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REVIEW

From vaccine practice to vaccine science: the contribution of human immunology to the prevention of infectious disease

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Over the past 50 years, the practice of vaccination has reached the important goal of reducing many of the diseases that afflicted humanity in past centuries. A better understanding of immunological mechanisms underlying the induction of immune protection and the advent of new technology led to improved vaccine preparations based on purified microbial antigens and new adjuvants able to boost both humoral and cellular immune responses. Despite these tremendous advances, much remains to be done. The emergence of new pathogens, the spread of strains resistant to antibiotics and the enormous increase in latent infections are urgently demanding more and more effective vaccines. Understanding the immunological mechanisms that mediate resistance against infections would certainly provide valuable information for the design of new candidate vaccines.
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In the 1700s, the British physician Edward Jenner noted that milkmaids exposed to cowpox rarely contracted the deadly disease of smallpox, which was rampant at the time. He hypothesized, and later proved experimentally, that exposure to the agent causing cowpox could be used to generate protective immunity against smallpox. This revolutionary idea marked the birth of the field of vaccinology, and Jenner's work is widely considered to have saved the lives of more people than that of any other person.[1]

By today's standards, Jenner's experimental approach might be considered rather empirical, but surprisingly much of modern vaccine science is still carried out in the absence of a complete understanding of how vaccines work. Nevertheless, many severe infectious diseases, including smallpox and poliomyelitis, have been eradicated and vaccination is certainly among the most successful medical practices.

So how is it that we still do not fully understand the mechanisms of action of vaccines? And why have we been unable to generate protective vaccines against major diseases such as tuberculosis (TB), malaria and human immuno-deficiency virus (HIV)? A significant factor is the complexity of the host–pathogen interaction. The specifics of the intricate interplay between an invading organism and its human host vary according to the pathogen, the genetic background and physical environment of the host, and how well the two parties have coevolved. Pathogens actively evade the host immune system, either by modulating it to their own advantage or by hiding themselves in the body, as is the case with Mycobacteria in the lungs. This coevolution and adaptation is continuously taking place and is why some diseases, such as aquired immuno-deficiency syndrome, have been so difficult to prevent. This means that what we know of one pathogen can rarely be applied to another in terms of successful vaccine design. Understanding the ongoing and changing nature of coevolution will require extensive immuno-monitoring on a large scale in conjunction with vaccination trials. Further complicating the matter, as the immune system of mammals has been shaped by its interaction with pathogens throughout evolution, we must define the aspects of the immune response, which provide an evolutionary advantage to the host versus the pathogen.

In this review, we will cover the most pertinent features of the immune response to pathogens, and how some of today's vaccines have harnessed these responses. We will review the commonly used adjuvants that enable vaccine designers to potentiate and direct immunity, and will highlight some of the most urgent challenges remaining in the field.

HOST IMMUNE RESPONSE TO PATHOGENS

Whenever an infectious organism breaches the body's barriers, it puts in motion a series of highly coordinated immunological events that aim to eliminate the pathogen (Figure 1). The first line of response is the innate immune system, which can be activated within a few minutes of the initial invasion. Innate immunity relies on the combined action of humoral (complement and cytokines) and cellular (macrophages, dendritic cells (DC) and natural killer cells) components.[2] Macrophages are important for their role in phagocytosing and lysing microorganisms, either directly or through recognition of complement or antibodies coating the pathogen. DC are professional

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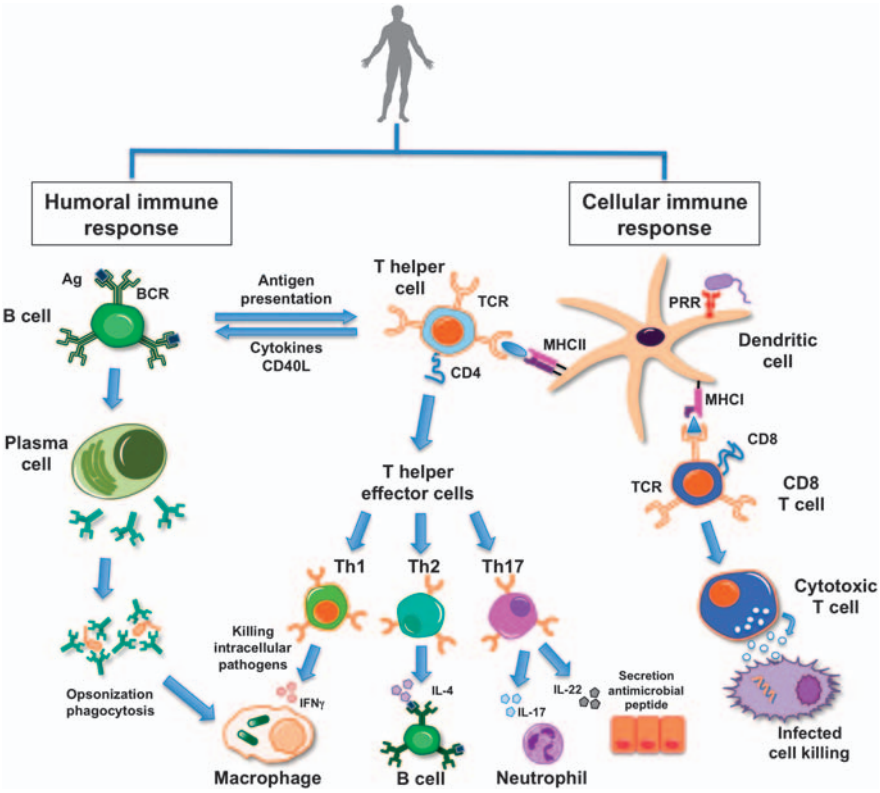


Figure 1 Host immune response. Humoral immune response is initiated when B cells are stimulated through B-cell receptor (BCR) by a particular antigen (Ag), followed by its differentiation into plasma cell. Plasma cells release high-affinity antibodies that opsonize antigens and promote their removal by macrophages. Professional antigen-presenting cells (APC), namely dendritic cells (DC), are activated by microbial-associated antigens through engagement of surface pattern recognition receptors (PPR). Intracellular and extracellular antigens are processed into peptide by APC and loaded onto Major histocompatibility complex class I (MHC I) and class II (MHC II) antigens presented to $CD8^+$ and $CD4^+$ T cells, respectively. DC activation results also in cytokine production and costimulation of T cells. T-helper cells produce multiple cytokines that promote the proliferation and activation of other lymphocytes and macrophages. There are three types of T-helper cells: Th1, Th2 and Th17 cells. Th1 cells produce cytokines as IFNγ that activate macrophages to kill antigen-containing cells. Th2 cells activate B cells to produce antibody. Th17 cells secrete IL-17 that activate neutrophils and IL-22 that induce secretion of antimicrobial peptides by epithelial cells. $CD8^+$ cytotoxic T cells (CTL) affect antigen clearance by directly killing cells, surface tissue antigens of which become altered by exposure to infectious organisms or damaging toxins. Like phagocytes, CTLs depend on T helper cell-produced cytokines.

