (Figure 20A). By using heat inactivated (HI)-serum in which complement activity is destroyed for the analysis, we found that HI-serum opsonized BPZE1 did not reduce the level of ROS production in neutrophils, which means that Fc-receptor-mediated opsonophagocytosis play a dominant role in triggering ROS production (Figure 20B). Further, we evaluated neutrophil killing activity following antibody-mediated bacterial opsonization. An enhanced killing activity by neutrophils was found when using BPZE1-induced antibodies to opsonize the bacteria. In contrast, aPV-induced antibodies did not show this effect consistent with their inability to stimulate ROS production (Figure 20C). One potential mechanism why the aPV-induced antibodies could not stimulate ROS production may be related to the release of ACT by *B. pertussis,* which is known to inhibit ROS production of neutrophils[166]. By ELISA we found that the BPZE1 vaccinees showed significantly increased titers of IgG1, IgG3 and IgA antibodies against ACT, which could potentially have resulted in neutralization of ACT and alleviating any inhibition on ROS production. In contrast, the aPV vaccinees did not show induction of ACT-specific antibodies (Figure 20D). This may, at least partly, explain the inability of aPV-induced antibodies to stimulate ROS production. Collectively, these data show that BPZE1 is superior over aPV at inducing effective opsonizing antibodies leading to an enhanced bactericidal activity of neutrophils.

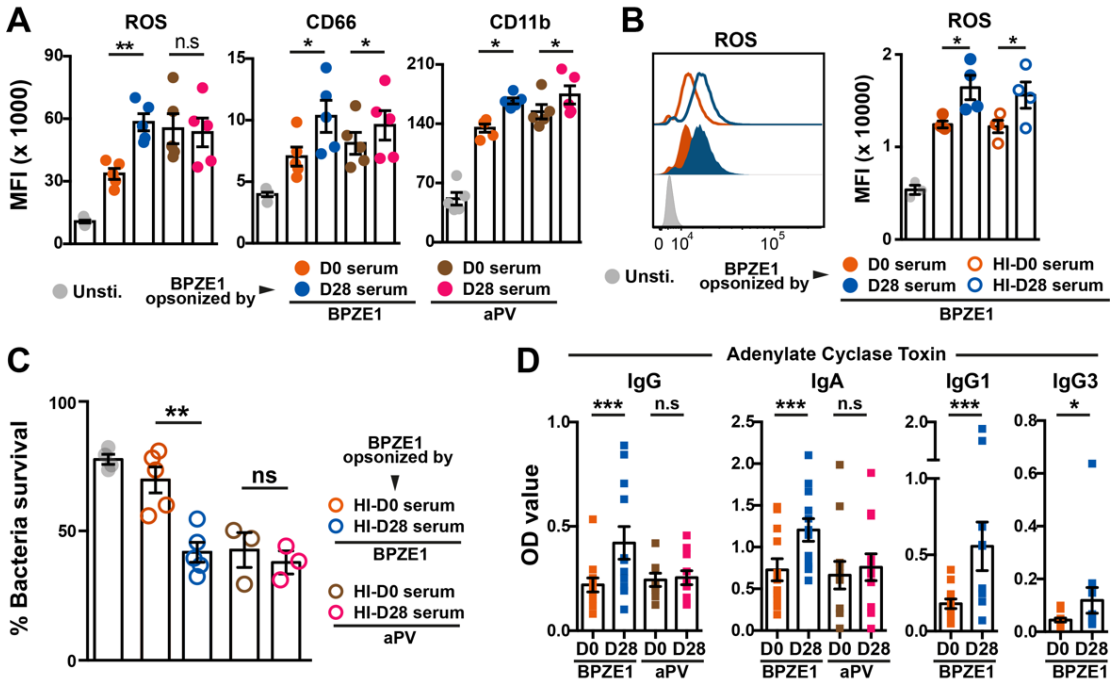


Figure 20. (A-C). BPZE1 is superior over aPV at inducing opsonizing antibodies leading to an enhanced ROS production and bactericidal activity by neutrophils. (D). BPZE1 elicits robust levels of IgG1, IgG3 and IgA in humans.

In conclusion, this study provides novel findings on increased antibody breadth and bacterial killing functions induced by BPZE1 compared to aPV. Importantly, novel immunogenic antigens in BPZE1 were identified, which may represent critical antigens to generate better protection against pertussis disease and transmission. An intranasal immunization with a live pertussis vaccine is likely in a better position to stimulate both effective mucosal and systemic protective responses comparable to what the natural infection generates. Upon intranasal delivery into the host (Figure 21), BPZE1 first colonizes at the nasal cavity, activates local resident cells, generates inflammation, and primes the innate immune system. Th1-polarized antibody responses and cellular responses are subsequently generated and constitute both mucosal and systemic line of defense. A well-established Th1 type immune system favors the clearance of infection and prevention from disease or transmission. This can be attributed to the highly efficient collaboration between Th1-type antibodies (IgG1, IgG3) and innate effector cells, as well as the strong ability of Th1 cytokines (IFN-γ, IL-12) in activating innate cells.

