The contribution of immunology to the rational design of novel antibacterial vaccines

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Abstract | In most cases, a successful vaccine must induce an immune response that is better than the response invoked by natural infection. Vaccines are still unavailable for several bacterial infections and vaccines to prevent such infections will be best developed on the basis of our increasing insights into the immune response. Knowledge of the signals that determine the best possible acquired immune response against a given pathogen — comprising a profound T- and B-cell memory response as well as long-lived plasma cells — will provide the scientific framework for the rational design of novel antibacterial vaccines.

"The specific antitoxins which represent the active principle of blood serum therapy have only been found in the blood of immunized animals." [1](Behring, 1894)

Every year, 4 million people die of acute respiratory infections, 2 million die of diarrhoeal disease — most of them children under 5 years of age — and another 2 million people die from tuberculosis (TB)[2]. Added to this list is the increasing threat of the nosocomial infections that are caused by staphylococci, enterococci, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and other bacterial pathogens[3–12]. Additionally, the increasing incidence of multidrug-resistant strains, notably meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), is becoming a major concern: in the US alone, 2 million people suffer from nosocomial infections annually, of whom 100,000 die; and up to US$20 billion are spent on nosocomial infections in the industrialized world per year. The various bacterial pathogens that cause food-borne diseases, which have diarrhoea as the most frequent outcome, are not covered here for reasons of brevity. Obviously, new vaccines against the bacterial infections that pose a threat in both the developing and industrialized world are urgently needed, and could save millions of lives every year and avert costs of billions of US dollars.

Wherever they have been implemented successfully by covering ~90% of the target population, vaccines have impressively proven their efficacy[13]. The oldest vaccines in use today were developed early in the last century and are directed against toxins, such as those produced during tetanus and diphtheria infections. After a gap of half a century, a subunit vaccine against *Bordetella pertussis* was produced and, as the most recent advance, conjugate vaccines against *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae* (pneumococci) and *Neisseria meningitidis* (meningococci) followed.

Why do we have vaccines for some, but not all, bacterial pathogens (BOX 1)? Current vaccines against bacterial pathogens or their toxins are based on pre-existing antibodies in the serum, which prevent disease but not infection[14]. In the case of tetanus and diphtheria, antibodies neutralize the toxins. In the case of pneumococci, meningococci and Hib, antibodies activate phagocytes and complement for bacterial destruction[15]. Impressive progress has been made in the field of vaccine development, which is highlighted by the success of conjugate vaccines that stimulate the production of immunoglobulin (Ig) G against encapsulated bacteria by conjugating carbohydrates to protein carriers[16–18]. Yet, until now, the contribution of immunology to the development of antibacterial vaccines has been minimal.

Vaccinology pre-existed immunology as a discipline, with the heroes of vaccinology being Edward Jenner (1749–1823) and Louis Pasteur (1822–1895): Jenner introduced vaccinia (cowpox; the name vaccinia comes from the Latin term for cow, *vacca*) as the first reliable vaccine, and Pasteur coined the generic term 'vaccine' in honour of this achievement (FIG. 1). Immunology was developed as a spin-off from infection biology and vaccinology at the end of the nineteenth century when Paul Ehrlich (1854–1915) and Emil Behring (1854–1917) joined forces to develop passive vaccination, which led to

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Box 1 | **Significant bacterial pathogens**

Streptococcus pneumoniae

Streptococcus pneumoniae mostly afflicts the youngest (<2 years) and the oldest (>65 years) among us[17,135]. Up to 1 million children worldwide die of pneumococcal disease each year and, in developing countries, 10–20% of all deaths among children are caused by pneumococci. Pneumococci cause bacterial pneumonia and influenza-like symptoms as well as otitis media, bacteraemia, sepsis and meningitis. Pneumococci are encapsulated and the carbohydrate components of the capsule are targets of protective immunity. Conjugate vaccines are already available, which cover a profound proportion of the 90 different pneumococcal serotypes that exist. These vaccines have achieved massive reduction of pneumococcal diseases; they also reduced pneumococcal colonization in vaccinated children and, consequently, the rate of transmission to non-vaccinated individuals of all ages[108,136]. This reduction includes antibiotic-resistant strains. Hence, vaccination will increase the success rates of chemotherapy in the long term.

Neisseria meningitidis

This commensal bacterium is found in up to 10% of the human population, who serve as asymptomatic carriers[18,137]. Thirteen meningococcal serotypes have been identified, five of which are responsible for 90% of meningococcal diseases — meningitis and septicaemia. Worst hit are countries of the so-called meningitis belt in central Africa where epidemics strike periodically, the last one in 1996/1997, which afflicted more than 200,000 people. In 2007, a new epidemic seems to be on the rise and has already afflicted more than 22,000 people in Burkina Faso alone, with a 7% fatality rate. A vaccine that covers approximately half of all meningococcal diseases is already available[18].

Group A streptococci

The typical purulent pathogens group A streptococci (*Streptococcus pyogenes*) cause different types of disease, which range from laryngitis/pharyngitis to invasive generalized disease[135,138,139]. Group A streptococci colonize the upper respiratory tract in 10% of all individuals as commensals, from where they can spread to immunocompromised patients or invade diverse tissue sites. In the United States alone, 10 million new cases occur annually, most of them mild, but 10,000 of these cases take severe forms that have a 20% mortality rate.

Group B streptococci

Serious invasive disease can be caused by group B streptococci (*Streptococcus agalactiae*) when newborns are infected during birth — typically by commensal streptococci from their mothers (puerperal fever)[109,135,140]. Group B streptococci are normal inhabitants of the vagina of up to one quarter of women in many parts of the world. Transmission of group B streptococci to newborns during labour and delivery is therefore a frequent event.

The major nosocomial pathogens

Staphylococcus aureus, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* represent the major nosocomial pathogens. They cause various diseases depending on the site of invasion, including pneumonia, urinary-tract infections and systemic infections, as well as skin and diverse tissue infections[3–12]. Although these microorganisms can be treated by chemotherapy, there has been a dramatic increase in the incidence of multidrug-resistant strains; in particular meticillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Vancomycin is the last active drug available for the treatment of MRSA. However, vancomycin treatment favours VRE, and strains of vancomycin-resistant MRSA have already been identified. Some strains of *S. aureus* produce a multitude of toxins, including the toxic-shock-syndrome toxin that is responsible for the shock syndromes associated with menses and the enterotoxins that are responsible for major food poisoning. Enterotoxins and toxic-shock-syndrome toxin are superantigens, which can cause a cytokine storm or septic-shock-like syndrome.

Mycobacterium tuberculosis

Mycobacterium tuberculosis is a member of the infamous triad of killer pathogens that comprises the infective agents responsible for AIDS, malaria and tuberculosis (TB)[123,124,141]. Although we have a vaccine available (bacillus Calmette–Guérin (BCG)) which prevents childhood TB, this vaccine is ineffective in preventing the major form of the disease, pulmonary TB, in adults[123,124]. TB is on the rise in numerous countries, and the situation is becoming increasingly exacerbated for two reasons: first, the increasing numbers of multidrug-resistant (MDR-TB) and even extensively drug resistant (XDR-TB) strains; and second, the deadly coalition between TB and HIV/AIDS. This makes the need for a new vaccine against TB more urgent than ever before, but also raises new complications.

Chlamydia trachomatis

Transmitted during sexual intercourse at a rate of 90 million times annually, two-thirds of *Chlamydia trachomatis* infections occur in developing countries[128,142]. *C. trachomatis* not only causes inflammation but also serves as a cofactor for the transmission of HIV and human papilloma virus, which are responsible for AIDS and cervical cancer, respectively. Furthermore, in the long term, *C. trachomatis* infection can also cause infertility and chronic pelvic inflammation.

Helicobacter pylori

The stomach-dwelling bacterium *Helicobacter pylori* has infected more than half of the world's population[129,130,143]. It can remain silent, or it can cause gastroduodenal disease — notably peptic ulcers — which can later transform into stomach cancer. However, recent studies have proved that there is an inverse correlation between the presence of *H. pylori* in the stomach and oesophageal cancer, which indicates that there might be a protective role for *H. pylori* against this other lethal malignancy.