antigen-presenting cells (APC) that have innate actions but also have a central role in translating messages from the innate immune system and converting them to initiation and direction of adaptive T- and B-cell responses.[3] The adaptive arm of immunity will progress to clear the pathogen and generate immunological memory with the potential to protect the host from reinfection by that pathogen for the rest of its life.[4,5]

A powerful property of the immune system is its ability to tailor its effector mechanisms to the nature of each threat. During infection, the type of microorganism, its route of entry into the host and the site within the body where it resides will all be taken into account in determining the type of immune response most suitable for its eradication (Figure 1). Humoral immunity mediated by antibody is important for neutralization of viruses and bacterial toxins, thus limiting both the damage and spread of infection. Cellular immunity is necessary to eradicate intracellular pathogens and to support and direct the humoral response. For example, in the case of the intracellular bacteria *Mycobacterium tuberculosis* and *Listeria monocytogenes*, the cellular response dominates, mediated by $CD4^+$ T-helper type 1 (Th1) cells secreting IFNγ and TNFα, as well as $CD8^+$ T cells.[6] Activated Th1 cells clonally expand and differentiate into effector cells with the ability to activate macrophages and granulocytes, which are important innate effectors in the clearance of pathogens. Importantly, activated $CD8^+$ T cells have the ability to kill infected cells directly. Extracellular bacteria and fungi also induce cell-mediated immunity, but this time dominated by $CD4^+$ T cells that secrete mainly interleukin (IL)-17, called Th17 cells in host defense.[7] In contrast, infections by helminthes elicit Th2 $CD4^+$ cells secreting IL-4, IL-5 and IL-13.

So, how is this tailoring of the immune response actually achieved? Many parameters of the host response, including the populations of lymphocytes stimulated, the amplitude and the eventual downregulation of effectors largely depend on myeloid DC.[8] These cells are able to interact with the pathogen and translate the information they gain into an appropriately polarized immune response through the activation of naïve lymphocytes (Figure 1). DC engulf pathogens or their components, classify the category of microbe using a system of receptors (discussed later) and degrade the microorganism into peptides loaded onto major histocompatibility complex I (MHC I) and II (MHC II). Peptide-major histocompatibility complexes are presented to antigen-specific T cells alongside the costimulatory molecules required for naïve T cells to become activated and differentiate into effectors. An important feature of DC is the expression of a panel of receptors designed to identify molecular patterns that are conserved within classes of microorganism. For example, Toll-like receptors (TLRs) that are found on the plasma membrane and in endosomal compartments,

recognize components of bacteria, viruses, protozoa and fungi.[9] In addition, DC possess cytosolic receptors such as the nucleotide-binding domain LRR-containing family (NLRs), that sense viral nucleic acids or bacterial products.[10] Recognition through these receptors initiates the process of DC maturation that is characterized by efficient uptake and processing of antigens, increased expression of T cell costimulatory molecules and secretion of soluble T-cell-activating factors. It is this complex process of initiation, maturation and specific stimulation that vaccines aim to mimic in order to harness the natural defense mechanisms that our body uses to protect against infections.

HUMAN VACCINES

Infectious disease vaccines currently licensed for human use have taken various approaches to achieving stimulation of the specific immune response. These can be broadly divided into four categories according to the type of antigen they contain: inactivated, live attenuated, subunit and virus-like particles (VLP).

Inactivated vaccines use the microorganism responsible for the disease, but in a killed form after treatment by chemical or physical agents like heat or radiation. Some of the first vaccines were made in this way; however, they carried the risk of incomplete inactivation, for example, in the Salk vaccine, which in 1955 caused a number of cases of paralytic polio. Modern inactivated vaccines include those against hepatitis A and influenza.[11] The vaccines are very safe but induce only relatively weak immune protection as they lack many of the innate-stimulating properties of the live organisms.

Some of the problems of inactivated vaccines can be overcome by using live attenuated organisms, such as in yellow fever, measles, rubella and mumps vaccines. By serially passaging the pathogen in tissue culture, strains emerge that have lost their genes relating to pathogenicity, virulence and immune evasion, as they are no longer needed. These strains are then safe to inject into a healthy host, where they retain their immunogenicity but are unable to cause disease. Using this strategy a robust immune response is usually achieved with just one or two administrations. However, attenuated vaccines run the small but significant risk of reversion to virulence, potential contamination of the cultures (as in the case of SV40 and polio vaccine) and a lack of safety in immunocompromised individuals.[12]

Subunit vaccines work by stimulating immunity towards just parts of the pathogen, with the hope that this will be sufficient to protect the host, while being safer or more widely applicable than attenuated vaccines.[13] Toxoid vaccines derive from inactivation of purified bacterial toxins (known as toxoids after inactivation), which ultimately cause the disease. Examples are tetanus and diphtheria vaccines containing toxoids purified from *Clostridium tetani* and *Clostridium diphtheriae,* respectively. As with whole-pathogen inactivated vaccines, the lack of live organism means that immunity tends to decline with time, making booster doses necessary but thus running the risk of inducing tolerance to the antigen.[14] This risk is countered by the inclusion of adjuvants, which will be discussed later.

An important development in the subunit vaccine field was the advent of conjugated vaccines, which aim to enhance responses to poorly immunogenic subunits. Such is the case for vaccines against life-threatening invasive meningitis caused by *Haemophilus influenzae* type b, *Streptococcus pneumoniae* or meningococci.[15,16] The first obstacle in immunizing against these organisms is that their pathogenic components are capsular polysaccharides. These are especially weak thymus-independent antigens that induce poor immune responses, especially in young children. To overcome their weak immunogenicity, the purified polysaccharides were conjugated to carrier proteins such as diphtheria or tetanus toxoids.[17] This aims to convert the thymus-independent antigen into a thymus-dependent form, which means that T cells will be stimulated and result in not only a better antibody response but also significantly improved immunological memory.

The progress of advanced molecular biology and genetic engineering techniques is opening new doors in vaccine science. Immunologically relevant proteins from many infectious agents have now been identified, cloned and expressed *in vitro.* This has removed the need to grow large amounts of dangerous pathogenic microorganisms in order to extract their components for inactivation, and so made vaccines safer for both the producer and the recipient. It remains to be seen whether these recombinant protein subunit vaccines combined with adjuvants will be sufficient to induce strong immune responses and long-term protection.

As well as engineered proteins, recombinant DNA can be administered directly as a vaccine. DNA vaccines are a small bacterial DNA plasmid that has been engineered to include the sequence encoding one or two proteins from a pathogen. By injecting DNA into muscle tissue, some cells take up the DNA and become 'antigen factories', producing the protein antigens within the plasmid. This has the major potential advantage that the DNA-delivered antigens are synthesized intracellularly and presented through MHC I, leading to the activation of $CD8^+$ T cells.[18] Thus, unlike conventional vaccines that generally induce predominantly antibody-mediated immunity, DNA vaccines can target the T-cell response.[19] Further advantages of DNA vaccines include their low cost and high thermal stability, which is important for their application in developing countries. Although the concept of DNA vaccination was discovered in the 1990s, many of the details underlying their mechanisms *in vivo* remain unclear.[20–22] For example, it is uncertain whether it is the cells that first take up the DNA are in fact the primary antigen-presenters, or whether bystander APC takes up these cells. It is not clear how long antigen presentation lasts, or how efficiently T-cell effector and memory responses are initiated in humans. DNA vaccines are effective in animal models, but this lack of mechanistic understanding has so far hindered their successful translation into the human setting.