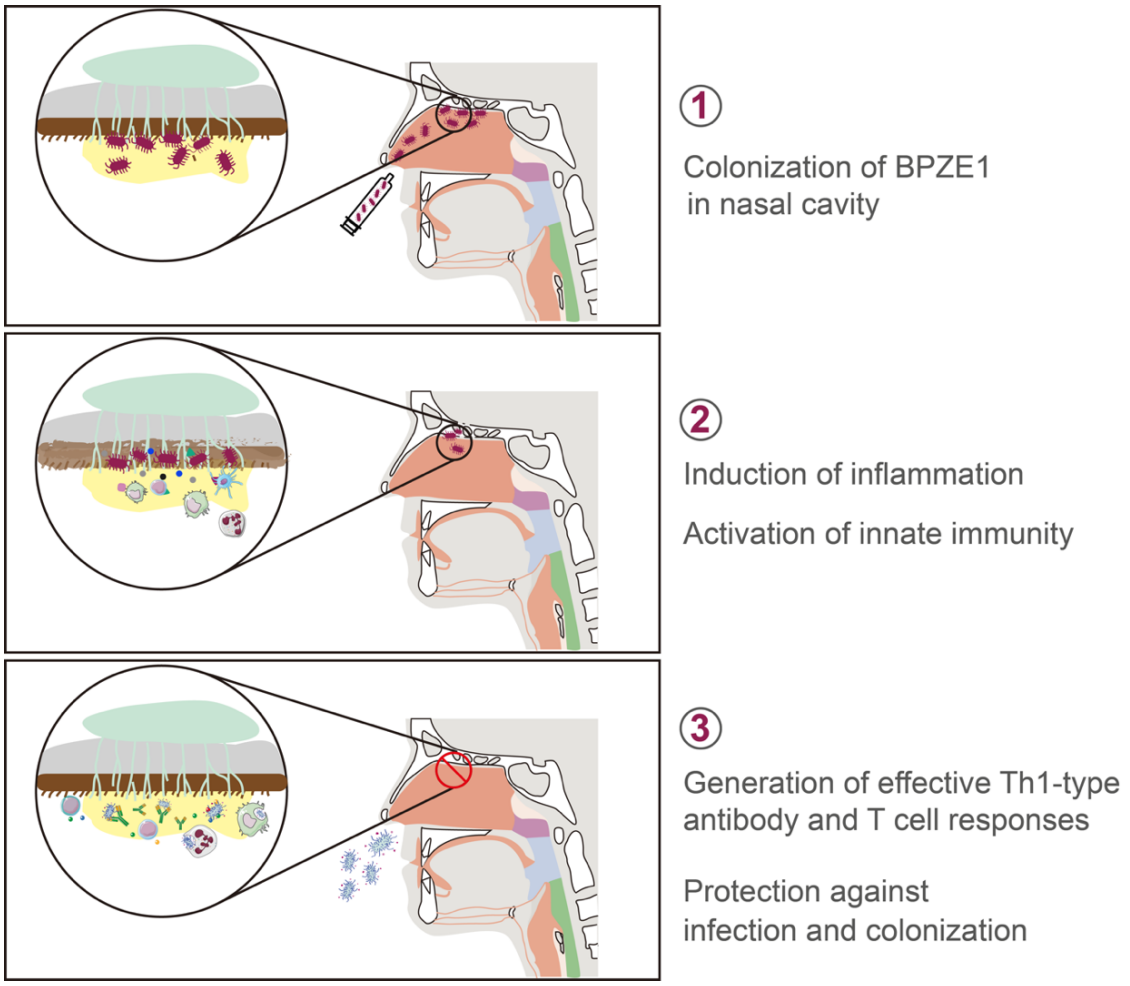


Figure 21. Steps in BPZE1 inducing immunity

This study does not only demonstrate promising improved immunity by a new vaccine candidate but will also hopefully increase the overall basic understanding of the immunological mechanisms critical for pertussis protection and control.

8 CONCLUDING REMARKS

Rational vaccine design relies on a comprehensive understanding of the immune mechanisms dictating vaccine responses. This thesis aiming to expand such knowledge has particularly focused on the myeloid cells and antibodies.

Innate immune system being the first unit interacting with vaccines is believed to fundamentally affect the later generation of adaptive responses, and ultimately determines the vaccine outcomes. **Neutrophils** being the major member of innate system are not only phagocytic cells eliminating pathogens, but also possess versatile abilities in orchestrating adaptive immunity, for example, the antigen-presenting function as described in this thesis. Although innate immune activation is critical for the induction of adaptive responses, "*Too much is as bad as not enough*." Activation of innate responses has to be well controlled to an appropriate degree that can lead to an efficient adaptive response and at the same time ensure the safety. To this end, negative immune regulators, such as **MDSCs**, are needed. Therefore, defining the role of different myeloid cells in vaccination may aid in the development of novel vaccination strategies through manipulating or targeting these cells.

Most vaccines so far work by inducing antibodies for the protection against infectious diseases. In the context of *B. pertussis* infection, Th1 type **antibodies** can efficiently interact with innate effector cells to facilitate their anti-bacterial functions and therefore are important for a successful pertussis vaccine. Both the magnitude (antibody titer) and the type of immune responses needed for the control and protection of *B. pertussis* infection must be taken into consideration for rational vaccine design.

The main conclusions of the three studies in this thesis are:

I. Neutrophils can present antigens to cognate memory T cells and thus are referred to as atypcial APCs (**Paper I**).

II. MDSCs that are elicited early after vaccine administration may play a counter-balancing role to prevent excessive inflammation (**Paper II**).

III. A live pertussis vaccine BPZE1 can elicit Th1 type antibodies with broad antigen-binding specificity and potent bacterial opsonizing activity (**Paper III**).

9 ACKNOWLEDGEMENTS

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10 REFERENCES

1 Gross, C. P. & Sepkowitz, K. A. The myth of the medical breakthrough: smallpox, vaccination, and Jenner reconsidered. *Int J Infect Dis* **3**, 54-60 (1998).

2 Riedel, S. Edward Jenner and the history of smallpox and vaccination. *Proc (Bayl Univ Med Cent)* **18**, 21-25, doi:10.1080/08998280.2005.11928028 (2005).

3 De Gregorio, E. & Rappuoli, R. From empiricism to rational design: a personal perspective of the evolution of vaccine development. *Nat Rev Immunol* **14**, 505-514, doi:10.1038/nri3694 (2014).

4 Rappuoli, R., Mandl, C. W., Black, S. & De Gregorio, E. Vaccines for the twenty-first century society. *Nat Rev Immunol* **11**, 865-872, doi:10.1038/nri3085 (2011).

5 Hajj Hussein, I. *et al.* Vaccines Through Centuries: Major Cornerstones of Global Health. *Front Public Health* **3**, 269, doi:10.3389/fpubh.2015.00269 (2015).

6 Pardi, N., Hogan, M. J., Porter, F. W. & Weissman, D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* **17**, 261-279, doi:10.1038/nrd.2017.243 (2018).

7 Wagner, V., Dullaart, A., Bock, A. K. & Zweck, A. The emerging nanomedicine landscape. *Nat Biotechnol* **24**, 1211-1217, doi:10.1038/nbt1006-1211 (2006).

8 Henderson, D. A. Smallpox eradication--the final battle. *J Clin Pathol* **28**, 843-849, doi:10.1136/jcp.28.11.843 (1975).

9 Hinman, A. Eradication of vaccine-preventable diseases. *Annu Rev Public Health* **20**, 211-229, doi:10.1146/annurev.publhealth.20.1.211 (1999).

10 Omer, S. B., Salmon, D. A., Orenstein, W. A., deHart, M. P. & Halsey, N. Vaccine refusal, mandatory immunization, and the risks of vaccine-preventable diseases. *N Engl J Med* **360**, 1981-1988, doi:10.1056/NEJMsa0806477 (2009).

11 Walter A. Orenstein, S. H., Melinda Wharton. Trends in vaccine-preventable diseases. *Seminars in Pediatric Infectious Diseases* **8**, 23-33, doi:https://doi.org/10.1016/S1045-1870(97)80006-4 (1997).

12 Barouch, D. H. Challenges in the development of an HIV-1 vaccine. *Nature* **455**, 613-619, doi:10.1038/nature07352 (2008).

13 Crompton, P. D., Pierce, S. K. & Miller, L. H. Advances and challenges in malaria vaccine development. *J Clin Invest* **120**, 4168-4178, doi:10.1172/JCI44423 (2010).

14 Voss, G. *et al.* Progress and challenges in TB vaccine development. *F1000Res* **7**, 199, doi:10.12688/f1000research.13588.1 (2018).

15 Consortium, P. PERISCOPE: road towards effective control of pertussis. *Lancet Infect Dis* **19**, e179-e186, doi:10.1016/S1473-3099(18)30646-7 (2019).

16 Ljungman, P. Vaccination of immunocompromised patients. *Clin Microbiol Infect* **18 Suppl 5**, 93-99, doi:10.1111/j.1469-0691.2012.03971.x (2012).

17 Linhart, B. & Valenta, R. Vaccines for allergy. *Curr Opin Immunol* **24**, 354-360, doi:10.1016/j.coi.2012.03.006 (2012).

18 Anderson, R. P. & Jabri, B. Vaccine against autoimmune disease: antigen-specific immunotherapy. *Curr Opin Immunol* **25**, 410-417, doi:10.1016/j.coi.2013.02.004 (2013).

19 Pulendran, B. Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. *Nat Rev Immunol* **9**, 741-747, doi:10.1038/nri2629 (2009).

20 Pulendran, B. & Ahmed, R. Translating innate immunity into immunological memory: implications for vaccine development. *Cell* **124**, 849-863, doi:10.1016/j.cell.2006.02.019 (2006).