Of course, the many bacterial pathogens that cause food-borne diseases — which, in most cases, result in diarrhoea — are of equal importance, but these pathogens are not covered here.

Figure 1 | **The fathers of immunology and vaccinology.** The figure shows (**a**) Edward Jenner (1749–1823), (**b**) Louis Pasteur (1822–1895), (**c**) Paul Ehrlich (1854–1915) and (**d**) Emil Behring (1854–1917).

the elucidation of the principles of acquired immunity (FIG. 1). Behring and Ehrlich started their work at the institute of Robert Koch (1843–1910), the founder of medical microbiology. At the institute of Louis Pasteur (who was the champion of vaccinology), Elie Metchnikoff (1845–1916) developed the principles of innate immunity. Thus, infection biology and vaccinology were instrumental in establishing immunology.

All the vaccines that can be developed by trial and error have now been developed, and a comprehensive understanding of the immune response is now needed for the rational design of vaccines that are directed at more complex pathogens. Such pathogens can often be controlled only if the vaccine induces a better immune response than natural infection. With the enormous progress that has been made in the field in recent years, immunology can now offer payback to vaccinology by providing guidelines for the development of new vaccines against bacterial infections.

What to induce: antibacterial immunity

Both the humoral immune response mediated by antibodies and the cellular immune response mediated by T cells are controlled by T helper (T_H) cells. T_H cells have therefore moved into the forefront of vaccinology[19–21]. The T-cell system can be segregated into distinct T-cell subsets, which perform different functions and therefore predominate in different types of infection. The innate immune system senses the type of infectious agent that has invaded the host and instructs the acquired immune system how to generate the appropriate response for defence against the invader[22]. This is regulated by a complex cytokine milieu and an equally complex combination of co-stimulatory molecules, which are expressed on the surface of professional antigen-presenting cells (APCs). Antigens that are presented by the appropriate major histocompatibility complex (MHC) molecules activate T cells, which then undergo further maturation from effector T cells into memory T cells. B cells are stimulated by direct recognition of the antigen and develop into plasma cells and memory B cells. A pathogen invading a vaccinated host is directly attacked by pre-existing antibodies that are produced by plasma cells[14]. In parallel, B- and T-cell memory is reactivated by the pathogen, producing a more robust and accelerated protective response[23,24].

Stimulation of long-lasting immunological memory remains a major goal of vaccine development. Once the pathogen has been eradicated, regulatory T cells (T_{Reg} cells) dampen protective immunity to minimize collateral damage[25,26]. Nevertheless, during chronic infections, this regulation can be misled; on the one hand, an ongoing immune response could cause damage, but on the other, it is required for the control of persistence and prevention of disease. Obviously, a better understanding of how to stimulate memory T cells and manipulate T_{Reg} cells will provide guidelines for the development of more efficacious vaccines[21,22,25,26].

One factor that is of increasing importance is the migration of T cells from immune organs to distant tissue sites, where most infectious diseases become manifest[27]. Of particular significance, obviously, is the mucosal system, which serves as a port of entry and site of disease manifestation for numerous diseases — notably, the respiratory tract and lung for pneumonia, and the gut for diarrhoea. It is not surprising, therefore, that the mucosa has its own immune system, which partly operates independently and partly interacts with the central immune response[28]. A greater understanding of mucosal immunity will help design mucosal vaccines that can be administered by the oral or aerogenic route.

How the innate immune system senses invaders

Until a decade ago, the innate immune system was considered solely as a first line of defence that rapidly attacks, with greater or lesser success, invading pathogens. Although the microbial components that activate the complement cascade had been known for some time, non-specific stimulation of host effector cells existed only in the minds of immunologists. This changed with the identification of the Toll-like receptors (TLRs) as sensors that specifically recognize microbial components or patterns[29]. Later, intracellular recognition molecules with similar functions to TLRs were described[30,31]. In contrast to the TLRs, which are membrane receptors, these molecules are soluble proteins that scavenge the host cell cytosol for foreign invaders. Members of this family are known as NLRs for Nod-like receptors. So far, more than 20 NLRs have been described, which can be grouped into three classes,

Passive vaccination
The provision of protective immunity by the transfer of immunoglobulins or T cells.

Plasma cell
A non-dividing, terminally differentiated, immunoglobulin-secreting cell of the B-cell lineage.

Regulatory T cell
(T_{Reg} cell). A population of $CD4^+$ T cells that naturally express high levels of CD25 (the interleukin-2 receptor α-chain) and the transcription factor forkhead box P3 (Foxp3), and that have suppressive regulatory activity towards effector T cells and other immune cells.

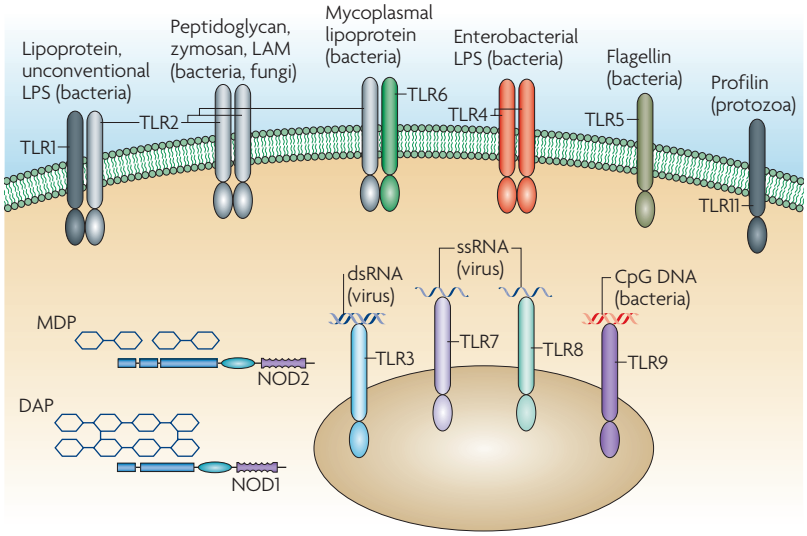


Figure 2 | **Pattern-recognition receptors: TLRs and NODs.** The figure focuses on the better-known pattern-recognition receptors — Toll-like receptors (TLRs) and NODs — leaving out the more recently described members of the expanding Nod-like receptor (NLR) family. The different specificity of each receptor is discussed in the main text. DAP, diaminopimelic acid; ds, double-stranded; MDP, muramyl dipeptide; LPS, lipopolysaccharide; LAM, lipoarabinomannan; ss, single-stranded.

Pattern-recognition receptor (PRR). A host receptor (such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs)) that can sense pathogen-associated molecular patterns and initiate signalling cascades that lead to an innate immune response. These can be membrane-bound (such as TLRs) or soluble cytoplasmic receptors (such as NLRs).

Dendritic cell (DC). 'Professional' antigen-presenting cells that are found in the T-cell areas of lymphoid tissues and as minor cellular components in most tissues. They have a branched or dendritic morphology and are the most potent stimulators of T-cell responses.

CD4$^+$ T cell A subpopulation of T cells that express the CD4 receptor. These cells aid in immune responses and are therefore referred to as T helper cells.

CD8$^+$ T cell A subpopulation of T cells that express the CD8 receptor. CD8$^+$ cells recognize antigens that are presented on the surface of host cells by MHC class I molecules, leading to their destruction, and are therefore also known as cytotoxic T lymphocytes (CTLs).

the NODs, the NALPs (NACHT-, leucine-rich-repeat (LRR)- and pyrin-domain-containing proteins) and a third more diverse family without a specific name. They are all characterized by a nucleotide-binding oligomerization domain (NOD) and a number of LRRs. Many of the NLRs have been associated with human genetic disorders. Because of their recent discovery, vaccinology has not yet extensively exploited their potential, and so in this article I will focus on the TLRs and NODs as they relate to bacterial infections[30–32].