One of the most exciting recent developments in vaccine science has been the use of VLP. VLP exist naturally as coproducts in viral replication, and are essentially viral particles that lack genetic material, while retaining the highly repetitive immunogenic structure of the native virus. By chemically linking desired antigens to the VLP surface, the antigen of choice is conferred the immunogenic properties of the VLP, with little or no need for additional adjuvants. Delivering antigens in this way induces strong humoral responses without the risks associated with live attenuated or inactivated viral vaccines.[23,24] VLP may also be engineered to allow induction of strong T-cell responses, including $CD8^+$ T cells, making them a flexible and powerful tool.[25] Unlike DNA vaccines, this technology has translated well from animal models into humans; VLP-based vaccines have been licensed for use against hepatitis B and human papilloma virus.[26,27] Current research shows promise in the development of VLP vaccines to prevent influenza and Chikungunya virus infection.[28,29]

ADJUVANTS: AN AID FOR INNATE CELLS

An ongoing trade-off in vaccine design is that of safety versus efficacy; most protein and DNA-based vaccines are safer and cheaper to produce compared with live-attenuated or inactivated whole pathogens, but struggle to elicit strong adaptive immune responses and long-term protection. To boost the activity of such vaccines they are

often administered in conjunction with substances known as adjuvants. Adjuvants are agents that, while having little or no antigenic effect of their own, can stimulate the immune system and thus potentiate the antigen-specific effects of a vaccine. Although it is known that the action of adjuvants often relies upon their structural characteristics, their precise molecular and cellular mechanisms are often poorly characterized, although this is beginning to change. As a generalization, many adjuvants are thought to exert their effects through APC, in particular DC. They promote DC maturation, upregulation of antigen-presenting functions and costimulatory molecules, coupled with cytokine secretion and migration into the T-cell area of the draining lymph nodes.[30–33]

Modern adjuvants belong to two main groups: the vehicles and the immunostimulants. Vehicles are substances that enable optimal presentation of the vaccine antigen to the immune system. They include: mineral salts (aluminum or calcium phosphate), emulsions, liposomes, virosomes and biodegradable polymeric microspheres. Immunostimulants differ in that they directly increase the immune response to antigens. Often they are microbial products such as TLR ligands (lipopolysaccharide (LPS), cytidine-phosphate-guanosine, flagellin, lipoproteins, zymosan and bacterial DNA) or bacterial exotoxins, but may also be cytokines or of plant origin (such as saponins).[34]

Because of their powerful immune-modulatory properties, adjuvants are the topic of much research and investment. However, relatively few are licensed for human use, and our knowledge of a selection of these will be summarized in this section.

Aluminum salts

The aluminum salts, known collectively as alum, are the most frequently used vaccine adjuvants and are included in inoculations against *C. diphtheriae* and *C. tetani*, hepatitis A, *S. pneumonia*, meningitis and human papilloma virus.[35] Alum-based adjuvants favor the development of a Th2-polarized response by inducing the production of IL-4, IL-5 and IL-10 by myeloid cells, leading in turn to significantly improved antibody responses.[36]

Remarkably, despite the widespread use of alum and the fact it has been licensed since 1934, until recently its precise mechanism of action was unknown. For over 50 years, it was thought that alum worked by forming a long-lived depot of antigen at the injection site, which provided a continuous supply for APC.[37,38] However, accumulating evidence began to hint that alum had more complex immunomodulatory properties. For example, there were contradictory reports of its ability to stimulate APC *in vitro*, whereas *in vivo* alum injected into the peritoneal cavity of mice induced monocyte recruitment and subsequent differentiation into functional DC.[30,39–41] Tissue phagocytes directly engulfed alum at the injection site. These cells (including macrophages, DC and B cells) responded by producing proinflammatory mediators such as CCL2, CCL3 and CCL11, which begin the cascade of events leading to antigen-specific immunity.[30,31]

The question remained concerning how interaction with this metal salt induced proinflammatory responses in APC. The major breakthrough in the understanding of alum's mechanism of action came in 2007, when it was demonstrated to induce caspase-1-dependent secretion of IL-1β and IL-18 in human and murine macrophages stimulated with LPS.[42] Soon after, B. Lambrecht's group made the discovery that many *in vivo* properties attributed directly to alum were in fact mediated by an intermediate, namely uric acid.[30] Immunization with alum causes a degree of necrotic cell death locally.[43] This leads to the sudden release of a number of potential inflammatory mediators (molecules possessing damage-associated molecular patterns), one of which is uric acid.[44] Once in the extracellular environment, uric acid forms crystals that have adjuvant properties *in vivo* in their own right and in the absence of microbial stimuli.[45–47] The importance of damage-associated molecular patterns in potentiating vaccine responses is an emerging field that should begin to shed light on the complex interaction of adjuvants with the immune system.

Emulsions

One of the most widely used emulsion adjuvants is MF59 (Novartis Vaccines and Diagnostics, Siena, Italy), formed of squalene particles no more than 250 nm diameter, in water.[48] Currently, MF59 is used in Europe in flu vaccines, but it has also previously been used for vaccines against herpes, hepatitis B and HIV.[49–51] Despite obvious biochemical differences with alum, in practical terms there are a number of similarities. MF59 also promotes antibody responses at the expense of Th1 and $CD8^+$ type cell-mediated immunity, and may act in part through the induction of local inflammation at the injection site.[52,53] *In vitro* at least, MF59 enhances the differentiation of monocytes into DC and (unlike alum) additionally acts as a granulocyte attractant.[33] Interestingly, muscle cells may also be specifically targeted by MF59, but currently its main mechanism of action is considered to be formation of an antigen depot that provides sustained APC stimulation and slow antigen release.[32] Another oil-in-water emulsion, called AS03 was developed by GlaxoSmithKline Biologicals (Rixensart, Belgium) and used in the prepandemic flu vaccine. AS03 seems to have the advantage of inducing high antibody responses with associated T-cell activation, but its efficacy in humans remains to be evaluated in depth.[54,55]

TLR ligands

As many subunit vaccines lack the original immunogenicity of the infectious organism they came from, it is a logical step to try and reinstate that property by coadministering microbial TLR agonists. Nonmethylated cytidine-phosphate-guanosine sequences are potent adjuvants in animal models and in some clinical trials.[32,56–58] However, although TLR pathways in general are well characterized, it is not yet clear how the TLR agonist-based vaccine adjuvants act *in vivo*. Intriguingly, it has been shown that antigen-mediated inflammatory responses in mice lacking important component of TLR pathways, such as MyD88 and Trif, were still able to mount specific humoral immune response against allergens injected with different kind of adjuvants (incomplete and complete Freund's adjuvant, alum and TLR agonists).[59,60] These observations suggest that, although TLR activation can lead to increased antibody response, TLR-mediated signaling may be dispensable to induce a greater response to antigens.