21 Iwasaki, A. & Medzhitov, R. Regulation of adaptive immunity by the innate immune system. *Science* **327**, 291-295, doi:10.1126/science.1183021 (2010).

22 Lu, L. L., Suscovich, T. J., Fortune, S. M. & Alter, G. Beyond binding: antibody effector functions in infectious diseases. *Nat Rev Immunol* **18**, 46-61, doi:10.1038/nri.2017.106 (2018).

23 Schumacher, T. N., Gerlach, C. & van Heijst, J. W. Mapping the life histories of T cells. *Nat Rev Immunol* **10**, 621-631, doi:10.1038/nri2822 (2010).

24 Bassler, K., Schulte-Schrepping, J., Warnat-Herresthal, S., Aschenbrenner, A. C. & Schultze, J. L. The Myeloid Cell Compartment-Cell by Cell. *Annu Rev Immunol* **37**, 269-293, doi:10.1146/annurev-immunol-042718-041728 (2019).

25 Kondo, M. Lymphoid and myeloid lineage commitment in multipotent hematopoietic progenitors. *Immunol Rev* **238**, 37-46, doi:10.1111/j.1600-065X.2010.00963.x (2010).

26 Liang, F. *et al.* Vaccine priming is restricted to draining lymph nodes and controlled by adjuvant-mediated antigen uptake. *Sci Transl Med* **9**, doi:10.1126/scitranslmed.aal2094 (2017).

27 Liang, F. & Lore, K. Local innate immune responses in the vaccine adjuvant-injected muscle. *Clin Transl Immunology* **5**, e74, doi:10.1038/cti.2016.19 (2016).

28 Kolaczkowska, E. & Kubes, P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* **13**, 159-175, doi:10.1038/nri3399 (2013).

29 Hong, C. W. Current Understanding in Neutrophil Differentiation and Heterogeneity. *Immune Netw* **17**, 298-306, doi:10.4110/in.2017.17.5.298 (2017).

30 Adrover, J. M., Nicolas-Avila, J. A. & Hidalgo, A. Aging: A Temporal Dimension for Neutrophils. *Trends Immunol* **37**, 334-345, doi:10.1016/j.it.2016.03.005 (2016).

31 Adrover, J. M. *et al.* A Neutrophil Timer Coordinates Immune Defense and Vascular Protection. *Immunity* **50**, 390-402 e310, doi:10.1016/j.immuni.2019.01.002 (2019).

32 Kruger, P. *et al.* Neutrophils: Between host defence, immune modulation, and tissue injury. *PLoS Pathog* **11**, e1004651, doi:10.1371/journal.ppat.1004651 (2015).

33 Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol* **18**, 134-147, doi:10.1038/nri.2017.105 (2018).

34 Mocsai, A. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J Exp Med* **210**, 1283-1299, doi:10.1084/jem.20122220 (2013).

35 Silvestre-Roig, C., Fridlender, Z. G., Glogauer, M. & Scapini, P. Neutrophil Diversity in Health and Disease. *Trends Immunol* **40**, 565-583, doi:10.1016/j.it.2019.04.012 (2019).

36 Rosales, C. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Front Physiol* **9**, 113, doi:10.3389/fphys.2018.00113 (2018).

37 Ng, L. G., Ostuni, R. & Hidalgo, A. Heterogeneity of neutrophils. *Nat Rev Immunol* **19**, 255-265, doi:10.1038/s41577-019-0141-8 (2019).

38 Martin, C. *et al.* Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* **19**, 583-593, doi:10.1016/s1074-7613(03)00263-2 (2003).

39 Bai, M. *et al.* CD177 modulates human neutrophil migration through activation-mediated integrin and chemoreceptor regulation. *Blood* **130**, 2092-2100, doi:10.1182/blood-2017-03-768507 (2017).

40 Clemmensen, S. N. *et al.* Olfactomedin 4 defines a subset of human neutrophils. *J Leukoc Biol* **91**, 495-500, doi:10.1189/jlb.0811417 (2012).

41 Welin, A. *et al.* The human neutrophil subsets defined by the presence or absence of OLFM4 both transmigrate into tissue in vivo and give rise to distinct NETs in vitro. *PLoS One* **8**, e69575, doi:10.1371/journal.pone.0069575 (2013).

42 Casanova-Acebes, M. *et al.* Neutrophils instruct homeostatic and pathological states in naive tissues. *J Exp Med* **215**, 2778-2795, doi:10.1084/jem.20181468 (2018).

43 Peters, A. M., Saverymuttu, S. H., Bell, R. N. & Lavender, J. P. Quantification of the distribution of the marginating granulocyte pool in man. *Scand J Haematol* **34**, 111-120, doi:10.1111/j.1600-0609.1985.tb02242.x (1985).

44 Devi, S. *et al.* Neutrophil mobilization via plerixafor-mediated CXCR4 inhibition arises from lung demargination and blockade of neutrophil homing to the bone marrow. *J Exp Med* **210**, 2321-2336, doi:10.1084/jem.20130056 (2013).

45 Puga, I. *et al.* B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat Immunol* **13**, 170-180, doi:10.1038/ni.2194 (2011).

46 Cerutti, A., Puga, I. & Magri, G. The B cell helper side of neutrophils. *J Leukoc Biol* **94**, 677-682, doi:10.1189/jlb.1112596 (2013).

47 Deniset, J. F. & Kubes, P. Neutrophil heterogeneity: Bona fide subsets or polarization states? *J Leukoc Biol* **103**, 829-838, doi:10.1002/JLB.3RI0917-361R (2018).

48 Lawrence, S. M., Corriden, R. & Nizet, V. The Ontogeny of a Neutrophil: Mechanisms of Granulopoiesis and Homeostasis. *Microbiol Mol Biol Rev* **82**, doi:10.1128/MMBR.00057-17 (2018).

49 Scapini, P. & Cassatella, M. A. Social networking of human neutrophils within the immune system. *Blood* **124**, 710-719, doi:10.1182/blood-2014-03-453217 (2014).

50 Carmona-Rivera, C. & Kaplan, M. J. Low-density granulocytes: a distinct class of neutrophils in systemic autoimmunity. *Semin Immunopathol* **35**, 455-463, doi:10.1007/s00281-013-0375-7 (2013).

51 Hsu, B. E. *et al.* Immature Low-Density Neutrophils Exhibit Metabolic Flexibility that Facilitates Breast Cancer Liver Metastasis. *Cell Rep* **27**, 3902-3915 e3906, doi:10.1016/j.celrep.2019.05.091 (2019).

52 Bronte, V. *et al.* Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* **7**, 12150, doi:10.1038/ncomms12150 (2016).

53 Condamine, T. *et al.* Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol* **1**, doi:10.1126/sciimmunol.aaf8943 (2016).

54 Marini, O. *et al.* Mature CD10(+) and immature CD10(-) neutrophils present in G-CSF-treated donors display opposite effects on T cells. *Blood* **129**, 1343-1356, doi:10.1182/blood-2016-04-713206 (2017).

55 Giese, M. A., Hind, L. E. & Huttenlocher, A. Neutrophil plasticity in the tumor microenvironment. *Blood* **133**, 2159-2167, doi:10.1182/blood-2018-11-844548 (2019).