In the domain of bacterial pathogens, TLR2 — sometimes in combination with other TLRs — senses lipoproteins, lipoarabinomannans and lipoteichoic acids; TLR4 senses different lipopolysaccharides of the Enterobacteriaceae; and TLR5 recognizes flagellin of flagellated bacteria (FIG. 2). TLR3, TLR7 and TLR8 are mostly responsible for detecting viral RNA, whereas TLR9 recognizes low-methylated DNA that contains CpG motifs, which are characteristic of bacterial DNA. Less is known about the NODs. However, NOD1 and NOD2 recognize diaminopimelic acid and muramyl dipeptides, both of which are prevalent components of the peptidoglycan that is found in the bacterial cell wall (FIG. 2). These and other pattern-recognition receptors (PRRs) provide the signalling which is ultimately translated by macrophages and dendritic cells (DCs) into a specific cocktail of secreted cytokines and a unique combination of co-stimulatory molecules on cell surfaces. These APCs then direct the acquired immune response[33]. Identification of the ligands for TLRs and NODs will allow the tailored design of adjuvants that stimulate the preferred immune response for a given bacterial pathogen[34]. Adjuvant design is described in detail elsewhere in this Focus issue.

The central role of T cells

The professional APCs comprise macrophages, B cells and DCs. Macrophages might become particularly important for the presentation of antigens from bacterial pathogens that are hard to digest. B cells are particularly interesting as 'specific' APCs as, by means of their specific surface Ig receptors, they can select antigen for presentation and therefore focus on the stimulation of a unique T-cell clone during the acquired immune response. The most effective APCs, however, are the DCs. They can directly engulf pathogens and subsequently process and present their components to T cells[33,35,36]. In addition, it is also possible that DCs take up antigens from bacteria that have been pre-digested by macrophages in the vicinity. Antigens that are processed through class II MHCs are recognized by CD4$^+$ T cells[37]. MHC-class-I-restricted presentation of antigenic peptides results in the stimulation of CD8$^+$ T cells. Some bacterial glycolipid antigens are presented to T cells by CD1 (REF. 38). All these T cells, namely MHC class I-restricted, MHC class II-restricted and CD1-restricted T cells, express a T-cell receptor that comprises an α/β-chain combination[39]. An alternative T-cell receptor that is composed of a γ/δ-chain configuration is used by the so-called $\gamma\delta$ T cells, which recognize phosphate-containing non-proteinaceous antigens without the need for a known presentation molecule[37,39]. Although $\gamma\delta$ T cells and CD1-restricted T cells can contribute to antibacterial immunity, and therefore should not be completely ignored in vaccine development, the major burden of protection rests on the so-called conventional T cells, namely, the MHC class II-restricted CD4$^+$ T cells and MHC class I-restricted CD8$^+$ T cells. Therefore, these conventional T cells are the major focus of vaccine design.

The CD4$^+$ T cells produce a plethora of cytokines that help other cells express their functional activities at full strength[19,22,40]. The CD8$^+$ T cells also produce cytokines, but in addition, they can directly lyse target cells by means of perforin and granzymes[41]. Although, for convenience, cytokine production is the more widely used measurement for CD8$^+$ T cells, the availability of specific monoclonal antibodies has made the analysis of specific cytolytic molecules feasible. Originally, a strict separation between CD4$^+$ T_H cells, as mediators of protection against bacterial infections, and CD8$^+$ cytolytic T lymphocytes (CTLs), as mediators of protection against viral infections, was proposed[37]. This segregation was strengthened by the finding that viral antigens have preferential access to MHC class I antigen processing and bacterial antigens have preferential access to MHC class II antigen processing. More recently, however, this picture has been modified from black and white to graduated gray shading. Some bacteria, such as shigellae and listeriae, can egress from the phagosome into the cytosol where their antigens reach the MHC class I antigen-processing pathway[37]. Accordingly, protection against *Listeria monocytogenes* strongly depends on CD8$^+$ T cells, at least in mice. Most intracellular bacteria, however, such as *Mycobacterium tuberculosis* and *Salmonella enterica*, remain in the phagosome[37], yet protection against chronic infectious diseases like TB and typhoid can benefit from CD8$^+$ T cells in addition to CD4$^+$ T cells.

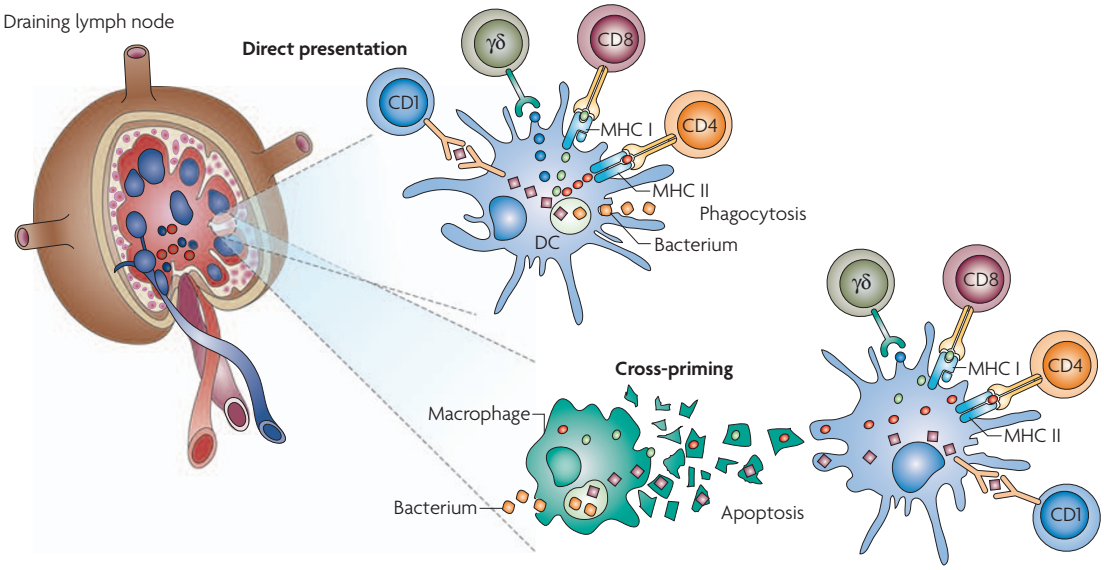


Figure 3 | **Antigen presentation to different T-cell subsets: direct presentation and cross-priming.** Direct antigen presentation leads to unrestricted stimulation of CD4$^+$ T cells, γδ T cells and CD1-restricted T cells. However, some bacteria have developed evasion mechanisms that impair direct antigen presentation. So, vaccine efficacy can be improved by avoiding or counteracting these mechanisms. As most bacterial pathogens reside in the phagosome, direct major histocompatability complex (MHC) class I presentation of antigen for CD8$^+$ T cells is impaired. Only bacterial pathogens that egress into the cytosol, such as *Listeria monocytogenes*, allow for direct antigen presentation to CD8$^+$ T cells. Novel vaccination strategies exploit such pathways to increase CD8$^+$ T-cell stimulation without affecting stimulation of the other T-cell subsets. Cross-priming was originally described as a pathway that allows MHC class I presentation of exogenous antigens to CD8$^+$ T cells. It was later extended to include antigen presentation from bacterial pathogens. As several bacterial pathogens impair direct antigen presentation for CD4$^+$ T cells, γδ T cells and CD1-restricted T cells, cross-priming can also facilitate antigen presentation to these T-cell populations. Novel vaccination strategies therefore exploit cross-priming as a mechanism for improved T-cell responses in general. DC, dendritic cell.

γδ T cell
A minor population of T cells that express the γδ T-cell receptor, and that are more abundant in epithelial-rich tissues such as skin and gut and reproductive tracts. Like NKT cells, γδ T cells can be cytolytic and produce high levels of cytokines and chemokines.

Perforin
A calcium-sensitive membrane-lytic protein that is found in cytoplasmic granules of cytotoxic T lymphocytes and natural killer cells.

Granzyme
A family of serine proteinases that are found primarily in the cytoplasmic granules of cytotoxic T lymphocytes and natural killer cells. These proteinases enter target cells through perforin pores, then cleave and activate intracellular caspases and induce apoptosis of target cells.