Despite the confusion concerning their mechanism of action, some TLR-ligand-related adjuvants are showing promise. The TLR4 ligand, LPS, is highly immune stimulatory but unfortunately to the point of toxicity, at least in animal models. However, a derivative of LPS, known as monophosphoryl lipid A (MPL), isolated from *S. minnesota* R595 was recently approved as vaccine adjuvant for papillomavirus. MPL retains the stimulatory properties of LPS but without its toxicity. Studies in mice suggest that by binding TLR2 and TLR4, MPL promotes DC maturation into potent APC that efficiently stimulate Th1 cells and IgG2a production, making MPL one of the better-understood novel adjuvants.[61–63]

WHY IN SOME CASES VACCINATION FAILS

The 1980s–1990s were debatably the golden decade of vaccine success; with increasing vaccination coverage leading to a significant decrease in the incidence of major vaccine-preventable diseases. Three million

children are saved from death each year as a result of vaccines alone. The most successful vaccines in terms of lives saved, are those against polio, measles, mumps, rubella and yellow fever. All of these vaccines are of the live-attenuated type, which allows them to induce specific, lifelong protective antibodies, memory B cells and plasma cells, as well as polyclonal $CD4^+$ and $CD8^+$ T cells. So why do not we employ the same strategy for all infectious diseases? Here, we will discuss the cases of TB and malaria, which are old enemies of human health and remain so today despite huge efforts to design and administer effective vaccines.

TB

TB is a mycobacterial disease that continues to claim approximately 2 million human lives each year.[64] Surprisingly, perhaps, a live-attenuated vaccine against TB has been widely administered for the last 75 years. This vaccine is derived from *M. bovis*, a primarily veterinary pathogen that was attenuated by tissue culture to give the vaccine strain 'bacillus Calmette-Guérin' (BCG).[65] BCG has undoubtedly helped to reduce TB outbreaks in developed countries, but is unreliable in endemic situations such as those of developing countries, where it is needed most.[66,67] Randomized controlled trials in adults show that the protective efficacy of BCG vaccination against pulmonary TB ranges from 0–80%. In children, the vaccine is generally effective against the most serious manifestations of the disease, such as meningitis or disseminated TB, but even then protection does not last a lifetime.

For many years, the reasons for the shortcomings of the BCG vaccine have been sought, and there are a number of hypotheses.[68–70] The nature of attenuating an organism carries with it the risk that important antigenic determinants of protection will be lost. In the case of BCG this is especially pertinent; first, because it was not originally derived from *M. tuberculosis*, but rather the homologous pathogen *M. bovis*. Second, the original BCG strain has undergone repeated passage over the last 75 years, so not only has it drifted genetically (and so antigenically) but also it is likely that today's strains of *M. tuberculosis* are also different than those BCG used to protect against. It is also difficult to accurately determine BCG's efficacy on a large scale, as before the advent of universally accepted world health organization guidelines each country implemented its own immunization and monitoring policy.[71]

The most common explanation for the variability in protective efficacy of BCG is differences in exposure to nonpathogenic environmental mycobacteria, which can mask or inhibit the protection induced by BCG.[69,70] Research to learn the lessons of BCG could hardly be more urgent. Coinfection of susceptible individuals with HIV and the emergence of multidrug resistant *M. tuberculosis* strains threaten to permit a resurgence of widespread TB death. With this in mind, new strategies for vaccination against TB are under development. For example, it may be possible to genetically modify BCG to increase immunogenicity, or to generate new attenuated strains of *M. tuberculosis* through the targeted deletion of genes that confer virulence to the bacterium.[72–74]

Malaria

Malaria is an infectious disease affecting almost 40% of the world's population, and is prevalent across Africa, South-East Asia, Latin and Central America. The disease kills one to two million people each year, mainly children under 5 years of age. Malaria is caused by the Plasmodium parasite, which is transmitted to humans through mosquito bites. Along with TB, malaria is one of the most pressing candidates for an effective vaccine. A large scale vaccine trial based on the dominant surface protein of the sporozoite, RTS,S is undergoing in Africa, but preliminary observations revealed that it offers only partial protection.[75–77] One of the reasons, why the development of malaria vaccines is so challenging is because of the complex life cycle of the parasite and its array of immune-evasion strategies. Malaria is caused by four types of Plasmodia (*P. falciparum*, *P. vivax*, *P. malariae* and *P. oval*), and in endemic areas, simultaneous mixed infections are common. Even within one Plasmodium species, the parasite expresses polymorphic or variant genes in different stages of the life cycle.[78–80] This means that immunity to a single antigen expressed during a particular life-stage is unlikely to protect against clinical disease. Specific antibodies that prevent binding of the parasite to erythrocytes would be certainly enough to achieve protection, but the mutation of the antigenic epitopes on the parasite surface render this strategy almost impossible.[81] The malaria parasite at the blood stage induces immune suppression, and this may be another important mechanism evolved by the parasite to evade host-immune response.[82–84]

Certainly, vaccination against malaria is a very promising approach. Infection of human volunteers with sporozoites attenuated by irradiation was successfully tested in humans.[85–87] Unfortunately, this method is not applicable as routine immunization because of the elevated number of sporozoites that have to be injected to achieve protective immunity. An alternative and promising approach successfully tested on humans was to expose healthy volunteers undergoing prophylactic treatment with chloroquine to a serie of *P. falciparum*-infected mosquito bites.[88] All vaccinated subjects developed immune protection. This represents the first proof-of-concept study in humans not previously exposed to malaria.

REMARKS FOR EFFICACIOUS VACCINE DESIGN

Vaccine delivery route represents an important variable in vaccination design. Virus and bacteria naturally penetrate through various anatomical sites. HIV is transmitted through genital and gastrointestinal mucosal surfaces, whereas influenza and bacterial pneumonia through the respiratory tract. In immunocompetent individuals, immune response generated at mucosal sites is crucial for effective clearance of the infection and long-term protection. The majority of vaccines currently used are administered through intramuscular, intradermal or subcutaneous injections.[89,90] Vaccine delivery should follow the natural route as possible. It could therefore be of vital importance to formulate vaccines administrable through natural routes of transmission. An example comes again from the polio vaccine. Polio vaccine was originally prepared from the inactivated virus (Salk type), administered through intramuscular or subcutaneous injections. Later, the oral polio vaccine (Sabin type) made of attenuated virus was developed. We must not forget that poliovirus infection occurs by fecal-oral route through ingestion of contaminated food or water, or by saliva droplets emitted by sick people or healthy carriers. Both Salk and Sabin vaccines are currently in use throughout the world. However, the benefits of oral polio vaccine are enormous: it is easily administered in young children, it guarantees immunity for life, it protect against viral reinfections through the digestive tract, and finally it is much more economic.[91,92] Similarly to Salk vaccine, Sabin oral vaccine induces IgG antibody production responsible for systemic protection, but more importantly, stimulates secretion of IgA in Peyer's patches and lamina propria. Intestinal production of IgA antibodies prevents both systemic and intestinal viral replication, minimizing the risk of latent infections due to viral persistence in the gut that could be potentially spread to other individuals.