56 Shaul, M. E. & Fridlender, Z. G. Tumour-associated neutrophils in patients with cancer. *Nat Rev Clin Oncol* **16**, 601-620, doi:10.1038/s41571-019-0222-4 (2019).

57 Leliefeld, P. H., Koenderman, L. & Pillay, J. How Neutrophils Shape Adaptive Immune Responses. *Front Immunol* **6**, 471, doi:10.3389/fimmu.2015.00471 (2015).

58 Lin, A. & Lore, K. Granulocytes: New Members of the Antigen-Presenting Cell Family. *Front Immunol* **8**, 1781, doi:10.3389/fimmu.2017.01781 (2017).

59 Gosselin, E. J., Wardwell, K., Rigby, W. F. & Guyre, P. M. Induction of MHC class II on human polymorphonuclear neutrophils by granulocyte/macrophage colony-stimulating factor, IFN-gamma, and IL-3. *J Immunol* **151**, 1482-1490 (1993).

60 Radsak, M., Iking-Konert, C., Stegmaier, S., Andrassy, K. & Hansch, G. M. Polymorphonuclear neutrophils as accessory cells for T-cell activation: major histocompatibility complex class II restricted antigen-dependent induction of T-cell proliferation. *Immunology* **101**, 521-530, doi:10.1046/j.1365-2567.2000.00140.x (2000).

61 Matsushima, H. *et al.* Neutrophil differentiation into a unique hybrid population exhibiting dual phenotype and functionality of neutrophils and dendritic cells. *Blood* **121**, 1677-1689, doi:10.1182/blood-2012-07-445189 (2013).

62 Oehler, L. *et al.* Neutrophil granulocyte-committed cells can be driven to acquire dendritic cell characteristics. *J Exp Med* **187**, 1019-1028, doi:10.1084/jem.187.7.1019 (1998).

63 Mudzinski, S. P. *et al.* Expression of HLA-DR (major histocompatibility complex class II) on neutrophils from patients treated with granulocyte-macrophage colony-stimulating factor for mobilization of stem cells. *Blood* **86**, 2452-2453 (1995).

64 Reinisch, W. *et al.* In vivo induction of HLA-DR on human neutrophils in patients treated with interferon-gamma. *Blood* **87**, 3068 (1996).

65 van den Elsen, P. J. Expression regulation of major histocompatibility complex class I and class II encoding genes. *Front Immunol* **2**, 48, doi:10.3389/fimmu.2011.00048 (2011).

66 Muller, I., Munder, M., Kropf, P. & Hansch, G. M. Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? *Trends Immunol* **30**, 522-530, doi:10.1016/j.it.2009.07.007 (2009).

67 Abi Abdallah, D. S., Egan, C. E., Butcher, B. A. & Denkers, E. Y. Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *Int Immunol* **23**, 317-326, doi:10.1093/intimm/dxr007 (2011).

68 Vono, M. *et al.* Neutrophils acquire the capacity for antigen presentation to memory CD4(+) T cells in vitro and ex vivo. *Blood* **129**, 1991-2001, doi:10.1182/blood-2016-10-744441 (2017).

69 Cassatella, M. A. Human mature neutrophils as atypical APC. *Blood* **129**, 1895-1896, doi:10.1182/blood-2017-02-767574 (2017).

70 Meinderts, S. M. *et al.* Neutrophils acquire antigen-presenting cell features after phagocytosis of IgG-opsonized erythrocytes. *Blood Adv* **3**, 1761-1773, doi:10.1182/bloodadvances.2018028753 (2019).

71 Gabrilovich, D. I. Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* **5**, 3-8, doi:10.1158/2326-6066.CIR-16-0297 (2017).

72 Gabrilovich, D. I. & Nagaraj, S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* **9**, 162-174, doi:10.1038/nri2506 (2009).

73 Umansky, V., Blattner, C., Gebhardt, C. & Utikal, J. The Role of Myeloid-Derived Suppressor Cells (MDSC) in Cancer Progression. *Vaccines (Basel)* **4**, doi:10.3390/vaccines4040036 (2016).

74 Peranzoni, E. *et al.* Myeloid-derived suppressor cell heterogeneity and subset definition. *Curr Opin Immunol* **22**, 238-244, doi:10.1016/j.coi.2010.01.021 (2010).

75 Lin, A. *et al.* Rhesus Macaque Myeloid-Derived Suppressor Cells Demonstrate T Cell Inhibitory Functions and Are Transiently Increased after Vaccination. *J Immunol* **200**, 286-294, doi:10.4049/jimmunol.1701005 (2018).

76 Cassetta, L. *et al.* Deciphering myeloid-derived suppressor cells: isolation and markers in humans, mice and non-human primates. *Cancer Immunol Immunother* **68**, 687-697, doi:10.1007/s00262-019-02302-2 (2019).

77 Dumitru, C. A., Moses, K., Trellakis, S., Lang, S. & Brandau, S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunol Immunother* **61**, 1155-1167, doi:10.1007/s00262-012-1294-5 (2012).

78 Brandau, S., Moses, K. & Lang, S. The kinship of neutrophils and granulocytic myeloid-derived suppressor cells in cancer: cousins, siblings or twins? *Semin Cancer Biol* **23**, 171-182, doi:10.1016/j.semcancer.2013.02.007 (2013).

79 Youn, J. I. & Gabrilovich, D. I. The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. *Eur J Immunol* **40**, 2969-2975, doi:10.1002/eji.201040895 (2010).

80 Kotsakis, A. *et al.* Myeloid-derived suppressor cell measurements in fresh and cryopreserved blood samples. *J Immunol Methods* **381**, 14-22, doi:10.1016/j.jim.2012.04.004 (2012).

81 Lechner, M. G., Liebertz, D. J. & Epstein, A. L. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. *J Immunol* **185**, 2273-2284, doi:10.4049/jimmunol.1000901 (2010).

82 Tcyganov, E., Mastio, J., Chen, E. & Gabrilovich, D. I. Plasticity of myeloid-derived suppressor cells in cancer. *Curr Opin Immunol* **51**, 76-82, doi:10.1016/j.coi.2018.03.009 (2018).

83 Wang, J. *et al.* Effect of TLR agonists on the differentiation and function of human monocytic myeloid-derived suppressor cells. *J Immunol* **194**, 4215-4221, doi:10.4049/jimmunol.1402004 (2015).

84 Poschke, I. & Kiessling, R. On the armament and appearances of human myeloid-derived suppressor cells. *Clin Immunol* **144**, 250-268, doi:10.1016/j.clim.2012.06.003 (2012).

85 Hoechst, B. *et al.* A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology* **135**, 234-243, doi:10.1053/j.gastro.2008.03.020 (2008).

86 Ostrand-Rosenberg, S., Sinha, P., Beury, D. W. & Clements, V. K. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Semin Cancer Biol* **22**, 275-281, doi:10.1016/j.semcancer.2012.01.011 (2012).

87 Gabitass, R. F., Annels, N. E., Stocken, D. D., Pandha, H. A. & Middleton, G. W. Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. *Cancer Immunol Immunother* **60**, 1419-1430, doi:10.1007/s00262-011-1028-0 (2011).

88 Meyer, C. *et al.* Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. *Cancer Immunol Immunother* **63**, 247-257, doi:10.1007/s00262-013-1508-5 (2014).

89 Gonda, K. *et al.* Myeloid-derived suppressor cells are increased and correlated with type 2 immune responses, malnutrition, inflammation, and poor prognosis in patients with breast cancer. *Oncol Lett* **14**, 1766-1774, doi:10.3892/ol.2017.6305 (2017).

