Immune mechanisms of protection: can adjuvants rise to the challenge?

Amy S McKeel†, Megan KL MacLeod†, John W Kappler1,2,3 and Philippa Marrack1,3,4*

**Abstract**

For many diseases vaccines are lacking or only partly effective. Research on protective immunity and adjuvants that generate vigorous immune responses may help generate effective vaccines against such pathogens.

The immune system is functionally diverse, able to make a refined response to hundreds of different types of infectious organisms. The initiation of an immune response to an infection requires collaboration between innate immune cells, which recognize general distinguishing features of pathogens, and the T lymphocytes of the adaptive immune system, whose highly variable antigen receptors are specific for a given pathogen. The activation of T lymphocytes depends on interactions with professional antigen-presenting cells (APCs), specialized cells of the innate immune system that are directly activated by the pathogens they engulf and regurgitate for presentation to, and activation of, T cells. The T cells then proliferate and are mobilized to protect the body by activating other immune cells or by killing infected cells. Among the immune cells activated by T lymphocytes, most importantly, are the B lymphocytes that produce antibodies. T lymphocytes direct the types of antibodies that B cells produce and the activity of other immune cells, thereby directing the immune response to optimally provide protection against different types of infections.

At the end of an immune response, the majority of activated B and T cells will undergo apoptosis, but a small number remain as memory cells, primed ready in case the host is exposed to the same infection [1,2]. Vaccines must work in a similar way, priming antigen-specific T and B cells, some of which convert to the memory cells that will control subsequent infections by the invader targeted by the vaccine. Moreover, like the infection itself, the vaccine must generate the optimal type of immune response to protect against a particular pathogen.

The different ways in which the immune system can respond to antigen are schematically summarized in Figure 1, which shows the two major classes of T lymphocyte, cytotoxic (or CD8) cells and helper (or CD4) cells, and their principal actions. For example, virus infections can be cleared by cytotoxic T cells (CTLs) or antibodies, whereas intracellular bacteria and parasites are most effectively controlled by T cells that produce cytokines specialized to activate particular groups of innate immune cells (Figure 1) [3-5].

The immune system is thought to distinguish the different kinds of pathogens through recognition by innate immune cells of pathogen-associated molecular patterns (PAMPs) on microorganisms, which enables responding cells to direct the adaptive responses along the pathway that will best help to reject the invader. PAMPs include, for example, characteristic cell wall components of bacteria, double-stranded RNA, which is found in some viruses, and CpG-rich DNA, which can be present in bacteria and viruses. These microbial components are detected by innate signaling pattern recognition receptors (PRRs), among the best known and most versatile of which are the Toll-like receptors (TLRs). Different members of the TLR family of membrane-bound receptors are specialized for detection of different classes of pathogens. In addition, many cytoplasmic proteins have recently been recognized to be important in innate immunity as PRRs [6-9]. Recognition of PAMPs by responding cells promotes recruitment of innate immune cells and APCs and activates APCs, increasing uptake of antigen and inducing cell-surface molecules and soluble mediators that are required for T cell activation. Together, these effects influence the magnitude of T and B cell responses and the numbers of memory cells that are produced. Not only do they serve to warn and activate the cells of the adaptive immune response but, importantly, they also control the type of immune response that follows.
Currently, three main types of vaccine are used in humans: live attenuated vaccines composed of a virus or bacterium that is similar to but less pathogenic than the real pathogen; inactivated vaccines that are heat-inactivated or chemically inactivated particles of the pathogen; or subunit vaccines that are made from components of the pathogen. Vaccines contain not only the antigen that is the target of the adaptive immune response, but either PAMPs or other substances that amplify or influence the adaptive response: these are known as adjuvants. In the live attenuated vaccines, the antigens that are recognized by the cells of the adaptive immune system are coupled to the PAMPs that activate professional APCs, just as they are in the pathogen itself, and these thus provide a natural adjuvant. Subunit vaccines, by contrast, consist of the purified antigens that are specifically recognized by lymphocytes, and although they are safer than whole-organism vaccines, they are unable, on their own, to activate the immune system optimally because they lack intrinsic PAMPs. Such vaccines require the addition of adjuvants that enhance their immunogenicity and influence the magnitude and nature of the response.