Phagosome
The functional definition of the organelle in which bacteria are internalized. Phagosomal and endosomal pathways undergo interconnected maturation and merge before fusion with lysosomes. Some bacterial pathogens inhibit the acidification of the phagosome and its fusion with lysosomes.

T helper 1 cell
(T_H1 cell). A type of activated T_H cell that promotes responses associated with the production of a particular set of cytokines, including interleukin (IL)-2 and interferon (IFN)-γ, the main function of which is to stimulate phagocytosis-mediated defences against intracellular pathogens.

T helper 2 cell
(T_H2 cell). A type of activated T_H cell that participates in phagocytosis-independent responses and downregulates pro-inflammatory responses that are induced by T_H1 cells. T_H2 cells secrete interleukin (IL)-4 and IL-5.

Until recently, the route taken by bacterial antigens from the phagosomal compartment to presentation by MHC class I molecules was incompletely understood. We now know that two pathways are of particular importance[42]. In the first, during chronic infection, the phagosome membrane becomes leaky, under the assault of bacterial cytolysins for example, which form small pores that allow the translocation of larger molecules but not of the complete bacterium (FIG. 3). Second, infected macrophages undergo apoptosis, which leads to the formation of apoptotic blebs that are filled with bacterial antigens (FIG. 3). These apoptotic vesicles can be taken up by DCs, which then process and present the antigens with high efficiency, both through the MHC class II and MHC class I pathways, resulting in CD4$^+$ and CD8$^+$ T-cell stimulation[43–45]. Originally, the mechanism underlying the loading of MHC class I molecules with exogenous antigens was known as cross-priming. In bacterial infections, this process frequently involves the apoptosis of infected host cells and the subsequent uptake of pre-processed antigenic cargo by APCs[43–45]. As many bacterial pathogens can impair antigen presentation by infected APCs, this pathway not only improves the stimulation of MHC class-I-restricted CD8$^+$ T cells, but also that of MHC class-II-restricted CD4$^+$ T cells, CD1-restricted T cells and γδ T cells. Accordingly, I extend the term cross-priming to encompass the broad antigen presentation to different sets of T cells that is caused by the same or similar events.

T_H cells occupy a central role in all immune responses because they help B cells to produce antibodies, activate macrophages to kill intracellular pathogens, and promote the development of CTLs, which kill infected target cells. With such multipotency it is no surprise that T_H cells undergo a further segregation, which, until recently, was thought to be binary: T helper 1 (T_H1) cells being responsible for cell-mediated immunity, with interferon γ (IFN-γ) and interleukin 2 (IL-2) as the lead cytokines; and T helper 2 (T_H2) cells being central for humoral immunity and defence against helminths, with IL-4 and IL-5 as the lead cytokines[40,46] (FIG. 4). More recently, a third T_H cell type, T_H17 cells, have been identified as a distinct entity[47–50]. These T cells produce IL-17 as a marker cytokine and are apparently highly pathogenic because they have mostly been found in subjects suffering from autoimmune diseases[51]. However, it is likely that they also have a role in antimicrobial defence and the initial evidence indicates that they might participate in immunity against extracellular bacteria by activating neutrophils[52]. More recent findings indicate that T_H17 cells might also contribute to protection against intracellular bacteria by directing T_H1 cells to the site of bacterial replication[53]. Another cell type that express the CD4 phenotype — T_{Reg} cells — control and counteract excessive immune responses[54]. Both natural T_{Reg} cells that are already active before antigen encounter and inducible T_{Reg} cells that must be activated by antigen exist[25,26].

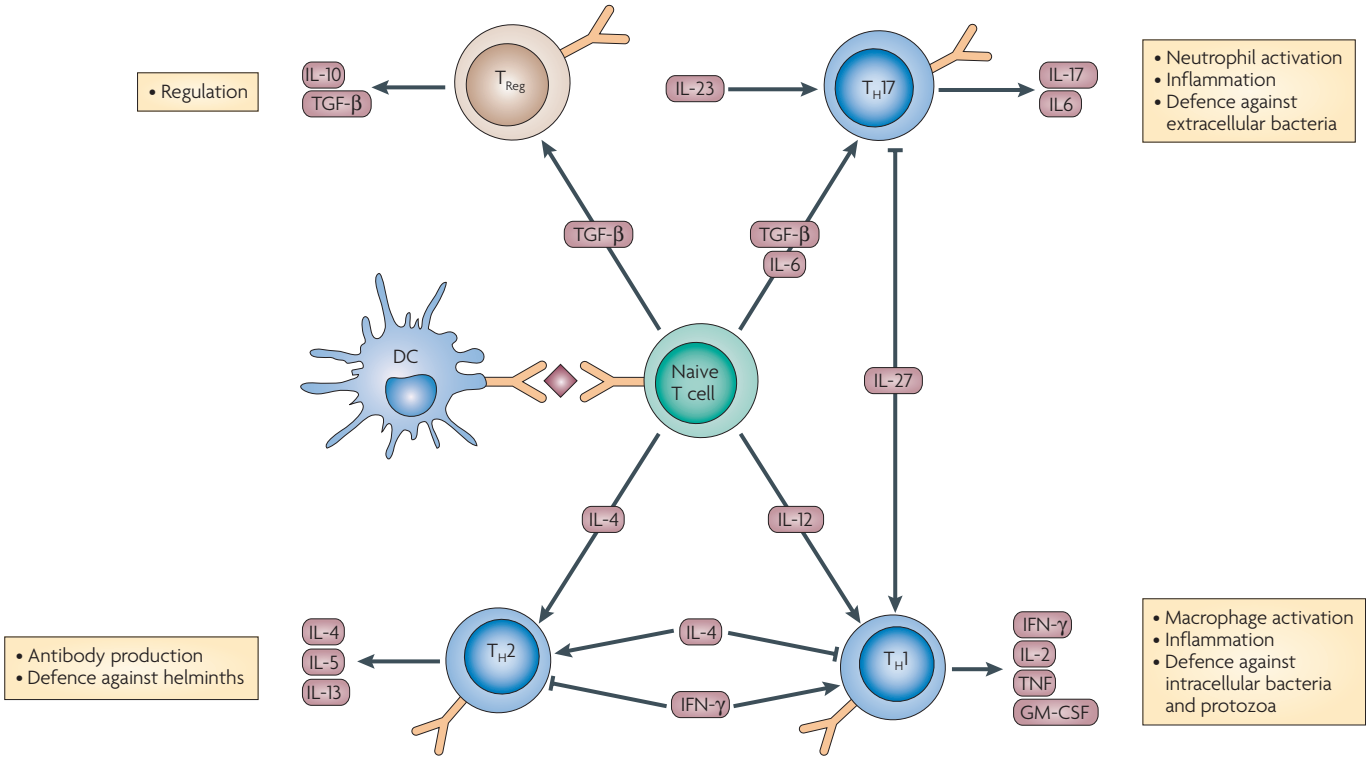


Figure 4 | **Different subpopulations of CD4⁺ T cells.** Four main populations of CD4⁺ T cells are shown. Regulatory T cells (T_{Reg} cells) control and counteract excessive immune responses. T helper 1 (T_H1) cells are responsible for cell-mediated immunity, with interferon γ (IFN-γ) and interleukin 2 (IL-2) the lead cytokines produced. T helper 2 (T_H2) cells are of central importance in humoral immunity (antibody production) and defence against helminths, with IL-4 and IL-5 the lead cytokines. T_H17 cells produce IL-17 and initial evidence indicates that T_H17 cells might be involved in defence against extracellular bacteria by activating neutrophils and intracellular bacteria by directing T_H1 cells to the site of bacterial replication. DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; TGF, transforming growth factor.