90 Karakasheva, T. A. *et al.* CD38+ M-MDSC expansion characterizes a subset of advanced colorectal cancer patients. *JCI Insight* **3**, doi:10.1172/jci.insight.97022 (2018).

91 Anani, W. & Shurin, M. R. Targeting Myeloid-Derived Suppressor Cells in Cancer. *Adv Exp Med Biol* **1036**, 105-128, doi:10.1007/978-3-319-67577-0_8 (2017).

92 Ko, J. S. *et al.* Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clin Cancer Res* **15**, 2148-2157, doi:10.1158/1078-0432.CCR-08-1332 (2009).

93 Degos, L. & Wang, Z. Y. All trans retinoic acid in acute promyelocytic leukemia. *Oncogene* **20**, 7140-7145, doi:10.1038/sj.onc.1204763 (2001).

94 Nefedova, Y. *et al.* Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. *Cancer Res* **67**, 11021-11028, doi:10.1158/0008-5472.CAN-07-2593 (2007).

95 Pawelec, G., Verschoor, C. P. & Ostrand-Rosenberg, S. Myeloid-Derived Suppressor Cells: Not Only in Tumor Immunity. *Front Immunol* **10**, 1099, doi:10.3389/fimmu.2019.01099 (2019).

96 Medina, E. & Hartl, D. Myeloid-Derived Suppressor Cells in Infection: A General Overview. *J Innate Immun* **10**, 407-413, doi:10.1159/000489830 (2018).

97 Dorhoi, A. *et al.* MDSCs in infectious diseases: regulation, roles, and readjustment. *Cancer Immunol Immunother* **68**, 673-685, doi:10.1007/s00262-018-2277-y (2019).

98 Pastula, A. & Marcinkiewicz, J. Myeloid-derived suppressor cells: a double-edged sword? *Int J Exp Pathol* **92**, 73-78, doi:10.1111/j.1365-2613.2010.00754.x (2011).

99 Chen, S. *et al.* Diminished immune response to vaccinations in obesity: role of myeloid-derived suppressor and other myeloid cells. *Obes Res Clin Pract* **9**, 35-44, doi:10.1016/j.orcp.2013.12.006 (2015).

100 Shah, K. H. *et al.* Myeloid Suppressor Cells Accumulate and Regulate Blood Pressure in Hypertension. *Circ Res* **117**, 858-869, doi:10.1161/CIRCRESAHA.115.306539 (2015).

101 Ostrand-Rosenberg, S. *et al.* Frontline Science: Myeloid-derived suppressor cells (MDSCs) facilitate maternal-fetal tolerance in mice. *J Leukoc Biol* **101**, 1091-1101, doi:10.1189/jlb.1HI1016-306RR (2017).

102 Sui, Y. *et al.* Vaccine-induced myeloid cell population dampens protective immunity to SIV. *J Clin Invest* **124**, 2538-2549, doi:10.1172/JCI73518 (2014).

103 Filipazzi, P. *et al.* Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *J Clin Oncol* **25**, 2546-2553, doi:10.1200/JCO.2006.08.5829 (2007).

104 Plotkin, S. A. Vaccines: correlates of vaccine-induced immunity. *Clin Infect Dis* **47**, 401-409, doi:10.1086/589862 (2008).

105 Melchers, F. Checkpoints that control B cell development. *J Clin Invest* **125**, 2203-2210, doi:10.1172/JCI78083 (2015).

106 Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M. & Corcoran, L. M. The generation of antibody-secreting plasma cells. *Nat Rev Immunol* **15**, 160-171, doi:10.1038/nri3795 (2015).

107 Treanor, B. B-cell receptor: from resting state to activate. *Immunology* **136**, 21-27, doi:10.1111/j.1365-2567.2012.03564.x (2012).

108 Jeurissen, A., Ceuppens, J. L. & Bossuyt, X. T lymphocyte dependence of the antibody response to 'T lymphocyte independent type 2' antigens. *Immunology* **111**, 1-7, doi:10.1111/j.1365-2567.2004.01775.x (2004).

109 Vos, Q., Lees, A., Wu, Z. Q., Snapper, C. M. & Mond, J. J. B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. *Immunol Rev* **176**, 154-170, doi:10.1034/j.1600-065x.2000.00607.x (2000).

110 Parker, D. C. T cell-dependent B cell activation. *Annu Rev Immunol* **11**, 331-360, doi:10.1146/annurev.iy.11.040193.001555 (1993).

111 Zubler, R. H. Naive and memory B cells in T-cell-dependent and T-independent responses. *Springer Semin Immunopathol* **23**, 405-419 (2001).

112 Kurosaki, T., Kometani, K. & Ise, W. Memory B cells. *Nat Rev Immunol* **15**, 149-159, doi:10.1038/nri3802 (2015).

113 Crotty, S. T Follicular Helper Cell Biology: A Decade of Discovery and Diseases. *Immunity* **50**, 1132-1148, doi:10.1016/j.immuni.2019.04.011 (2019).

114 Mesin, L., Ersching, J. & Victora, G. D. Germinal Center B Cell Dynamics. *Immunity* **45**, 471-482, doi:10.1016/j.immuni.2016.09.001 (2016).

115 Victora, G. D. & Nussenzweig, M. C. Germinal centers. *Annu Rev Immunol* **30**, 429-457, doi:10.1146/annurev-immunol-020711-075032 (2012).

116 Forthal, D. N. Functions of Antibodies. *Microbiol Spectr* **2**, 1-17 (2014).

117 Hoffman, W., Lakkis, F. G. & Chalasani, G. B Cells, Antibodies, and More. *Clin J Am Soc Nephrol* **11**, 137-154, doi:10.2215/CJN.09430915 (2016).

118 Onoue, K., Grossberg, A. L., Yagi, Y. & Pressman, D. Immunoglobulin M antibodies with ten combining sites. *Science* **162**, 574-576, doi:10.1126/science.162.3853.574 (1968).

119 Chen, K. & Cerutti, A. The function and regulation of immunoglobulin D. *Curr Opin Immunol* **23**, 345-352, doi:10.1016/j.coi.2011.01.006 (2011).

120 Vidarsson, G., Dekkers, G. & Rispens, T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* **5**, 520, doi:10.3389/fimmu.2014.00520 (2014).

121 Boyaka, P. N. Inducing Mucosal IgA: A Challenge for Vaccine Adjuvants and Delivery Systems. *J Immunol* **199**, 9-16, doi:10.4049/jimmunol.1601775 (2017).

122 Kubo, S., Nakayama, T., Matsuoka, K., Yonekawa, H. & Karasuyama, H. Long term maintenance of IgE-mediated memory in mast cells in the absence of detectable serum IgE. *J Immunol* **170**, 775-780, doi:10.4049/jimmunol.170.2.775 (2003).

123 Brioen, P., Dekegel, D. & Boeye, A. Neutralization of poliovirus by antibody-mediated polymerization. *Virology* **127**, 463-468, doi:10.1016/0042-6822(83)90159-9 (1983).

124 Thomas, A. A., Vrijsen, R. & Boeye, A. Relationship between poliovirus neutralization and aggregation. *J Virol* **59**, 479-485 (1986).