Adjuvants may promote immune responses by recruiting professional APCs to the vaccination site, by increasing the delivery of antigens to APCs, or by activating APCs to produce cytokines and provide activating signals to T cells. One adjuvant that has a long historical use in human vaccines is aluminum salt (sometimes referred to as alum). Proteins from the pathogen are adsorbed onto the aluminum salt, creating a suspension that is injected intramuscularly. Despite its long-standing and widespread use in human vaccines, it is still not clear exactly how this adjuvant works. Although it was widely believed that aluminum adjuvants promote their effects by maintaining a slow-releasing depot of antigen to the immune system, it is now clear that they promote multiple effects on the innate immune system. In addition, excision of aluminum adjuvant nodules after immunization has no impact on the magnitude of the immune response, which has brought the role of the depot into question [10].

Adjuvants were first deliberately introduced into vaccines after it was shown that aluminum salts and other particles could enhance immune responses [11]. At present, there are very few licensed vaccine adjuvants for clinical use. In the USA, aluminum salts have been, for many years, the only adjuvants that could be added to human vaccines. Recently, monophosphoryl lipid A (MPL), a derivative of the highly immunogenic bacterial cell wall component lipopolysaccharide (LPS), has been approved for use in the GlaxoSmithKline vaccine for
Human papillomavirus in combination with aluminum hydroxide. In Europe several additional adjuvants are used, including the oil-in-water adjuvants MF59 and ASO3, made by Novartis and GlaxoSmithKline, respectively (Table 1). As well as these, a large number of novel vaccine adjuvants have been studied in the laboratory, and some of them have also been used in clinical trials in humans (Tables 1 and 2). Whether these adjuvants will pass the two major criteria required for general use (effectiveness and safety) remains to be determined.

**Adjuvants and antibody production**

Most current vaccines act by inducing long-lived plasma cells - terminally differentiated B cells - that continuously secrete antibody over a considerable period of time [5]. Antibodies act quickly by binding to and thus stopping the pathogen, or products of the pathogen, in their tracks before damage to the host occurs. They are thus ideal for control of many diseases, including infection by viruses and intoxication by a number of bacterial products, including tetanus and diphtheria toxins [5]. Many of the viral vaccines use attenuated viruses as agents and generate good, long-lasting antibody production [12]. However, this is not so true for the subunit vaccines. For example, the tetanus vaccine, which is composed of a toxoid (an inactivated toxin that retains its antigenic properties) adsorbed to aluminum adjuvant, leads to the generation of plasma cells that make anti-tetanus-toxin antibody. However, this vaccine is routinely given to individuals every 10 to 15 years as the specific plasma antibodies are predicted to have a half-life of 3,014 years [12]. This is in contrast to the plasma cells that make the specific plasma antibody eventually die off [13].

Table 1. Adjuvants in use or being tested for use in human vaccines

| Adjuvant | Composition | Current status | References |
|----------|-------------|----------------|------------|
| Aluminum salts (alum) | Aluminum hydroxide or aluminum phosphate non-crystalline gels | In use in vaccines against DT, DPT, HBV, Hib, Streptococcus pneumoniae, meningococcal and HPV infections | [94] |
| MF59 | Oil (squalene)-in-water emulsion | In use in influenza vaccine (Europe); in trials for malarial, hepatitis C and HIV vaccine systems | [18,95-99] |
| MPL | Non-toxic derivative of LPS | Used in various trials in combination with oil (squalene)-in-water emulsions for malaria and leishmaniasis or in liposomal formulation | [87,100] |
| QS21 | Purified fraction of Quil A | Trialed alone and in combination with MPL (AS02, AS01) for malaria, influenza and cancers | [87] |
| ISCOMS | Liposomes containing QS21 |Trials for influenza vaccines | [101,102] |
| AS01 | Liposomal formulation containing MPL and QS21 | Trials for malaria vaccines (a more effective formulation than AS03 and AS04) | [87] |
| AS02 | Oil (squalene)-in-water emulsion of MPL and QS21 |Trials for malaria, HBV and TB vaccines | [103] |
| AS03 | Oil (squalene)-in-water emulsion |Trials for influenza vaccines | [20,21] |
| AS04 | Aluminum hydroxide and MPL |Trials for HBV and HPV vaccines | [104,105] |
| MPL-SE | MPL in a oil (squalene)-in-water emulsion |Trials for leishmaniasis vaccines | [100] |

Abbreviations: DPT, *Diphtheria* pertussis tetanus toxoid; DT, *Diphtheria* toxoid; HBV, Hepatitis B virus; Hib, *Haemophilus influenzae* ; HIV, human immunodeficiency virus; HPV, human papilloma virus; ISCOMs, immune stimulating complexes; LPS, lipopolysaccharide; MPL, monophosphoryl lipid A; TB, tuberculosis.