Despite of the numerous advantages of oral vaccines, only a few are used because they are extremely difficult to produce. Indeed, oral vaccines must overcome the physical, chemical and enzymatic barriers of the oral-gastrointestinal tract. To make more effective mucosal vaccines, a number of specific adjuvants based on bacterial toxins acting at intestinal level, such as cholera toxin or *E. coli* labile toxin, have been successfully developed.[93,94]

For viral diseases transmitted through the respiratory tract, such as influenza, the development of a vaccine administered intranasally by aerosolization is promising.[95,96] Several formulations of intranasal vaccines for seasonal and pandemic influenza were developed, they seem to be safe and effective but evaluation of protection remains to be defined.

The ability of the immune system to respond to infective agents is very different in children than in adults or the elderly. Over the years, the immune system undergoes a process known as immune senescence, characterized by a series of changes leading to a weakening or partial loss of the body's defensive capabilities.[97] The absolute number of B cells, $CD4^+$ and $CD8^+$ T cells is significantly reduced, accompanied by an increase in natural killer cells.[98,99] Thymic involution results in a decline in the number of naive T cells, but memory T-cells pool increases.[100] Interestingly, increased levels of inflammatory cytokines, such as TNFα, IL-1 and IL-6, were observed that might contribute to deregulation of cell-mediated responses in aged people.[101] Indeed, expansion of Th1 cells producing IFNγ was observed at the expense of IL-4-secreting Th2 cells.[102]

It is therefore necessary to protect this cohort of subjects with highly immunogenic vaccines. The pulmonary infections are especially important targets for novel vaccines as a result of the high social impact of such diseases in an ageing population. The paradox is created. The aim of vaccination is to induce and boost immune protection against infections. However, in aged people, the number of T-cell clones is limited. To what extent does the flu vaccine, that every autumn mobilizes the media, protect the eldest? Generally, the effectiveness of vaccines is evaluated as serum-antibody titer, and this correlates with changes in the pool of T cells. However, in recent years, it was found that the cell-mediated response better correlates with protection in older adults.[103] The lack of effective assay that can measure the cell-mediated T cell response has greatly limited the ability to fully assess the efficacy of vaccines in humans. Adjuvated vaccine formulations based on TLR agonists may offer a good opportunity to elicit cellular immunity in the elderly. The use of poly I:C, an agonist for TLR3, as adjuvant, have shown promising results in aged animals.[104]

More and more randomized controlled studies are showing that there is no major evidence for the effectiveness of the influenza vaccine in over 65s, despite vaccination has been recommended for high-risk groups for the last 40 years.[105,106] It is not impossible that behind the wide advertising campaign that is made each year for anti-flu vaccines there is a reason dictated largely by economic purposes.[107]

Similarly to natural immunity, vaccine-induced immunity may vary among individuals depending on their genetic characteristics.[108,109] Genetic factors in fact explain, at least in part, why some people resist infection more successfully than others. The system of human leukocyte antigens involved in antigen presentation is highly polymorphic and influences significantly the variation in immune response to vaccination. Several studies pointed out a clear association between human leukocyte antigens haplotypes and immune response to vaccines against hepatitis B and C, papilloma viruses and influenza.[110,111] Approximately 5% of subjects receiving vaccines against hepatitis B are not able to generate a specific antibody response. *In vitro* studies showed that T-cell proliferation and IL-2 secretion in response to hepatitis B antigens were impaired in nonresponder individuals. Haplotype association analysis in related and unrelated populations identified several other genes coding for cytokines and adhesion molecules, such as CD44, CD58, CDC42, IL-19 and IL-1RI.[112,113]

There is no doubt that a deeper understanding of the genetic factors that determine susceptibility to infections may provide the key for optimal vaccine formulation to prevent the most common and deadly infectious diseases, such as aquired immuno-deficiency syndrome, malaria and TB.

CONCLUSIONS AND FUTURE DIRECTIONS

In the last 200 years, the practice of vaccination was an empirical science, which used approaches that do not require a detailed knowledge of individual antigens or the cellular immune responses elicited. Despite that, very successful vaccines have been developed using conventional culture methods. However, these strategies are no longer enough to rapidly address novel and emerging infectious diseases, as HIV, pandemic influenza, malaria and TB. The past decades of animal and human studies taught us that, to make a good vaccine several aspects should be considered. Humoral response sometimes is not sufficient, but cell-mediated immunity, which depends on $CD4^+$ T-cell help and cytotoxic T cells, is required for effective immune protection. Nevertheless, the only biomarker of vaccine efficacy is currently antibody titer. It is clear that this is inadequate as a measure especially in the case of the very young, old or immunecompromised individuals. Moreover, in many cases, we do not even know whether antibody production is the key to protection from human disease, and therefore in future studies it will be imperative to more fully characterize all the facets of immunity to the pathogen. Moreover, immune escape mechanisms can not be ignored any longer.

An improvement in rational vaccine design can only follow more detailed knowledge of both the disease process and the mechanisms of action of current vaccines and adjuvants. To study correlates of immune protection, we should invest heavily in development of *in vitro* functional assays and *in vivo* animal models that better mimic the human disease, but also in clinical immunomonitoring of vaccine trials.

Recent advances in molecular biology, genomics transcriptomic and proteomics have opened up new frontiers in the interface between microbiology, immunology and vaccinology. Indeed, integration of the traditional disciplines with these novel approaches has begun to shed light on the mechanisms underlying the immune protection to infections, but much remains to be done for a rational design of new and improved vaccines. The benefits derived from these new approaches may be enormous, considering that they may be broadened to vaccination against noninfectious chronic diseases, such as cancer and autoimmunity.

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1 Plotkin SL, Plotkin SA. *A Short History of Vaccination*, 4th edn. W.B. Saunders: Philadelphia, PA, 2004.
2 Parkin J, Cohen B. An overview of the immune system. *Lancet* 2001; **357**: 1777–1789.