125 Bishop, A. L., Schild, S., Patimalla, B., Klein, B. & Camilli, A. Mucosal immunization with Vibrio cholerae outer membrane vesicles provides maternal protection mediated by antilipopolysaccharide antibodies that inhibit bacterial motility. *Infect Immun* **78**, 4402-4420, doi:10.1128/IAI.00398-10 (2010).

126 LaRocca, T. J. *et al.* The bactericidal effect of a complement-independent antibody is osmolytic and specific to Borrelia. *Proc Natl Acad Sci U S A* **106**, 10752-10757, doi:10.1073/pnas.0901858106 (2009).

127 Zhang, Y., Hoppe, A. D. & Swanson, J. A. Coordination of Fc receptor signaling regulates cellular commitment to phagocytosis. *Proc Natl Acad Sci U S A* **107**, 19332-19337, doi:10.1073/pnas.1008248107 (2010).

128 Uribe-Querol, E. & Rosales, C. Control of Phagocytosis by Microbial Pathogens. *Front Immunol* **8**, 1368, doi:10.3389/fimmu.2017.01368 (2017).

129 Sorman, A., Zhang, L., Ding, Z. & Heyman, B. How antibodies use complement to regulate antibody responses. *Mol Immunol* **61**, 79-88, doi:10.1016/j.molimm.2014.06.010 (2014).

130 Plotkin, S. A. Correlates of protection induced by vaccination. *Clin Vaccine Immunol* **17**, 1055-1065, doi:10.1128/CVI.00131-10 (2010).

131 Reiling, L. *et al.* Targets of complement-fixing antibodies in protective immunity against malaria in children. *Nat Commun* **10**, 610, doi:10.1038/s41467-019-08528-z (2019).

132 Melvin, J. A., Scheller, E. V., Miller, J. F. & Cotter, P. A. Bordetella pertussis pathogenesis: current and future challenges. *Nat Rev Microbiol* **12**, 274-288, doi:10.1038/nrmicro3235 (2014).

133 Klein, N. P. Licensed pertussis vaccines in the United States. History and current state. *Hum Vaccin Immunother* **10**, 2684-2690, doi:10.4161/hv.29576 (2014).

134 van Amersfoorth, S. C. *et al.* Analysis of Bordetella pertussis populations in European countries with different vaccination policies. *J Clin Microbiol* **43**, 2837-2843, doi:10.1128/JCM.43.6.2837-2843.2005 (2005).

135 Kuchar, E., Karlikowska-Skwarnik, M., Han, S. & Nitsch-Osuch, A. Pertussis: History of the Disease and Current Prevention Failure. *Adv Exp Med Biol* **934**, 77-82, doi:10.1007/5584_2016_21 (2016).

136 Romanus, V., Jonsell, R. & Bergquist, S. O. Pertussis in Sweden after the cessation of general immunization in 1979. *Pediatr Infect Dis J* **6**, 364-371, doi:10.1097/00006454-198704000-00005 (1987).

137 Morgan, L. G. Pertussis immunization: an update. *The Journal of the Canadian Chiropractic Association* **41**, 86-90 (1997).

138 Syed, M. A. Choosing from Whole Cell and Acellular Pertussis Vaccines-Dilemma for the Developing Countries. *Iran J Public Health* **46**, 272-273 (2017).

139 Schwartz, K. L. *et al.* Effectiveness of pertussis vaccination and duration of immunity. *CMAJ* **188**, E399-E406, doi:10.1503/cmaj.160193 (2016).

140 Ausiello, C. M. & Cassone, A. Acellular pertussis vaccines and pertussis resurgence: revise or replace? *MBio* **5**, e01339-01314, doi:10.1128/mBio.01339-14 (2014).

141 Witt, M. A., Arias, L., Katz, P. H., Truong, E. T. & Witt, D. J. Reduced risk of pertussis among persons ever vaccinated with whole cell pertussis vaccine compared to recipients of acellular pertussis vaccines in a large US cohort. *Clin Infect Dis* **56**, 1248-1254, doi:10.1093/cid/cit046 (2013).

142 Cherry, J. D. Why do pertussis vaccines fail? *Pediatrics* **129**, 968-970, doi:10.1542/peds.2011-2594 (2012).

143 Diavatopoulos, D. A. & Edwards, K. M. What Is Wrong with Pertussis Vaccine Immunity? Why Immunological Memory to Pertussis Is Failing. *Cold Spring Harb Perspect Biol* **9**, doi:10.1101/cshperspect.a029553 (2017).

144 Mielcarek, N. *et al.* Live attenuated B. pertussis as a single-dose nasal vaccine against whooping cough. *PLoS Pathog* **2**, e65, doi:10.1371/journal.ppat.0020065 (2006).

145 Locht, C. *et al.* Live Attenuated Pertussis Vaccine BPZE1 Protects Baboons Against Bordetella pertussis Disease and Infection. *J Infect Dis* **216**, 117-124, doi:10.1093/infdis/jix254 (2017).

146 Feunou, P. F., Kammoun, H., Debrie, A. S., Mielcarek, N. & Locht, C. Long-term immunity against pertussis induced by a single nasal administration of live attenuated B. pertussis BPZE1. *Vaccine* **28**, 7047-7053, doi:10.1016/j.vaccine.2010.08.017 (2010).

147 Thorstensson, R. *et al.* A phase I clinical study of a live attenuated Bordetella pertussis vaccine--BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. *PLoS One* **9**, e83449, doi:10.1371/journal.pone.0083449 (2014).

148 Higgs, R., Higgins, S. C., Ross, P. J. & Mills, K. H. Immunity to the respiratory pathogen Bordetella pertussis. *Mucosal Immunol* **5**, 485-500, doi:10.1038/mi.2012.54 (2012).

149 Hewlett, E. L. *et al.* Pertussis pathogenesis--what we know and what we don't know. *J Infect Dis* **209**, 982-985, doi:10.1093/infdis/jit639 (2014).

150 Mattoo, S. & Cherry, J. D. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. *Clin Microbiol Rev* **18**, 326-382, doi:10.1128/CMR.18.2.326-382.2005 (2005).

151 Gillard, J., van Schuppen, E. & Diavatopoulos, D. A. Functional Programming of Innate Immune Cells in Response to Bordetella pertussis Infection and Vaccination. *Adv Exp Med Biol*, doi:10.1007/5584_2019_404 (2019).

152 Jongerius, I., Schuijt, T. J., Mooi, F. R. & Pinelli, E. Complement evasion by Bordetella pertussis: implications for improving current vaccines. *J Mol Med (Berl)* **93**, 395-402, doi:10.1007/s00109-015-1259-1 (2015).

153 Barnes, M. G. & Weiss, A. A. BrkA protein of Bordetella pertussis inhibits the classical pathway of complement after C1 deposition. *Infect Immun* **69**, 3067-3072, doi:10.1128/IAI.69.5.3067-3072.2001 (2001).

154 Higgins, S. C. *et al.* Toll-like receptor 4-mediated innate IL-10 activates antigen-specific regulatory T cells and confers resistance to Bordetella pertussis by inhibiting inflammatory pathology. *J Immunol* **171**, 3119-3127, doi:10.4049/jimmunol.171.6.3119 (2003).

155 Warfel, J. M. & Merkel, T. J. Bordetella pertussis infection induces a mucosal IL-17 response and long-lived Th17 and Th1 immune memory cells in nonhuman primates. *Mucosal Immunol* **6**, 787-796, doi:10.1038/mi.2012.117 (2013).