Table 2. Proposed mechanisms of adjuvant activity of major adjuvant components

| Adjuvant | Composition | Adjuvant activity | Mechanism of adjuvant action | References |
|----------|-------------|------------------|-----------------------------|------------|
| Aluminum salts | AlOH or AlPO<sub>4</sub>, non-crystalline gels | Antibody and T<sub>2</sub> cells | Chemokine/cytokine production, recruitment of monocytes and differentiation to DC; antigen uptake by DC | [31,34,46, 106,107] |
| MF59 | Oil (squalene)-in-water emulsion | ↑ Ab titre; ↑ Ab cross-reactivity; drives T<sub>2</sub> cells | Chemokine/cytokine production; recruitment of monocytes to injection site; antigen uptake by DCs | [34, 108-110] |
| TLR ligands | MPL, GpG, imiquimod, resiquimod (both imidazoquinolinamines) or poly(I:C) | Drives T<sub>1</sub> and CTL cells; ↑ T cell memory | TLR signaling in DCs promotes antigen presentation on MHC I and MHC II; enhanced migration of DCs to lymph nodes and DCs cytokine production; may have direct impacts on lymphocytes | [111] |
| QS21 | Purified fraction of Quil A that has lower toxicity and retains adjuvant effects | Antibody, T<sub>1</sub> and CTL responses | Enhances protective responses through poorly understood mechanisms; has lytic capacity and local reactogenicity | [97] |

Abbreviations: Ab, antibody;CTL, cytotoxic T lymphocyte; MPL, monophosphoryl lipid A; poly(I:C), a synthetic analog of double-stranded RNA; Th, T helper cell; TLR, Toll-like receptor.
The type of antibody produced is also affected by the adjuvant. There are five major classes of antibody with different properties and, ideally, vaccines should be designed to induce the antibody class that would be most effective in dealing with the pathogen. Immunoglobulin A (IgA) is highly effective against agents that infect through mucosal surfaces (see, for example, [14]). This factor may be responsible for the overall greater effectiveness of the Sabin (live attenuated) than the Salk (heat killed) polio vaccine. This is because the oral, live vaccine induces IgA secretion in the gut and respiratory tract, whereas the inactivated intramuscular Salk vaccine does not [15]. It is possible that adjuvants can be selected to enhance secretary IgA production, probably through their effects on APCs and T cell differentiation (see below).

**Antibodies are sometimes not enough**

Influenza vaccines operate by inducing antibodies against the two main surface proteins from the virus, hemagglutinin and neuraminidase. In so doing they effectively protect against infection by influenza strains expressing versions of these proteins present in the vaccine. However, these two proteins change as a consequence of mutation and re-assembly and the vaccine must be reformulated each year to contain the hemagglutinin and neuraminidase of the expected strain. Moreover, the vaccine has historically been, and in the US is currently, administered in the absence of an adjuvant. This means that larger doses must be given and immunity has been difficult to induce against the proteins found in emerging strains, such as those in H5N1 viruses that cause avian flu [16]. This may be partly because individuals have memory cells that can recognize annual but not emerging strains of the virus. Memory cells can respond in the absence of high levels of co-stimulation [17] (Figure 1) and, therefore, can be activated in the absence of an adjuvant. A primary response is required, however, to protect against newly emerging virus strains as they are more antigenically distinct from annual influenza strains. This primary response cannot be activated in the absence of the inflammation induced by added adjuvant.

Addition of adjuvants (MF59, ASO3 or aluminum salts; Table 1) to influenza vaccines increases antibody titers and persistence [18-21]. However, these approaches do not provide cross-reactivity to distinct subtypes of the virus. The same is true for the attenuated influenza vaccine Flu-Mist, which is also modified each year, although this vaccine may activate cross-reactive CD8+ T cells, at least in children [22]. CD8+ T cells recognize less variable parts of the virus - for example, in the core proteins [23-30] - and may provide a more cross-reactive response that could be induced by new vaccines.

Besides influenza there are clearly many other infections, HIV and malaria, for example, for which antibodies are not at all, or are insufficiently, protective. In these cases, both humoral immunity, mediated by antibodies, and cell-mediated immunity, which depends on cytotoxic T cells or T cells that activate immune cells by means of cytokines, may be required for effective protection.