3 Hoebe K, Janssen E, Beutler B. The interface between innate and adaptive immunity. *Nat Immunol* 2004; **5**: 971–974.
4 Sprent J, Surh CD. T cell memory. *Annu Rev Immunol* 2002; **20**: 551–579.
5 Castellino F, Galli G, Del Giudice G, Rappuoli R. Generating memory with vaccination. *Eur J Immunol* 2009; **39**: 2100–2105.
6 Lehar SM, Bevan MJ. Immunology: polarizing a T-cell response. *Nature* 2004; **430**: 150–151.
7 Zelante T, De Luca A, D'Angelo C, Moretti S, Romani L. IL-17/Th17 in anti-fungal immunity: what's new? *Eur J Immunol* 2009; **39**: 645–648.
8 Mortellaro A, Conforti-Andreoni C, Fric J, Ricciardi-Castagnoli P. Dendritic cells as sensors of environmental perturbations. *Microbes Infect* 2008; **10**: 990–994.
9 Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; **4**: 499–511.
10 Wilmanski JM, Petnicki-Ocwieja T, Kobayashi KS. NLR proteins: integral members of innate immunity and mediators of inflammatory diseases. *J Leukoc Biol* 2008; **83**: 13–30.
11 Nichol KL. The efficacy, effectiveness and cost-effectiveness of inactivated influenza virus vaccines. *Vaccine* 2003; **21**: 1769–1775.
12 Cann AJ, Stanway G, Hughes PJ, Minor PD, Evans DM, Schild GC *et al.* Reversion to neurovirulence of the live-attenuated Sabin type 3 oral poliovirus vaccine. *Nucleic Acids Res* 1984; **12**: 7787–7792.
13 Liljeqvist S, Stahl S. Production of recombinant subunit vaccines: protein immunogens, live delivery systems and nucleic acid vaccines. *J Biotechnol* 1999; **73**: 1–33.
14 Schneerson R, Barrera O, Sutton A, Robbins JB. Preparation, characterization, and immunogenicity of Haemophilus influenzae type b polysaccharide-protein conjugates. *J Exp Med* 1980; **152**: 361–376.
15 Cutts FT, Zaman SM, Enwere G, Jaffar S, Levine OS, Okoko JB *et al.* Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005; **365**: 1139–1146.
16 Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS *et al.* Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009; **360**: 244–256.
17 Datta K, Lees A, Pirofski LA. Therapeutic efficacy of a conjugate vaccine containing a peptide mimotope of cryptococcal capsular polysaccharide glucuronoxylomannan. *Clin Vaccine Immunol* 2008; **15**: 1176–1187.
18 McConkey SJ, Reece WH, Moorthy VS, Webster D, Dunachie S, Butcher G *et al.* Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat Med* 2003; **9**: 729–735.
19 Arrode-Bruses G, Sheffer D, Hegde R, Dhillon S, Liu Z, Villinger F *et al.* Characterization of T-cell responses in macaques immunized with a single dose of HIV DNA vaccine. *J Virol* 2010; **84**: 1243–1253.
20 Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A *et al.* Direct gene transfer into mouse muscle *in vivo*. *Science* 1990; **247**: 1465–1468.
21 Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992; **356**: 152–154.
22 Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, Dwarki VJ *et al.* Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 1993; **259**: 1745–1749.
23 Jegerlehner A, Tissot A, Lechner F, Sebbel P, Erdmann I, Kundig T *et al.* A molecular assembly system that renders antigens of choice highly repetitive for induction of protective B cell responses. *Vaccine* 2002; **20**: 3104–3112.
24 Jennings GT, Bachmann MF. Designing recombinant vaccines with viral properties: a rational approach to more effective vaccines. *Curr Mol Med* 2007; **7**: 143–155.
25 Mizukoshi F, Yamamoto T, Mitsuki YY, Terahara K, Kawana-Tachikawa A, Kobayashi K *et al.* Activation of HIV-1 Gag-specific CD8+ T cells by yeast-derived VLP-pulsed dendritic cells is influenced by the level of mannose on the VLP antigen. *Microbes Infect* 2009; **11**: 191–197.
26 Vietheer PT, Boo I, Drummer HE, Netter HJ. Immunizations with chimeric hepatitis B virus-like particles to induce potential anti-hepatitis C virus neutralizing antibodies. *Antivir Ther* 2007; **12**: 477–487.
27 Slupetzky K, Gambhira R, Culp TD, Shafti-Keramat S, Schellenbacher C, Christensen ND *et al.* A papillomavirus-like particle (VLP) vaccine displaying HPV16 L2 epitopes induces cross-neutralizing antibodies to HPV11. *Vaccine* 2007; **25**: 2001–2010.
28 Kang SM, Yoo DG, Lipatov AS, Song JM, Davis CT, Quan FS *et al.* Induction of long-term protective immune responses by influenza H5N1 virus-like particles. *PLoS One* 2009; **4**: e4667.
29 Akahata W, Yang ZY, Andersen H, Sun S, Holdaway HA, Kong WP *et al.* A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. *Nat Med* 2010; **16**: 334–338.
30 Kool M, Soullie T, van Nimwegen M, Willart MA, Muskens F, Jung S *et al.* Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J Exp Med* 2008; **205**: 869–882.
31 Sharp FA, Ruane D, Claass B, Creagh E, Harris J, Malyala P *et al.* Uptake of particulate vaccine adjuvants by dendritic cells activates the NALP3 inflammasome. *Proc Natl Acad Sci USA* 2009; **106**: 870–875.
32 Mosca F, Tritto E, Muzzi A, Monaci E, Bagnoli F, Iavarone C *et al.* Molecular and cellular signatures of human vaccine adjuvants. *Proc Natl Acad Sci USA* 2008; **105**: 10501–10506.
33 Seubert A, Monaci E, Pizza M, O'Hagan DT, Wack A. The adjuvants aluminum hydroxide and MF59 induce monocyte and granulocyte chemoattractants and enhance monocyte differentiation toward dendritic cells. *J Immunol* 2008; **180**: 5402–5412.