156 Dunne, P. J., Moran, B., Cummins, R. C. & Mills, K. H. CD11c+CD8alpha+ dendritic cells promote protective immunity to respiratory infection with Bordetella pertussis. *J Immunol* **183**, 400-410, doi:10.4049/jimmunol.0900169 (2009).

157 Dunne, A. *et al.* Inflammasome activation by adenylate cyclase toxin directs Th17 responses and protection against Bordetella pertussis. *J Immunol* **185**, 1711-1719, doi:10.4049/jimmunol.1000105 (2010).

158 Higgins, S. C., Jarnicki, A. G., Lavelle, E. C. & Mills, K. H. TLR4 mediates vaccine-induced protective cellular immunity to Bordetella pertussis: role of IL-17-producing T cells. *J Immunol* **177**, 7980-7989, doi:10.4049/jimmunol.177.11.7980 (2006).

159 Mahon, B. P. & Mills, K. H. Interferon-gamma mediated immune effector mechanisms against Bordetella pertussis. *Immunol Lett* **68**, 213-217, doi:10.1016/s0165-2478(99)00070-x (1999).

160 Gueirard, P., Druilhe, A., Pretolani, M. & Guiso, N. Role of adenylate cyclase-hemolysin in alveolar macrophage apoptosis during Bordetella pertussis infection in vivo. *Infect Immun* **66**, 1718-1725 (1998).

161 Khelef, N., Zychlinsky, A. & Guiso, N. Bordetella pertussis induces apoptosis in macrophages: role of adenylate cyclase-hemolysin. *Infect Immun* **61**, 4064-4071 (1993).

162 Lamberti, Y. A., Hayes, J. A., Perez Vidakovics, M. L., Harvill, E. T. & Rodriguez, M. E. Intracellular trafficking of Bordetella pertussis in human macrophages. *Infect Immun* **78**, 907-913, doi:10.1128/IAI.01031-09 (2010).

163 Eby, J. C., Hoffman, C. L., Gonyar, L. A. & Hewlett, E. L. Review of the neutrophil response to Bordetella pertussis infection. *Pathog Dis* **73**, ftv081, doi:10.1093/femspd/ftv081 (2015).

164 Andreasen, C. & Carbonetti, N. H. Role of neutrophils in response to Bordetella pertussis infection in mice. *Infect Immun* **77**, 1182-1188, doi:10.1128/IAI.01150-08 (2009).

165 Kirimanjeswara, G. S., Agosto, L. M., Kennett, M. J., Bjornstad, O. N. & Harvill, E. T. Pertussis toxin inhibits neutrophil recruitment to delay antibody-mediated clearance of Bordetella pertussis. *J Clin Invest* **115**, 3594-3601, doi:10.1172/JCI24609 (2005).

166 Cerny, O., Anderson, K. E., Stephens, L. R., Hawkins, P. T. & Sebo, P. cAMP Signaling of Adenylate Cyclase Toxin Blocks the Oxidative Burst of Neutrophils through Epac-Mediated Inhibition of Phospholipase C Activity. *J Immunol* **198**, 1285-1296, doi:10.4049/jimmunol.1601309 (2017).

167 Lamberti, Y., Perez Vidakovics, M. L., van der Pol, L. W. & Rodriguez, M. E. Cholesterol-rich domains are involved in Bordetella pertussis phagocytosis and intracellular survival in neutrophils. *Microb Pathog* **44**, 501-511, doi:10.1016/j.micpath.2008.01.002 (2008).

168 Fukasawa, M., Campeau, J. D., Yanagihara, D. L., Rodgers, K. E. & diZerega, G. S. Regulation of proliferation of peritoneal tissue repair cells by peritoneal macrophages. *J Surg Res* **49**, 81-87, doi:10.1016/0022-4804(90)90114-h (1990).

169 Lenz, D. H., Weingart, C. L. & Weiss, A. A. Phagocytosed Bordetella pertussis fails to survive in human neutrophils. *Infect Immun* **68**, 956-959, doi:10.1128/iai.68.2.956-959.2000 (2000).

170 Liang, F. *et al.* Efficient Targeting and Activation of Antigen-Presenting Cells In Vivo after Modified mRNA Vaccine Administration in Rhesus Macaques. *Mol Ther* **25**, 2635-2647, doi:10.1016/j.ymthe.2017.08.006 (2017).

171 Bagley, K. C., Abdelwahab, S. F., Tuskan, R. G., Fouts, T. R. & Lewis, G. K. Pertussis toxin and the adenylate cyclase toxin from Bordetella pertussis activate human monocyte-derived dendritic cells and dominantly inhibit cytokine production through a cAMP-dependent pathway. *J Leukoc Biol* **72**, 962-969 (2002).

172 Schnoeller, C. *et al.* Attenuated Bordetella pertussis vaccine protects against respiratory syncytial virus disease via an IL-17-dependent mechanism. *Am J Respir Crit Care Med* **189**, 194-202, doi:10.1164/rccm.201307-1227OC (2014).

173 Mills, K. H., Barnard, A., Watkins, J. & Redhead, K. Cell-mediated immunity to Bordetella pertussis: role of Th1 cells in bacterial clearance in a murine respiratory infection model. *Infect Immun* **61**, 399-410 (1993).

174 Mills, K. H., Ryan, M., Ryan, E. & Mahon, B. P. A murine model in which protection correlates with pertussis vaccine efficacy in children reveals complementary roles for humoral and cell-mediated immunity in protection against Bordetella pertussis. *Infect Immun* **66**, 594-602 (1998).

175 Granstrom, M., Olinder-Nielsen, A. M., Holmblad, P., Mark, A. & Hanngren, K. Specific immunoglobulin for treatment of whooping cough. *Lancet* **338**, 1230-1233, doi:10.1016/0140-6736(91)92101-7 (1991).

176 Wendelboe, A. M., Van Rie, A., Salmaso, S. & Englund, J. A. Duration of immunity against pertussis after natural infection or vaccination. *Pediatr Infect Dis J* **24**, S58-61, doi:10.1097/01.inf.0000160914.59160.41 (2005).

177 Storsaeter, J., Hallander, H. O., Gustafsson, L. & Olin, P. Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. *Vaccine* **16**, 1907-1916, doi:10.1016/s0264-410x(98)00227-8 (1998).

178 Cassone, A., Mastrantonio, P. & Ausiello, C. M. Are only antibody levels involved in the protection against pertussis in acellular pertussis vaccine recipients? *J Infect Dis* **182**, 1575-1577, doi:10.1086/315898 (2000).

179 Raeven, R. H. *et al.* Immunoproteomic Profiling of Bordetella pertussis Outer Membrane Vesicle Vaccine Reveals Broad and Balanced Humoral Immunogenicity. *J Proteome Res* **14**, 2929-2942, doi:10.1021/acs.jproteome.5b00258 (2015).

180 Hendrikx, L. H. *et al.* Different IgG-subclass distributions after whole-cell and acellular pertussis infant primary vaccinations in healthy and pertussis infected children. *Vaccine* **29**, 6874-6880, doi:10.1016/j.vaccine.2011.07.055 (2011).

181 Hendrikx, L. H. *et al.* Serum IgA responses against pertussis proteins in infected and Dutch wP or aP vaccinated children: an additional role in pertussis diagnostics. *PLoS One* **6**, e27681, doi:10.1371/journal.pone.0027681 (2011).