**Contribution of adjuvants to T cell priming**

Dendritic cells (DCs) are key antigen-presenting cells in the initiation of T cell responses, and are thus likely to be a major target of adjuvant effects. In the absence of infection, DCs are distributed throughout the tissues as phagocytic cells. The presence of infection is signaled to these cells both directly, by pattern-recognition receptors (PRRs) for microbial constituents, and indirectly, by inflammatory cytokines released by other innate immune cells that recognize microbial constituents. These signals activate the DCs to undergo a process known as maturation and to migrate into secondary lymphoid organs where they activate naïve T cells. DC maturation involves increased processing of microbial proteins, portions of which are presented to T cells on major histocompatibility complex (MHC) molecules (discussed below). This serves as a required first activation signal. In addition, activation of DCs by PRRs results in expression on the surface of the DCs of so-called accessory and co-stimulatory molecules and the secretion of cytokines. Co-stimulatory signals are secondary signals required for DCs to activate naïve T cells, and cytokines offer a third signal to direct their differentiation along different pathways (Figure 1, stage 2). One way in which adjuvants such as aluminum salts and MF59 act is by promoting inflammation and infiltration of DCs into the site of inoculation and improving the uptake of associated antigens by DCs [31-34].

Adjuvant effects are relatively well understood for signals that induce T helper cell 1 (T_{h1}) responses, which are characterized by T helper cells that produce high levels of IFNγ, and other cytokines that activate antimicrobial effects at the effector site. These T_{h1} driving signals are known to operate through TLRs to induce secretion of interleukin (IL)-12, which drives differentiation of T_{h1} cells [35-38]. Adjuvants such as QS21 or other saponins drive T_{h1} responses and are thought to work by the induction of IL-12 in DCs [39]. Aluminum salts, however, do not directly induce signaling through TLRs and do not stimulate IL-12 production by DCs. Instead, aluminum adjuvants drive T_{h2} responses [40], by mechanisms that are much less well understood.

The requirements for antigen presentation to CD8+ T cells, which give rise to cytotoxic cells, are distinct from those for the CD4 helper T cells. CD8+ T cells are specialized for detection of agents, such as viruses, that invade the cytoplasm, and the pathway by which antigen
measuring the effectiveness of different antigen-adjuvant characteristic markers [50-52] that may be useful in the memory pool. Memory cells can be identified by designated early in the response to survive and generate, or a selective process, in which a subset of cells is which a percentage of cells are randomly selected to of memory T cells. This may be a stochastic process, in which little idea of which signals are required for the generation Establishment of T cell memory

Despite many years of research, immunologists still have little idea of which signals are required for the generation of memory T cells. This may be a stochastic process, in which a percentage of cells are randomly selected to survive, or a selective process, in which a subset of cells is designated early in the response to survive and generate the memory pool. Memory cells can be identified by characteristic markers [50-52] that may be useful in measuring the effectiveness of different antigen-adjuvant combinations. In some cases, the generation of memory cells that express lymphoid homing markers is associated with long-term survival and thus protection [53]. In contrast, other investigators argue that memory T cells that migrate into non-lymphoid organs, where re-infections are likely to occur, provide the most effective protection [54]. Therefore, measurements of protective capacity (for example, reduced viral titers or bacterial loads following challenge) are more useful indicators of a successful vaccine than the phenotype of the memory cells.

Many variables can affect the number and phenotype of memory cells. For example, a large dose of antigen can activate a larger number of cells, but a low dose may be preferable in a vaccine, if it activates only cells with high-affinity receptors, which may be more effective in some infections [55,56]. This seems to be true in mouse models of Mycobacterium tuberculosis in which low-dose priming induces highly sensitive T cells that can make a broad cytokine response that is associated with protection [55].

Likewise, the amount of inflammation, which in the case of a vaccine can be influenced by the addition of an adjuvant, affects the phenotype and number of the memory cells generated, partly because inflammatory signals are required for the efficient expansion and survival of T cells [57]. The speed with which memory cells are generated, however, can be increased by reducing inflammation during priming, resulting in the more rapid generation of memory cells [58,59]. It may be critical, therefore, to adjust the amount of antigen and adjuvant depending on how many and what type of memory cells are required to provide protection. This leads us to the question of how important the two major classes of T cells - CD4 cells and CD8 cells - are in providing protection.