34 Brunner R, Jensen-Jarolim E, Pali-Scholl I. The ABC of clinical and experimental adjuvants—a brief overview. *Immunol Lett* 2010; **128**: 29–35.
35 Clements CJ, Griffiths E. The global impact of vaccines containing aluminium adjuvants. *Vaccine* 2002; **20**: S24–33.
36 Brewer JM, Conacher M, Hunter CA, Mohrs M, Brombacher F, Alexander J. Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. *J Immunol* 1999; **163**: 6448–6454.
37 Hem SL, Hogenesch H. Relationship between physical and chemical properties of aluminum-containing adjuvants and immunopotentiation. *Expert Rev Vaccines* 2007; **6**: 685–698.
38 Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. *Nat Rev Immunol* 2009; **9**: 287–293.
39 Ulanova M, Tarkowski A, Hahn-Zoric M, Hanson LA. The common vaccine adjuvant aluminum hydroxide up-regulates accessory properties of human monocytes via an interleukin-4-dependent mechanism. *Infect Immun* 2001; **69**: 1151–1159.
40 Sun H, Pollock KG, Brewer JM. Analysis of the role of vaccine adjuvants in modulating dendritic cell activation and antigen presentation *in vitro*. *Vaccine* 2003; **21**: 849–855.
41 Sokolovska A, Hem SL, HogenEsch H. Activation of dendritic cells and induction of CD4(+) T cell differentiation by aluminum-containing adjuvants. *Vaccine* 2007; **25**: 4575–4585.
42 Li H, Nookala S, Re F. Aluminum hydroxide adjuvants activate caspase-1 and induce IL-1beta and IL-18 release. *J Immunol* 2007; **178**: 5271–5276.
43 Yang YW, Wu CA, Morrow WJ. Cell death induced by vaccine adjuvants containing surfactants. *Vaccine* 2004; **22**: 1524–1536.
44 Rock KL, Kono H. The inflammatory response to cell death. *Annu Rev Pathol* 2008; **3**: 99–126.
45 Shi Y, Zheng W, Rock KL. Cell injury releases endogenous adjuvants that stimulate cytotoxic T cell responses. *Proc Natl Acad Sci USA* 2000; **97**: 14590–14595.
46 Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 2003; **425**: 516–521.
47 Behrens MD, Wagner WM, Krco CJ, Erskine CL, Kalli KR, Krempski J *et al.* The endogenous danger signal, crystalline uric acid, signals for enhanced antibody immunity. *Blood* 2008; **111**: 1472–1479.
48 Ott G, Barchfeld GL, Van Nest G. Enhancement of humoral response against human influenza vaccine with the simple submicron oil/water emulsion adjuvant MF59. *Vaccine* 1995; **13**: 1557–1562.
49 Heineman TC, Clements-Mann ML, Poland GA, Jacobson RM, Izu AE, Sakamoto D *et al.* A randomized, controlled study in adults of the immunogenicity of a novel hepatitis B vaccine containing MF59 adjuvant. *Vaccine* 1999; **17**: 2769–2778.
50 Banzhoff A, Gasparini R, Laghi-Pasini F, Staniscia T, Durando P, Montomoli E *et al.* MF59-adjuvanted H5N1 vaccine induces immunologic memory and heterotypic antibody responses in non-elderly and elderly adults. *PLoS One* 2009; **4**: e4384.
51 McFarland EJ, Borkowsky W, Fenton T, Wara D, McNamara J, Samson P *et al.* Human immunodeficiency virus type 1 (HIV-1) gp120-specific antibodies in neonates receiving an HIV-1 recombinant gp120 vaccine. *J Infect Dis* 2001; **184**: 1331–1335.
52 Valensi JP, Carlson JR, Van Nest GA. Systemic cytokine profiles in BALB/c mice immunized with trivalent influenza vaccine containing MF59 oil emulsion and other advanced adjuvants. *J Immunol* 1994; **153**: 4029–4039.
53 Dupuis M, Denis-Mize K, LaBarbara A, Peters W, Charo IF, McDonald DM *et al.* Immunization with the adjuvant MF59 induces macrophage trafficking and apoptosis. *Eur J Immunol* 2001; **31**: 2910–2918.
54 Li Y, Svehla K, Mathy NL, Voss G, Mascola JR, Wyatt R. Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary isolate trimeric and monomeric envelope glycoproteins in selected adjuvants. *J Virol* 2006; **80**: 1414–1426.
55 Schwarz TF, Horacek T, Knuf M, Damman HG, Roman F, Drame M *et al.* Single dose vaccination with AS03-adjuvanted H5N1 vaccines in a randomized trial induces strong and broad immune responsiveness to booster vaccination in adults. *Vaccine* 2009; **27**: 6284–6290.
56 McCluskie MJ, Weeratna RD, Krieg AM, Davis HL. CpG DNA is an effective oral adjuvant to protein antigens in mice. *Vaccine* 2000; **19**: 950–957.
57 Nystrom-Asklin J, Adamsson J, Harandi AM. The adjuvant effect of CpG oligodeoxynucleotide linked to the non-toxic B subunit of cholera toxin for induction of immunity against H. *pylori in mice. Scand J Immunol* 2008; **67**: 431–440.
58 Cooper CL, Davis HL, Morris ML, Efler SM, Adhami MA, Krieg AM *et al.* CPG 7909, an immunostimulatory TLR9 agonist oligodeoxynucleotide, as adjuvant to Engerix-B HBV vaccine in healthy adults: a double-blind phase I/II study. *J Clin Immunol* 2004; **24**: 693–701.
59 Gavin AL, Hoebe K, Duong B, Ota T, Martin C, Beutler B *et al.* Adjuvant-enhanced antibody responses in the absence of toll-like receptor signaling. *Science* 2006; **314**: 1936–1938.
60 Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 2008; **453**: 1122–1126.
61 Ismaili J, Rennesson J, Aksoy E, Vekemans J, Vincart B, Amraoui Z *et al.* Monophosphoryl lipid A activates both human dendritic cells and T cells. *J Immunol* 2002; **168**: 926–932.
62 Thompson BS, Chilton PM, Ward JR, Evans JT, Mitchell TC. The low-toxicity versions of LPS, MPL adjuvant and RC529, are efficient adjuvants for CD4+ T cells. *J Leukoc Biol* 2005; **78**: 1273–1280.