182 Berstad, A. K. *et al.* A nasal whole-cell pertussis vaccine induces specific systemic and cross-reactive mucosal antibody responses in human volunteers. *J Med Microbiol* **49**, 157-163, doi:10.1099/0022-1317-49-2-157 (2000).

183 Boehm, D. T. *et al.* Intranasal acellular pertussis vaccine provides mucosal immunity and protects mice from Bordetella pertussis. *NPJ Vaccines* **4**, 40, doi:10.1038/s41541-019-0136-2 (2019).

184 Leef, M., Elkins, K. L., Barbic, J. & Shahin, R. D. Protective immunity to Bordetella pertussis requires both B cells and CD4(+) T cells for key functions other than specific antibody production. *J Exp Med* **191**, 1841-1852, doi:10.1084/jem.191.11.1841 (2000).

185 Fedele, G., Cassone, A. & Ausiello, C. M. T-cell immune responses to Bordetella pertussis infection and vaccination. *Pathog Dis* **73**, doi:10.1093/femspd/ftv051 (2015).

186 Ross, P. J. *et al.* Relative contribution of Th1 and Th17 cells in adaptive immunity to Bordetella pertussis: towards the rational design of an improved acellular pertussis vaccine. *PLoS Pathog* **9**, e1003264, doi:10.1371/journal.ppat.1003264 (2013).

187 da Silva Antunes, R. *et al.* Th1/Th17 polarization persists following whole-cell pertussis vaccination despite repeated acellular boosters. *J Clin Invest* **128**, 3853-3865, doi:10.1172/JCI121309 (2018).

188 Mahon, B. P., Sheahan, B. J., Griffin, F., Murphy, G. & Mills, K. H. Atypical disease after Bordetella pertussis respiratory infection of mice with targeted disruptions of interferon-gamma receptor or immunoglobulin mu chain genes. *J Exp Med* **186**, 1843-1851, doi:10.1084/jem.186.11.1843 (1997).

189 Ryan, M. *et al.* Bordetella pertussis respiratory infection in children is associated with preferential activation of type 1 T helper cells. *J Infect Dis* **175**, 1246-1250, doi:10.1086/593682 (1997).

190 Wilk, M. M. *et al.* Lung CD4 Tissue-Resident Memory T Cells Mediate Adaptive Immunity Induced by Previous Infection of Mice with Bordetella pertussis. *J Immunol* **199**, 233-243, doi:10.4049/jimmunol.1602051 (2017).

191 Wilk, M. M. *et al.* Immunization with whole cell but not acellular pertussis vaccines primes CD4 TRM cells that sustain protective immunity against nasal colonization with Bordetella pertussis. *Emerg Microbes Infect* **8**, 169-185, doi:10.1080/22221751.2018.1564630 (2019).

192 Solans, L. *et al.* IL-17-dependent SIgA-mediated protection against nasal Bordetella pertussis infection by live attenuated BPZE1 vaccine. *Mucosal Immunol* **11**, 1753-1762, doi:10.1038/s41385-018-0073-9 (2018).

193 Schiavoni, I. *et al.* Live attenuated B. pertussis BPZE1 rescues the immune functions of Respiratory Syncytial virus infected human dendritic cells by promoting Th1/Th17 responses. *PLoS One* **9**, e100166, doi:10.1371/journal.pone.0100166 (2014).

194 Dirix, V. *et al.* Both CD4(+) and CD8(+) lymphocytes participate in the IFN-gamma response to filamentous hemagglutinin from Bordetella pertussis in infants, children, and adults. *Clin Dev Immunol* **2012**, 795958, doi:10.1155/2012/795958 (2012).

195 Rieber, N. *et al.* Acellular pertussis booster in adolescents induces Th1 and memory CD8+ T cell immune response. *PLoS One* **6**, e17271, doi:10.1371/journal.pone.0017271 (2011).

196 Liang, F. *et al.* Dissociation of skeletal muscle for flow cytometric characterization of immune cells in macaques. *J Immunol Methods* **425**, 69-78, doi:10.1016/j.jim.2015.06.011 (2015).

197 Lahoz-Beneytez, J. *et al.* Human neutrophil kinetics: modeling of stable isotope labeling data supports short blood neutrophil half-lives. *Blood* **127**, 3431-3438, doi:10.1182/blood-2016-03-700336 (2016).

198 Tak, T., Tesselaar, K., Pillay, J., Borghans, J. A. & Koenderman, L. What's your age again? Determination of human neutrophil half-lives revisited. *J Leukoc Biol* **94**, 595-601, doi:10.1189/jlb.1112571 (2013).

199 Abadie, V. *et al.* Neutrophils rapidly migrate via lymphatics after Mycobacterium bovis BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. *Blood* **106**, 1843-1850, doi:10.1182/blood-2005-03-1281 (2005).

200 Reis e Sousa, C. Dendritic cells in a mature age. *Nat Rev Immunol* **6**, 476-483, doi:10.1038/nri1845 (2006).

201 Reinisch, W. *et al.* Donor dependent, interferon-gamma induced HLA-DR expression on human neutrophils in vivo. *Clin Exp Immunol* **133**, 476-484, doi:10.1046/j.1365-2249.2003.02245.x (2003).

202 Yang, C. W., Strong, B. S., Miller, M. J. & Unanue, E. R. Neutrophils influence the level of antigen presentation during the immune response to protein antigens in adjuvants. *J Immunol* **185**, 2927-2934, doi:10.4049/jimmunol.1001289 (2010).

203 Zilio, S. & Serafini, P. Neutrophils and Granulocytic MDSC: The Janus God of Cancer Immunotherapy. *Vaccines (Basel)* **4**, doi:10.3390/vaccines4030031 (2016).

204 Sui, Y. *et al.* Paradoxical myeloid-derived suppressor cell reduction in the bone marrow of SIV chronically infected macaques. *PLoS Pathog* **13**, e1006395, doi:10.1371/journal.ppat.1006395 (2017).

205 Zahorchak, A. F., Ezzelarab, M. B., Lu, L., Turnquist, H. R. & Thomson, A. W. In Vivo Mobilization and Functional Characterization of Nonhuman Primate Monocytic Myeloid-Derived Suppressor Cells. *Am J Transplant* **16**, 661-671, doi:10.1111/ajt.13454 (2016).

206 Warfel, J. M., Zimmerman, L. I. & Merkel, T. J. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. *Proc Natl Acad Sci U S A* **111**, 787-792, doi:10.1073/pnas.1314688110 (2014).

207 Chit, A. *et al.* Acellular pertussis vaccines effectiveness over time: A systematic review, meta-analysis and modeling study. *PLoS One* **13**, e0197970, doi:10.1371/journal.pone.0197970 (2018).

208 Tefon, B. E. *et al.* A comprehensive analysis of Bordetella pertussis surface proteome and identification of new immunogenic proteins. *Vaccine* **29**, 3583-3595, doi:10.1016/j.vaccine.2011.02.086 (2011).

209 Altindis, E. *et al.* Immunoproteomic analysis of Bordetella pertussis and identification of new immunogenic proteins. *Vaccine* **27**, 542-548, doi:10.1016/j.vaccine.2008.11.020 (2009).

210 Yilmaz, C., Apak, A., Ozcengiz, E. & Ozcengiz, G. Immunogenicity and protective efficacy of recombinant iron superoxide dismutase protein from Bordetella pertussis in mice models. *Microbiol Immunol* **60**, 717-724, doi:10.1111/1348-0421.12445 (2016).