Activation of the different CD4⁺ T cells is regulated by the surface expression of co-stimulatory molecules and by the cytokine milieu that is created by APCs[22,55,56] (FIG. 3). So, the secretion of transforming growth factor (TGF)-β alone favours the development of T_{Reg} cells, which later control immune responses through IL-10 and TGF-β[57]. Together with IL-6, however, TGF-β also promotes the development of T_H17 cells, which are further sustained by IL-23, but counter-regulated by IL-27 (REFS 57–59). IL-12, with the help of IL-27, favours the development of T_H1 cells[58,59]. T_H1 cells are characterized by the production of IFN-γ, tumour necrosis factor (TNF), IL-2 and granulocyte–monocyte colony-stimulating factor (GM-CSF). IL-4 encourages T_H2 cells to become potent producers of IL-4, IL-5 and IL-13. T_H2-derived IL-4 and T_H1-derived IFN-γ inhibit T_H1 and T_H2 cells, respectively.

Several of these findings in basic immunology are currently exploited in vaccinology, notably in cancer vaccination. These include: targeting of antigens to DCs by means of monoclonal antibodies that are specific for DC markers; vaccination with vectors that induce cross-priming or with vectors that co-express immune-stimulating cytokines and growth factors to improve conditions for T-cell stimulation; and concurrent stimulation or inhibition of co-stimulatory molecules that improve or impair T-cell responses, respectively[34,60–66].

Memories are made of…

Obviously, the induction of immunological memory is central to all vaccination regimens. In this context, immunological memory is defined as a response that persists in the absence of the homologous antigen, that is, it can be maintained for a prolonged time period after clearance of the vaccine material[22–24]. Immunological memory comprises both memory B cells and memory T cells, which are cellular clones that are ready to respond promptly to pathogen or toxin encounter, as well as long-lived plasma cells, which are the main source of pre-existing antibodies that are ready to neutralize pathogens or toxins directly. As is often the case in immunology, memory is a highly complex homeostatic system comprising different developmental stages that involve different lymphocyte subsets.

T-cell memory. Frequently, T-cell memory comprises T cells that are capable of producing multiple cytokines. For example, T_H1 cells produce IL-2, IFN-γ, TNF-α and GM-CSF, whereas effector T cells that are stimulated after primary infection often produce a restricted number of cytokines and are therefore less potent[67]. T-cell memory is accomplished by two main T-cell subsets that develop from effector T cells after a primary antigen encounter (be it a vaccine or a pathogen), namely central memory

T cells and effector memory T cells[22,24,68–72] (FIG. 5a). These two subsets can be distinguished on the basis of their surface markers, which also reflect their different biological functions. In particular, these include CC chemokine receptor-7 (CCR7) and L-selectin (CD62L), which are both critical for T-cell homing to lymph nodes because they interact with high endothelial venules[73]. Central memory T cells express a high abundance of both molecules and therefore reside in lymph nodes, whereas effector memory T cells express few of these molecules and so reside in tissues[68]. Central memory T cells replicate efficiently and, on second antigen encounter, develop into terminally differentiated effector T cells, which probably undergo apoptosis once they have done their job[68]. Effector memory T cells replicate less efficiently, and it has been shown that $CD8^+$ T cells can develop into central memory T cells or perform effector functions directly. So, a protective immune response that is induced by a vaccine, be it an antigen–adjuvant formulation or a live carrier, will induce memory T cells of both types, with a preponderance towards central memory T cells in the long term.

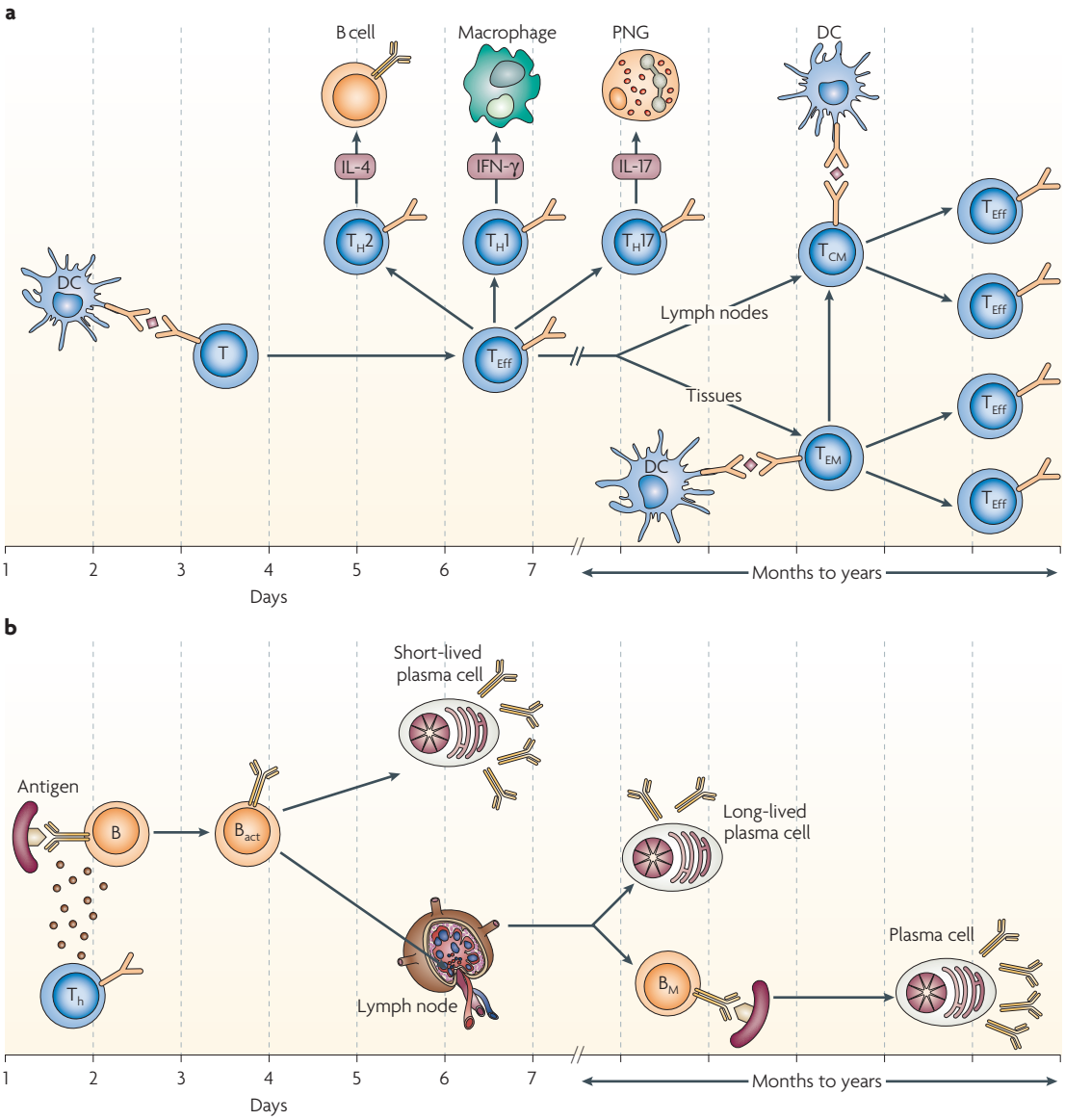


Figure 5 | **Memory generation in cells of the immune system. a** | T cells. Effector T cells (T_{Eff}) respond to a primary antigen encounter by differentiating into the various T cell subsets shown (T helper (T_H) 1, T_H2 and T_H17 cells). Additionally, two main T-cell subsets develop from T_{Eff} cells: central memory T cells (T_{CM}) in lymph nodes and effector memory T cells (T_{EM}) in tissues. T_{CM} cells replicate efficiently and, on second antigen encounter, develop into terminally differentiated T_{Eff} cells. T_{EM} cells replicate less efficiently than T_{CM} cells. **b** | B cells. Antigen-activated B cells (B_{act}), with assistance from $CD4^+$ T_H cells develop directly into short-lived plasma cells, which are responsible for the first burst of antibodies that target the invading pathogen. In secondary lymphoid organs, B_{act} cells develop into memory B cells (B_M) in germinal centres, resulting in high-affinity memory B cells and long-lived plasma cells that are responsible for continuous secretion of pre-existing antibodies. DC, dendritic cell; IFN, interferon; IL, interleukin; PNG, polymorphonuclear granulocyte.