63 Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TC. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. *Science* 2007; **316**: 1628–1632.
64 World Health Organization. *WHO Report 009: Global Tuberculosis Control—Epidemiology, Strategy, Financing*. WHO, Geneva, 2009.
65 Calmette A GrC, Boquet A, Ne'gre L. Prophylactic Vaccination Against Tuberculosis Using "BCG". Masson Paris, 1927.
66 Verma I, Grover A. Antituberculous vaccine development: a perspective for the endemic world. *Expert Rev Vaccines* 2009; **8**: 1547–1553.
67 Black GF, Weir RE, Floyd S, Bliss L, Warndorff DK, Crampin AC *et al.* BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. *Lancet* 2002; **359**: 1393–1401.
68 Behr MA, Small PM. Has BCG attenuated to impotence? *Nature* 1997; **389**: 133–134.
69 Demangel C, Garnier T, Rosenkrands I, Cole ST. Differential effects of prior exposure to environmental mycobacteria on vaccination with Mycobacterium bovis BCG or a recombinant BCG strain expressing RD1 antigens. *Infect Immun* 2005; **73**: 2190–2196.
70 Lozes E, Denis O, Drowart A, Jurion F, Palfliet K, Vanonckelen A *et al.* Cross-reactive immune responses against Mycobacterium bovis BCG in mice infected with non-tuberculous mycobacteria belonging to the MAIS-Group. *Scand J Immunol* 1997; **46**: 16–26.
71 Hanekom WA, Dockrell HM, Ottenhoff TH, Doherty TM, Fletcher H, McShane H *et al.* Immunological outcomes of new tuberculosis vaccine trials: WHO panel recommendations. *PLoS Med* 2008; **5**: e145.
72 Hinchey J, Lee S, Jeon BY, Basaraba RJ, Venkataswamy MM, Chen B *et al.* Enhanced priming of adaptive immunity by a proapoptotic mutant of Mycobacterium tuberculosis. *J Clin Invest* 2007; **117**: 2279–2288.
73 Aguilar D, Infante E, Martin C, Gormley E, Gicquel B, Hernandez Pando R. Immunological responses and protective immunity against tuberculosis conferred by vaccination of Balb/C mice with the attenuated Mycobacterium tuberculosis (phoP) SO2 strain. *Clin Exp Immunol* 2007; **147**: 330–338.
74 Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A *et al.* Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guerin mutants that secrete listeriolysin. *J Clin Invest* 2005; **115**: 2472–2479.
75 Bojang KA, Milligan PJ, Pinder M, Vigneron L, Alloueche A, Kester KE *et al.* Efficacy of RTS,S/AS02 malaria vaccine against Plasmodium falciparum infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet* 2001; **358**: 1927–1934.
76 Bojang KA, Olodude F, Pinder M, Ofori-Anyinam O, Vigneron L, Fitzpatrick S *et al.* Safety and immunogenicty of RTS,S/AS02A candidate malaria vaccine in Gambian children. *Vaccine* 2005; **23**: 4148–4157.
77 Abdulla S, Oberholzer R, Juma O, Kubhoja S, Machera F, Membi C *et al.* Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. *N Engl J Med* 2008; **359**: 2533–2544.
78 Lavazec C, Sanyal S, Templeton TJ. Expression switching in the stevor and Pfmc-2TM superfamilies in Plasmodium falciparum. *Mol Microbiol* 2007; **64**: 1621–1634.
79 Pasternak ND, Dzikowski R. PfEMP1: an antigen that plays a key role in the pathogenicity and immune evasion of the malaria parasite Plasmodium falciparum. *Int J Biochem Cell Biol* 2009; **41**: 1463–1466.
80 Petter M, Bonow I, Klinkert MQ. Diverse expression patterns of subgroups of the rif multigene family during Plasmodium falciparum gametocytogenesis. *PLoS One* 2008; **3**: e3779.
81 Persson KE, McCallum FJ, Reiling L, Lister NA, Stubbs J, Cowman AF *et al.* Variation in use of erythrocyte invasion pathways by Plasmodium falciparum mediates evasion of human inhibitory antibodies. *J Clin Invest* 2008; **118**: 342–351.
82 Elliott SR, Spurck TP, Dodin JM, Maier AG, Voss TS, Yosaatmadja F *et al.* Inhibition of dendritic cell maturation by malaria is dose dependent and does not require Plasmodium falciparum erythrocyte membrane protein 1. *Infect Immun* 2007; **75**: 3621–3632.
83 Torcia MG, Santarlasci V, Cosmi L, Clemente A, Maggi L, Mangano VD *et al.* Functional deficit of T regulatory cells in Fulani, an ethnic group with low susceptibility to Plasmodium falciparum malaria. *Proc Natl Acad Sci USA* 2008; **105**: 646–651.
84 Walther M, Tongren JE, Andrews L, Korbel D, King E, Fletcher H *et al.* Upregulation of TGF-beta, FOXP3, and CD4+CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. *Immunity* 2005; **23**: 287–296.
85 Clyde DF, Most H, McCarthy VC, Vanderberg JP. Immunization of man against sporozite-induced falciparum malaria. *Am J Med Sci* 1973; **266**: 169–177.
86 Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. *Nature* 1967; **216**: 160–162.
87 Mellouk S, Lunel F, Sedegah M, Beaudoin RL, Druilhe P. Protection against malaria induced by irradiated sporozoites. *Lancet* 1990; **335**: 721.
88 Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ *et al.* Protection against a malaria challenge by sporozoite inoculation. *N Engl J Med* 2009; **361**: 468–477.
89 Belshe RB, Newman FK, Cannon J, Duane C, Treanor J, Van Hoecke C *et al.* Serum antibody responses after intradermal vaccination against influenza. *N Engl J Med* 2004; **351**: 2286–2294.
90 Kenney RT, Frech SA, Muenz LR, Villar CP, Glenn GM. Dose sparing with intradermal injection of influenza vaccine. *N Engl J Med* 2004; **351**: 2295–2301.
91 John TJ. Immune response of neonates to oral poliomyelitis vaccine. *Br Med J (Clin Res Ed)* 1984; **289**: 881.
92 Grassly NC, Wenger J, Durrani S, Bahl S, Deshpande JM, Sutter RW *et al.* Protective efficacy of a monovalent oral type 1 poliovirus vaccine: a case-control study. *Lancet* 2007; **369**: 1356–1362.
93 Dickinson BL, Clements JD. Dissociation of Escherichia coli heat-labile enterotoxin adjuvanticity from ADP-ribosyltransferase activity. *Infect Immun* 1995; **63**: 1617–1623.
94 Pizza M, Giuliani MM, Fontana MR, Monaci E, Douce G, Dougan G *et al.* Mucosal vaccines: non toxic derivatives of LT and CT as mucosal adjuvants. *Vaccine* 2001; **19**: 2534–2541.
95 Boyce TG, Gruber WC, Coleman-Dockery SD, Sannella EC, Reed GW, Wolff M *et al.* Mucosal immune response to trivalent live attenuated intranasal influenza vaccine in children. *Vaccine* 1999; **18**: 82–88.
96 Bernstein DI, Yan L, Treanor J, Mendelman PM, Belshe R. Effect of yearly vaccinations with live, attenuated, cold-adapted, trivalent, intranasal influenza vaccines on antibody responses in children. *Pediatr Infect Dis J* 2003; **22**: 28–34.
97 Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F *et al.* Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev* 2007; **128**: 92–105.
98 Kang I, Hong MS, Nolasco H, Park SH, Dan JM, Choi JY *et al.* Age-associated change in the frequency of memory CD4+ T cells impairs long term CD4+ T cell responses to influenza vaccine. *J Immunol* 2004; **173**: 673–681.
99 Alter-Wolf S, Blomberg BB, Riley RL. Deviation of the B cell pathway in senescent mice is associated with reduced surrogate light chain expression and altered immature B cell generation, phenotype, and light chain expression. *J Immunol* 2009; **182**: 138–147.
100 Yager EJ, Ahmed M, Lanzer K, Randall TD, Woodland DL, Blackman MA. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J Exp Med* 2008; **205**: 711–723.
101 Haynes L, Eaton SM, Burns EM, Rincon M, Swain SL. Inflammatory cytokines overcome age-related defects in CD4 T cell responses *in vivo*. *J Immunol* 2004; **172**: 5194–5199.
102 Saurwein-Teissl M, Lung TL, Marx F, Gschosser C, Asch E, Blasko I *et al.* Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. *J Immunol* 2002; **168**: 5893–5899.
103 Goronzy JJ, Fulbright JW, Crowson CS, Poland GA, O'Fallon WM, Weyand CM. Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. *J Virol* 2001; **75**: 12182–12187.
104 Maue AC, Eaton SM, Lanthier PA, Sweet KB, Blumerman SL, Haynes L. Proinflammatory adjuvants enhance the cognate helper activity of aged CD4T cells. *J Immunol* 2009; **182**: 6129–6135.
105 Jefferson T, Rivetti D, Rivetti A, Rudin M, Di Pietrantonj C, Demicheli V. Efficacy and effectiveness of influenza vaccines in elderly people: a systematic review. *Lancet* 2005; **366**: 1165–1174.
106 Ortqvist A, Granath F, Askling J, Hedlund J. Influenza vaccination and mortality: prospective cohort study of the elderly in a large geographical area. *Eur Respir J* 2007; **30**: 414–422.
107 Jefferson T. Influenza vaccination: policy versus evidence. *BMJ* 2006; **333**: 912–915.
108 Segal S, Hill AV. Genetic susceptibility to infectious disease. *Trends Microbiol* 2003; **11**: 445–448.
109 Hill AV. Aspects of genetic susceptibility to human infectious diseases. *Annu Rev Genet* 2006; **40**: 469–486.
110 Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ *et al.* Genetic prediction of nonresponse to hepatitis B vaccine. *N Engl J Med* 1989; **321**: 708–712.
111 Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol* 2007; **13**: 1770–1787.
112 Ryckman KK, Fielding K, Hill AV, Mendy M, Rayco-Solon P, Sirugo G *et al.* Host genetic factors and vaccine-induced immunity to HBV infection: haplotype analysis. *PLoS One* 2010; **5**: e12273.
113 Davila S, Froeling FE, Tan A, Bonnard C, Boland GJ, Snippe H *et al.* New genetic associations detected in a host response study to hepatitis B vaccine. *Genes Immun* 2010; **11**: 232–238.