CD4 T cell-mediated protection

It is clear that CD4+ T cells are critical directors of both cellular and humoral memory. It has been established for many years that CD4+ T cells provide help to B cells [60], but CD4+ T cells are also crucial for the generation of effective CD8 memory T cells [61]. Any vaccine, regardless of its intended action, must therefore activate helper CD4+ T cells. Perhaps the most important consideration for deciding what adjuvant to use in a vaccine is what type of CD4+ T cell response is required to direct the ensuing ideal immune response. At least five subsets of CD4+ T helper are now recognized: T_{h}1, T_{h}2, cells, which activate macrophages in distinct ways and induce production of different classes of antibodies in B cells; T_{h}17 cells, which are inflammatory; T follicular cells, which are specialized for activating B cells; and regulatory T cells, which are thought to prevent autoimmunity (Figure 2). These subsets have been reviewed extensively elsewhere [62,63]; here we will mainly discuss T_{h}1 cells as
these have been most associated with protection following vaccination.

Although it is clear that CD4+ T cells must be activated following vaccination, the importance of generating CD4 memory cells is less obvious. We have recently discussed the subject in some detail [64] and so will not go into specifics here, but a careful analysis of the available evidence suggests that relatively few protective immune responses depend on CD4+ T cell memory. Protection from \textit{M. tuberculosis} is, however, a good example of how CD4 memory cells can act. CD4+ T cells producing the important cytokine interferon (IFN)γ provide protection to \textit{M. tuberculosis} by activating macrophages in infected lungs [4]. The current \textit{M. tuberculosis} vaccine, Bacille Calmette-Guérin (BCG), protects young children from the worse forms of the disease [65,66], but it is of limited use in adults [4]. Therefore, much \textit{M. tuberculosis} vaccine research is focused on a prime-boost approach, a series of two vaccines, with BCG as the primary vaccine and a second experimental vaccine designed to re-activate and increase the protective memory response. As the boost several substances have been tried. For example, a modified vaccinia virus (MVA) that expresses a protein from \textit{M. tuberculosis}, 85A, has been tested in animals and humans. By using a vaccinia vector, a broad immune response, including IL-12 production by DCs and IFNγ production by CD4 cells, is induced [67,68]. In mouse studies, boosting with MVA85A resulted in reduced levels of bacteria in challenged animals [69,70]. The vaccine also successfully boosts antigen-specific cells in humans and the consequent memory cells produce a range of cytokines, including IFNγ and tumor necrosis factor (TNF)α [71-73]. Such multifunctional cells, which also make cytokines at higher levels, have been shown to provide protection against infections, including \textit{M. tuberculosis}, in mouse models [55,73,74].

\textbf{CD8 T cell-mediated protection}

Although it has been difficult to demonstrate direct protective effects of CD4 memory T cells, the differentiation of CD8+ T cells into CTLs has long been a measure of their protective efficacy (Figure 1). Following activation and clonal expansion in lymphoid organs, CTLs migrate to sites of inflammation, where they kill infected cells by inducing apoptosis, thus limiting and eventually clearing the infection. CTLs have been shown to provide protection in various mouse infection models [3,75-78], and CTL activity has been demonstrated in assays \textit{in vitro} using human

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{CD4+ T helper subsets. CD4+ T cells can differentiate into different subsets depending on the cytokine milieu present during T cell activation. Th1 cells, activated in the presence of IL-12 and IL-18 produced by activated DCs, make IFNγ, which is important in activating macrophages to kill intracellular bacteria, such as \textit{M. tuberculosis}. IL-4 made by Th2 cells activates macrophages to expel parasites (the cellular source of the IL-4 that promotes Th2 development is currently poorly defined). T follicular (Tfh) cells can make the canonical cytokines that Th1 or Th2 cells produce, but they also make IL-21 and express cell-surface molecules, such as CD40 ligand and inducible T cell co-simulator (ICOS), that are required for effective B cell responses and production of high-affinity, class-switched antibodies. The more recently described Th17 cells can produce IL-17 and IL-22 and are generated in the presence of IL-6 and TGFβ. IL-17 and IL-22 are important for promoting the influx of neutrophils to inflamed sites and the production of antimicrobial peptides, respectively. Th17 cells are thought to be important in defense against extracellular bacteria and fungi. Activated T cells can also differentiate into regulatory T cells (Tregs) in the presence of TGFβ and/or retinoic acid (RA). These cells can inhibit and control immune responses to prevent excessive inflammation through cell-surface molecules (such as CTLA-4) or cytokines, such as IL-10.}
\end{figure}
CD8+ T cells [23,79,80]. CTLs are also correlated with protection in humans infected with influenza [79,81,82].