Central memory T cell
A memory T cell that lacks immediate effector function but can mediate rapid recall responses and has the capacity to circulate from the blood to the secondary lymphoid organs. Rapidly develops the phenotype and function of effector cells after restimulation with antigen.

Effector memory T cell
A memory T cell that homes to inflamed tissues. Can exert immediate effector functions without the need for further differentiation.

High endothelial venule
(HEV). A specialized venule with a cuboidal endothelial lining that occurs in peripheral lymph nodes and Peyer's patches. HEVs allow continuous transmigration of lymphocytes as a consequence of the constitutive expression of adhesion molecules and chemokines at their luminal surface.

The situation is more complicated in chronic infections, in which the persistent presence of antigens can cause continuous stimulation of effector T cells and perhaps also of T_{Reg} cells and therefore impair the development of an appropriate memory response. In theory, central memory T cells would be particularly suited to controlling infectious agents after their entry into lymphoid organs, whereas effector memory T cells produce a response directly at the tissue site where they reside. In the real world, the differences between these T-cell subsets are not as stringent as could be concluded from this generalized description and the memory-T-cell response possesses a high degree of plasticity, which allows it to respond to a given pathogen in the best possible way. The identification of such differences will help to define the optimum vaccination strategy for distinct pathogens. In particular, vaccination strategies that target T-cell immunity have attempted to improve vaccine efficacy by heterologous prime–boost strategies[74–76]. In addition to TB, HIV/AIDS is the target of such strategies[77].

Stimulation and differentiation of T cells is controlled by the cytokine milieu[40,73]. Of equal importance and complexity are the co-stimulatory molecules (most of which are members of the B7 family of professional antigen-presenting molecules) and their ligands on T cells at different developmental stages[22,55,56]. The impact of different cytokines on the development of distinct T_H-cell populations (T_H1, T_H2 and T_H17 cells) has been described above. The cytokines IL-7 and IL-15 are important factors for the development of T-cell memory[78].

The B7-1 (CD80) and B7-2 (CD86) molecules on professional APCs are involved in stimulating naive T cells that express the co-receptor CD28 (REF. 56). CD28 signalling also promotes, but is not critical for, memory-T-cell responses. CTLA-4 serves as a second co-receptor for B7-1 and B7-2, and modulates CD28 signalling in T-cell activation in both the primary and secondary responses — mostly in an inhibitory way[79,80]. The inducible T-cell co-stimulator (ICOS) molecule is found on memory T cells and effector T cells and, therefore, by interacting with its co-receptor ICOS-L on professional APCs, has an important role in T-cell activation and differentiation[81–84]. Programmed death-1 (PD-1; also known as PDCD1) and its co-receptors are thought to control the effector functions of T cells, after secondary antigen encounter or during chronic infections, by exhausting T-cell responses[85–89]. Professional APCs express the PD co-receptors PD-L1 (also known as B7-H1 and CD274) and PD-L2 (also known as B7-DC and PDCD1LG2), and activated T cells, particularly effector memory T cells, express the PD-1 receptor. In addition to the B7 family, the CD40–CD40L (also known as CD154) signalling pathway increases T-cell activation after both primary and secondary encounters with antigens[90,91]. In summary, T-cell stimulation is a complex event that is primarily directed by surface molecules, which stimulate different responses depending on qualitative and quantitative differences in surface-molecule composition. Memory-T-cell responses are generally more resistant to these differences than primary T-cell responses, which facilitates more robust and accelerated responses after secondary antigen encounter (or primary pathogen encounter in vaccinated individuals).

B-cell memory. Even though memory-B-cell responses have been exploited since the beginning of vaccinology, it is only now that we are beginning to understand their complexity[14,15,24,92–96]. B-cell memory for T-cell-independent antigens — such as the capsular carbohydrates of pneumococci and meningococci — exists. Recent data indicate that the responsible cells (B1b cells) might differentiate, not only into IgM-producing plasma cells, but also into B1b memory cells with the phenotype $B220^{low}$, $CD19^+$, $CD11b^+$ (REFS 92,97,98). These B1b memory cells rapidly develop into plasma cells after a second encounter with the same antigen.

Current conjugate vaccines, as well as novel vaccine candidates for conserved protein antigens of pneumococci and meningococci, however, are aimed at stimulating T-cell-dependent B-cell responses. With the help of $CD4^+$ T_H cells, antigen-activated B cells develop directly into short-lived plasma cells, which are responsible for the first burst of antibodies that target the invading pathogen (FIG. 5b). Whereas T_H2 cells have a general role in B-cell maturation to antibody-producing plasma cells, T_H1 cells are required for the switch to opsonizing immunoglobulin classes. The B-cell follicles in secondary lymphoid organs provide the appropriate histological framework for close interactions between B cells and T_H cells and, again, co-stimulatory molecules as well as cytokines have an important role, including ICOS, ICOS-L and CD40 (REFS 14,99,100). Subsequently, activated B cells develop into memory B cells, with the phenotype $B220^+$, $CD19^+$, $CD11b^-$ in germinal centres[99,100]. This germinal-centre reaction results in high-affinity memory B cells and long-lived plasma cells. Whereas naive B cells are restricted to the B-cell follicles, the memory B cells recirculate in the periphery ready to directly sense antigens.

Memory B cells are important mediators of vaccine-induced protection because, following an encounter with their target pathogen, they will develop into plasma cells that produce protective antibodies. Of equal or even more importance, however, are the long-lived plasma cells that produce pre-existing antibodies which immediately attack invading pathogens[14,94,101]. These long-lived plasma cells migrate from the germinal centre into the bone marrow, which provides the appropriate milieu for their maintenance and for antibody production[14,102–104].

Recent studies in both mice and humans revealed a role for TLRs in the activation and maintenance of B-cell responses[95,105,106]. The constitutive expression of different TLRs on human memory B cells allows the maintenance of B-cell memory in an antigen-independent manner due to the continuous TLR stimulation that is caused by encounters with commensal or pathogenic microorganisms of different types. So, protective antibodies are produced over long periods of time through TLR sensing of microorganisms. 'Generic' stimulation of specific antibody production can be exploited for long-lived maintenance of vaccine-induced immunity through intermittent boosters with TLR ligands. This

Heterologous prime–boost strategies
When a single application of a vaccine is insufficient, repeated vaccinations are carried out using different vaccine preparations, allowing the sequential stimulation of a better immune response.

Germinal centre
A highly specialized and dynamic microenvironment in the follicles of secondary lymphoid tissues (spleen, Peyer's patches and lymph nodes) that gives rise to secondary B-cell follicles during an immune response. The main site of B-cell maturation, leading to the generation of memory B cells and plasma cells that produce high-affinity antibody.

could facilitate the improvement of vaccines that induce weak and short-lived immune responses.

Vaccination and memory. The development of new vaccines against bacterial infections can benefit enormously from our increased knowledge of the ways in which the different types of immunological memory are stimulated. By designing adjuvants that provide the appropriate milieu — notably the cytokine mix and the composition of cell-surface co-stimulatory molecules — the required lymphocyte populations can be generated. First of all, however, we need to understand which antibody classes, cytokines and lymphocyte populations are required for protection against a given pathogen. Whilst it goes without saying that T_H1 cells are the main focus of interest for vaccines against many bacterial infections, T_H2 cells will be an important effector that should be considered wherever protective antibodies are needed. The role of T_H17 and T_{Reg} cells remains elusive at this stage, but elucidation of the biological functions of these T-cell subsets and how they can be harnessed for vaccine design would be of great value.

New approaches towards active vaccination

In the following section, I describe three levels of increasing complexity for future vaccine design from an immunological perspective. Vaccine development at Level 1 is technically feasible with our current knowledge; vaccine design at Level 2 is based on the latest state-of-the-art research, but could be ready to enter the developmental pipeline; and vaccine design at Level 3 is a more conjectural endeavour that still requires more basic research.

Level 1: T-cell-dependent antibody responses. Vaccines at Level 1 aim to stimulate pre-existing antibodies and B-cell memory. Therefore, they can build on experience with previously developed successful vaccines. To further increase their success rates, new adjuvants that stimulate T_H cells in addition to B cells will be of help. The finding that memory B cells express TLRs opens up possibilities for a generic booster vaccine that has custom-made TLR ligands to stimulate different B cells that have specificity for a multitude of pathogens.

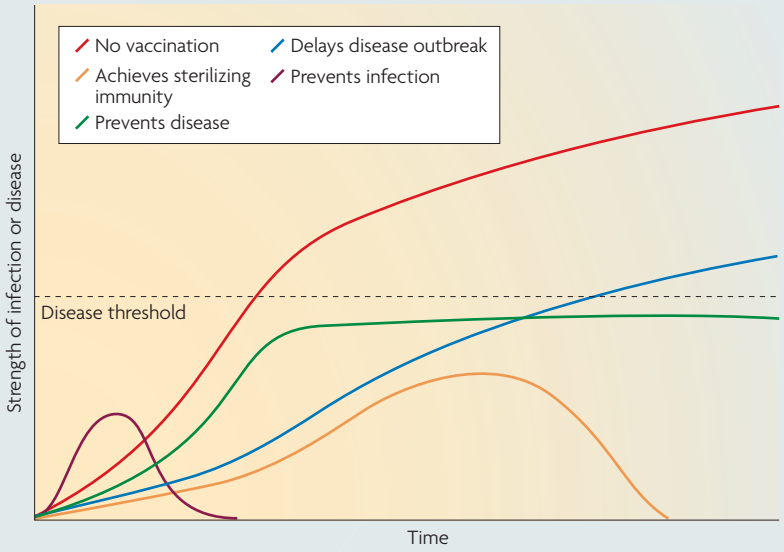
Vaccination against meningococci and pneumococci is achieved by conjugate vaccines directed against carbohydrates that are specific for distinct serotypes[17,18]. The multivalent conjugate meningococcal vaccine covers four of the five serogroups, which are responsible for most meningococcal disease. A conjugate vaccine exists for each of the major meningococcal strains, except for group B[18]. The group B meningococci are responsible for up to half of all meningococcal disease, however, use of a conjugate vaccine against group B meningococci is not feasible because of crossreactivity between the carbohydrate and human tissue, notably the neural cellular-adhesion molecule (N-CAM). This is a serious obstacle. A vaccine against the outer-membrane protein of meningococci type B has been developed and shows good protective responses[18,107]. But a vaccine that comprises a conserved protein — which would cover all meningococcal strains — would be ideal.

Incomplete coverage of all pneumococcal serotypes is mostly a technical and financial issue, which could be solved if necessary[17]. The pneumococcal strains not covered by vaccination were considered a minor fraction of disease-causing agents. However, more recent evidence indicates that these pneumococci can fill the niche generated by the vaccine and therefore new serotypes (replacement strains) are currently replacing the prevalent pneumococcal strains[108]. In both cases, the best strategy would be to identify conserved protein antigens that are shared by all meningococcal or all pneumococcal strains, and which, in an appropriate adjuvant, induce sufficiently broad antibody responses to cover all strains. Some meningococcal and pneumococcal proteins penetrate the bacterial capsule and are accessible for pre-existing antibodies, and would therefore make ideal targets for such vaccines.

A similar scenario holds true for nosocomial pathogens that have increasing multidrug-resistance profiles, including *S. aureus* (notably MRSA) and *S. epidermidis*, *P. aeruginosa, Enterococcus faecalis* (notably VRE), *E. coli* and *K. pneumoniae* as well as group A and group B streptococci, which are all controlled by antibodies[12,109–113]. Vaccines against group A streptococci have to contend with crossreactivity between the streptococcal M protein and human heart, kidney and cartilage tissue[112–114]. This crossreactivity is pivotal for the development of rheumatic fever subsequent to group A streptococcal infections. Because of the risk of autoimmune disease caused by this crossreactivity, a vaccine that is composed of conserved epitopes that are shared by most streptococcal A types, but not by human tissue antigens, would be desirable[114]. Alternatively, antigens that are distinct from the M protein should be evaluated. Active vaccination against group B streptococci has been tested using carbohydrate conjugate vaccine candidates. The high variation of serotypes of group B streptococci in different parts of the world ultimately demands a vaccine that is composed of a conserved antigen, probably a protein[109,115].

In conclusion, pre-existing protective antibodies and their antigens are the focus of Level 1 vaccine design. Accordingly, the identification of protective antigens provides the first and critical step in development. The focus has shifted back to protein antigens, even for encapsulated bacteria in which carbohydrate antigens induce protective immunity, driven by the unique specificity of antibodies directed against carbohydrates. The identification of protective protein antigens can be pursued by reverse vaccinology, which is primarily based on *in silico* analyses of the microbial genome in question[116]. Therefore, reverse vaccinology allows candidate vaccine antigens to be predicted independently of performing experiments with the pathogen in the wet laboratory, followed by testing of the immunogenicity of the predicted target antigens in experimental animal models[107,115,117]. Both vaccines against pneumococci and meningococci have benefited from this approach[107,117]. More sophisticated forms of vaccine design further exploit the immune response against natural infection to select relevant target antigens. These include immunomics,

Box 2 | **Different types of vaccines – how much is needed?**



The figure illustrates possible outcomes of infection in vaccinated individuals. The red line shows an unvaccinated individual in whom infection progresses until it exceeds the threshold above which clinical signs of disease develop. Current vaccines often delay the outbreak of disease by neutralizing or inactivating the invading pathogen or its toxins with the help of pre-existing antibodies. Such a delay often allows the immune response that is induced by natural infection to evolve. The subsequent cooperation of pre-existing antibodies with vaccine- and infection-induced immunity often results in pathogen eradication (orange line). In some cases, however, this immune response fails to eradicate the pathogen, and the vaccine only delays the outbreak of disease. As long as infection remains latent — that is, it does not exceed the threshold of clinical disease — this can be satisfactory (green line). However, the risk remains that the immune response that actively prevents disease breaks down — with age, or because of immunosuppressive insults for example — and then disease can evolve (blue line). So, a vaccine that prevents disease but not infection always bears the risk of disease outbreak at a later time point. A vaccine that prevents infection, however, would eradicate the pathogen before it has established itself in a secluded niche (purple line).

which applies algorithms that predict binding sites for B- and T-cell epitopes[118]. A number of relatively reliable algorithms have been developed for the definition of T-cell epitopes, mostly based on MHC binding, whereas the development of algorithms predicting B-cell epitopes is less advanced. The anti-genome approach exploits the specificity of antibodies that develop during natural infection, preferably without causing severe disease, to identify protective antigens from random genomic libraries[119]. Ideally, antigens that perform vital functions in the pathogen will be selected, so that protective antibodies by themselves will be bactericidal. Functional genomics can help to assign biological functions to hypothetical gene products and thereby facilitate the identification of protective antigens. The first promising results have been obtained with this strategy for a vaccine against *S. aureus*[120,121].

Level 2: T-cell-mediated responses. Future vaccination strategies at this level will exploit our increasing knowledge about antigen targeting to the different MHC pathways, stimulation of APCs through PRRs and stimulation and development of T cells. This can be achieved by manipulation of both the cytokine milieu and the surface expression of co-stimulatory molecules and their co-receptors on the cell surface of APCs or T cells, respectively. Obviously, the major targets of such Level 2 vaccines are intracellular bacteria, for which efficacious vaccines have not yet been developed — not least because they can hide from antibodies by virtue of their intracellular location.

M. tuberculosis is a highly robust pathogen that survives in the early phagosome of macrophages[122–124]. Even activated macrophages fail to eradicate this pathogen and cause only growth inhibition. Accordingly, in immunocompetent individuals, *M. tuberculosis* exhibits lifelong persistence without causing disease in 90% of those who are infected[122,124,125]. The bacteria are not eliminated, but are controlled by an active T-cell response. In 10% of infected individuals, disease will develop after an incubation time that can range from weeks to decades. Current vaccine design against TB is mostly directed at stimulating a potent immune response, which reduces the proportion of individuals who will develop active disease. No evidence exists that these vaccination strategies will achieve sterile eradication of the pathogen. Rather, they will delay disease outbreak, that is, they will contain the pathogen, hopefully for the lifetime of the host (BOX 2).