There has been a shift in the focus of influenza vaccine development towards generating memory CD8+ T cells that may be able to provide more cross-reactive protection; this is because, as mentioned above, the antigens that CD8+ T cells recognize are found in less variable portions of the virus [23-30]. Several approaches have been developed, and perhaps the most interesting are those that target the lung, generating memory cells in the correct location to provide the most rapid protection. For example, peptides recognized by CD8+ T cells have been combined with a lipid moiety, Pam-2-Cys, that activates a TLR on DCs to successfully prime protective CD8+ T cells [83]. When delivered intranasally, this vaccine generates CD8+ T cells that migrate to the lung to provide immediate protection.

The use of peptide fragments rather than whole antigens is a limitation for the outbred human population because different fragments are recognized by the T cells of different individuals, and a very large number of different fragments would need to be identified and included. As an alternative, whole detergent-inactivated influenza virus can be combined with ISCOMs, which can deliver enclosed antigen directly to DCs and activate a range of innate cells, generating a T<sub>1</sub> and CTL response [84]. ISCOMs containing inactivated influenza virus have been used to generate an intranasal vaccine that includes all the viral proteins and can induce cross-reactive protection [85]. This protection required both CTL and antibodies, indicating that the ISCOM vaccine induced an effective cell-mediated and humoral response.

The killing of infected cells by CTLs and T<sub>1</sub> cells is an effective way to clear an infection with an intracellular pathogen. However, in some cases, such as infection of the liver by the hepatitis B virus, IFNγ-producing CD8+ T cells [86]. In a similar vein, IFNγ-producing CD8+ T cells are associated with protection in individuals vaccinated with the RTS,S malaria vaccine. This vaccine contains a protein from the parasite fused to a surface protein from the hepatitis B virus [87]. Although not enough is known about the mechanisms by which immune individuals resist infection, it is believed that both humoral and cell-mediated immunity directed against multiple antigens expressed at different stages of the parasite’s lifecycle are required for protection during malarial infection [88]. The adjuvant system used in the most successful malarial vaccine is AS02, a preparation that contains both a saponin component and the TLR agonist MPL formulated in a particulate system. Notably, both saponin and MPL were required to induce a modest level of protection in immunized individuals [89]. In contrast, vaccines using the same antigen with aluminum hydroxide and MPL (AS04) or in an oil-in-water emulsion (AS03) induced high levels of antibody but failed to protect against infection. A greater understanding of the responses in protected individuals may help to efficiently identify more effective antigen-adjuvant combinations. For example, the successful adjuvant, AS02, promotes CD8 responses, T<sub>1</sub> differentiation and broad antibody responses [90]. This suggests that both antibody- and cell-mediated immunity have important roles in defense against this complex pathogen.

**In pursuit of the ideal adjuvant**

The immune system has a diverse range of mechanisms at its disposal to deal with infectious organisms (Figures 1 and 2). Successful vaccines should aim to activate several of these, creating a redundant protective response that can cope with mutations and pathogen escape strategies. Although live attenuated viral and bacterial vaccines can activate all arms of the immune system [67,91,92], adjuvants have so far not reached this goal. By combining adjuvants, such as aluminum salts with MPL, or using prime-boost strategies using DNA and then viral or bacterial vectors, both humoral and cell-mediated responses can be activated, and some successes, as discussed above, have been reported. Yellow fever and smallpox (vaccinia) viruses are highly effective live vaccines that promote B and T cell memory and promote lifelong protection [91,93]. Recent work from the groups of Rafi Ahmed and Bali Pulendran into why the yellow fever and vaccinia vaccines work so well may provide markers of both innate activation and early adaptive responses, providing biomarkers to evaluate the success of new vaccine and adjuvant strategies [91,92].

**Author details**

1. Howard Hughes Medical Institute and Integrated Department of Immunology, National Jewish Health, Denver, CO 80206, USA. 2. Program in Biomolecular Structure, University of Colorado Denver, School of Medicine, Aurora, CO 80045, USA. 3. Department of Medicine, University of Colorado Denver, School of Medicine, Aurora, CO 80045, USA. 4. Department of Biochemistry and Molecular Genetics, University of Colorado Denver, School of Medicine, Aurora, CO 80045, USA.