With the advent of HIV, however, the situation has changed, and it is hard to envisage that in individuals co-infected with *M. tuberculosis* and HIV — that is, in more than 15 million individuals globally, with the incidence increasing — the vaccine-induced immune response will remain efficacious[77,124]. Rather, we must assume that HIV, which harms and destroys $CD4^+$ T_H1 cells — critical mediators of protective immunity against TB — undermines or even nullifies this protective immunity, allowing for the reactivation of active disease. New strategies are therefore needed. A first solution to this would be a combination of novel vaccination schedules in heterologous prime–boost schemes comprising novel vaccine candidates that are capable of inducing sterile eradication of *M. tuberculosis*[122]. The most likely scenario is a prime with a recombinant *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) that induces a stronger immune response than the present BCG vaccine, followed by a boost with a mycobacterial adjuvant formulation or a recombinant modified vaccinia virus Ankara expressing a dominant mycobacterial antigen[125]. The demonstration of increased protective efficacy of various subunit vaccines given on top of BCG support this proposal[74–76]. Once proof of principle has been achieved, future refinements can be considered, such as expression of stimulatory cytokines by recombinant BCG, measures to interfere with stimulation of T_{Reg} cells and genetic manipulation to restrict the survival of a live vaccine in the host within a limited window of time[61,126,127].

Chlamydia trachomatis is an intracellular bacterial pathogen and protective immunity primarily depends on $CD4^+$ T_H1 cells, with further help from $CD8^+$ T cells and antibodies[128]. So, despite subtle differences, protective immunity against *C. trachomatis* shares characteristics with protective immunity against *M. tuberculosis*.

Accordingly, vaccine design against *C. trachomatis* largely follows the strategies that are being exploited for a TB vaccine.

Current evidence strongly suggests that protection against *Helicobacter pylori* mainly relies on T_H2 cells, whereas T_H1 cells mostly contribute to pathogenesis[129,130]. Despite this dependency on T_H2 cells, it appears that antibodies are dispensable for protection against *H. pylori*. Careful analysis of the immune response that is operative in the stomach will be needed to elucidate the precise mechanisms that underlie protective immunity against *H. pylori*, as it is only once this knowledge has been acquired that rational vaccine design against *H. pylori* will become possible.

In summary, stimulation of a protective T-cell response that performs better than the response induced by natural infection is central to Level 2 vaccine design. Although, so far, this endeavour has virtually no precedent in vaccinology, our knowledge of the immune response has made it feasible.

Level 3: antibody responses. Vaccines at Level 3 will benefit from our increasing knowledge about the different pathways that control B-cell stimulation, such as the co-stimulatory molecules and cytokines that mediate T-cell help, the microbial patterns that are capable of maintaining antibody production, and the means by which B cells and their progenitors are directed to niches that facilitate long-lasting antibody production. Current vaccines mostly prevent disease outbreak rather than infection (BOX 2). In other words, the vaccine-induced immune response, which typically consists of pre-existing antibodies, immediately attacks pathogens, impairs their growth and, consequently, ensures that the bacterial load remains below the threshold required for disease to evolve[94]. Therefore, the outbreak of disease is delayed, which gives the infection-induced immune response time to become fully active, whereupon the response evoked by a combination of vaccination and natural infection ultimately eradicates the pathogen. So, vaccines that delay the outbreak of disease are sufficient to control acute infectious diseases such as pertussis, pneumonia and diarrhoeal diseases.

By contrast, in chronic infections that have long incubation times before disease reactivation, this can cause problems. A good example is TB, in which most infected individuals can control the pathogen without eradicating it, as discussed above. Therefore, as an alternative to sterile pathogen eradication, prevention of infection would be of paramount value[123]. The lung is the main port of entry for *M. tuberculosis*. Therefore, the generation of high levels of IgG and IgA antibodies in the alveolar space would be desirable. This pre-existing IgG/IgA could attack *M. tuberculosis* immediately after infection and kill the pathogen directly by inactivating vital bacterial components or by opsonization, either directly or via complement activation. Subsequently, induction of a highly efficacious response in alveolar phagocytes would be required with the help of T_H17 cells. In such a scenario, infection is prevented and, therefore, the risk of disease reactivation after the development of an immunocompromised state is abolished. In addition, measures might be required to rapidly down-modulate effector cells after pathogen eradication to avoid damage to the highly susceptible lung tissue.

Vaccine design at Level 3 returns to pre-existing antibodies[14,15,94]. At this level, however, pre-existing antibodies must immediately eradicate the pathogen before it can find a protective niche in the host, even before the immune response that is induced by natural infection has developed. These antibodies require high affinity for the target antigen and must mobilize highly efficacious effector mechanisms that kill the pathogen before it can hide. It is likely that, as the preferred port of entry for pathogens, the mucosa will be the combat site. Should this strategy turn out to be successful, it could provide the blueprint for a second generation of vaccines for numerous pathogens for which current vaccines prevent disease outbreak, but not infection.

Back to passive vaccination

Several therapeutic vaccines are currently being developed following the original strategy of Emil Behring, Paul Ehrlich, Shibasaburo Kitasato and Fritz Wernicke, combined with modern technologies for custom-made humanized monoclonal antibodies[14,15,95,131,132]. Recent advances in cell-culture technology and molecular genetics have allowed the production of human monoclonal antibodies as alternatives to hyperimmune sera. These include: humanization of murine monoclonal antibodies by molecular genetics; immunization of transgenic mice bearing human Ig genes, and the subsequent cloning of antibody-producing cells; production of antibodies by phage-display libraries; and production of monoclonal antibodies by human memory B cells *in vitro*. Such monoclonal antibodies could provide novel and safe therapeutics for different infectious agents, including selected bacterial pathogens. The vaccines in the most advanced stage of development target *S. aureus*, *Bacillus anthracis*, *Clostridium difficile* and enterotoxic *E. coli* strains[131]. Custom-made antibodies can be further improved by combining antibody specificities for different epitopes or combinations of antibody-binding sites with antibiotics. One far-advanced approach combines antigen specificity for *S. aureus* with specificity for complement receptor-1 on different blood cells, thereby promoting rapid bacterial clearance[131].

Immunotherapeutic vaccination will gain increasing importance for diseases of low risk but extraordinary consequences, such as those caused by pathogens on the CDC list of dual-use agents. Among bacteria, *B. anthracis* is best known. The extremely low risk of anthrax does not provide grounds for preventive vaccination protocols. However, the increasing incidence of resistant strains and the need for prompt action if an anthrax attack occurs makes immunological-intervention strategies attractive. First, passive vaccination for therapy can be envisaged. The alternative would be harnessing the capacity of the immune system to target pathogens and/or their toxins. It has been shown recently that α-defensins can neutralize the anthrax lethal toxin and thereby profoundly ameliorate anthrax disease in

animal models[133,134]. The α-defensins are well-known natural antibiotics of the mammalian host that have the capacity to perforate bacterial membranes. This more recent finding — that they can also neutralize different bacterial toxins, including anthrax and diphtheria toxin — has opened a new avenue for immunotherapeutic intervention in infectious diseases caused by bacterial toxins[133].

Concluding remarks

Even though immunology was the child of vaccinology and infection biology, it soon went its own way, mostly approaching basic research issues with great success. Vaccinology remained empirical, but equally successful. Vaccination is the most cost-effective intervention measure in medicine; it is generally assumed that for US$1 spent on a vaccine, US$5–10 are saved in health care. The trial-and-error approach, however, has now been exploited to the maximum. We are increasingly beginning to realize that infectious diseases for which we do not have vaccines are best controlled if vaccines are developed that induce a better immune response than that stimulated by the natural infection. A precise understanding of the relevant immune mechanisms can provide guidelines for the rational design of such vaccines. So, in the future, immunology will compensate its application-based partner vaccinology for helping facilitate its creation. This would be a worthwhile endeavour — after all, more than 5 million deaths are caused by bacterial infections annually.

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Competing interests statement
The author declares competing financial interests: see web version for details.

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