**Published:** 12 April 2010

**References**

1. Spiertz J, Suth CD: T cell memory, *Annu Rev Immunol* 2002, 20:551-579.
2. Ahmed R, Gray D: Immunological memory and protective immunity: understanding their relation, *Science* 1996, 272:54-60.
3. Harty JT, Tivrinereim AR, White DW: CD8+ T cell effector mechanisms in resistance to infection, *Annu Rev Immunol* 2000, 18:275-308.
4. Hoft DF: Tuberculosis vaccine development: goals, immunological design, and evaluation, *Lancet* 2006, 367:164-175.
5. Plotkin SA: Vaccines: correlates of vaccine-induced immunity, *Clin Infect Dis* 2008, 47:401-409.
6. Kawai T, Akira S: Toll-like receptor and RIG-I-like receptor signaling, *Ann N Y Acad Sci* 2008, 1143:1-20.
21. Schwarz TF, Horacek T, Knuf M, Damman HG, Roman F, Drame M, Gillard P, Hutagalung Y, Tang H, Teoh YL, Ballou RW; H5N1 Flu Study Group for Hong Kong, Singapore, Taiwan and Thailand: Dendritic cells internalize vaccine adjuvant after intramuscular injection. Cell Immunol 1998, 186:18-27.

22. Mosca F, Tittoto E, Muzii A, Monaci E, Bagnoli F, Iavarone C, O'Hagan D, Rappuoli R, De Gregorio E: Molecular and cellular signatures of human vaccine adjuvants. Proc Natl Acad Sci USA 2008, 105:10501-10506.

23. Seubert A, Monaci E, Piazza M, O'Hagan D, Schultze J, Rappuoli R, De Gregorio E: The adjuvants aluminum hydroxide and MF59 induce monocyte and granulocyte chemoattractants and enhance monocyte differentiation toward dendritic cells. J Immunol 2008, 180:5402-5412.

24. Vyas JM, Van der Veen AG, Ploegh HL: The known unknowns of antigen processing and presentation. Nat Rev Immunol 2006, 8:607-618.

25. Trinchieri G: Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 2003, 3:133-146.

26. Jakob T, Walker PS, Krieg AM, Udey MC, Vogel JC: Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of T1 responses by immunostimulatory DNA. J Immunol 1998, 161:3042-3049.

27. Martin M, Michalek SM, Katz J: Role of innate immune factors in the adjuvant activity of monophosphoryl lipid A. Infect Immun 2003, 71:2498-2507.

28. Robson NC, Beacox-Harpin H, Donachie AM, Mowat AM: The role of antigen-presenting cells and interleukin-12 in the priming of antigen-specific CD4+ T cells by immune stimulating complexes. Immunology 2003, 110:95-104.

29. De Gregorio E, D'Oro U, Wark A: Immunology of TLR-independent vaccine adjuvants. Curr Opin Immunol 2009, 21:339-345.

30. Schnorrer P, Behrens GM, Wilson NS, Pooley JL, Smith CM, El-Sukkari D, Davey G, Kupresanin F, Li M, Maraskovsky E, Belz GT, Carbone FR, Shortman K, Heath WR, Villadangos JA: The dominant role of CD8+ dendritic cells in cross-presentation is not dictated by antigen capture. Proc Natl Acad Sci USA 2006, 103:10729-10734.

31. Burdorf D, Scholz C, Kautz A, Tampe R, Kunts C: Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. Nat Immunol 2008, 9:558-566.

32. Schnurr M, Orban M, Robson NC, Shin A, Braley H, Airey D, Cebon J, Maraskovsky E, Endres S: ISCOMATRIX adjuvant induces efficient cross-presentation of tumor antigen by dendritic cells via rapid cytokopic antigen delivery and processing via tripeptidyl peptide II. J Immunol 2009, 182:1253-1259.

33. L H, Willingham SB, Ting JP, Re F: Cutting edge: inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. J Immunol 2008, 181:17-21.

34. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA: Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminum adjuvants. Nature 2008, 453:1122-1126.

35. Seubert A, Munks MW, MacLeod MK, Fleenor CL, Van Rooijen N, Kappler JW, Marrack P: Alum induces innate immune responses through macrophage and mast cell sensors, but these sensors are not required for alum to act as an adjuvant for specific immunity. J Immunol 2009, 183:4403-4414.

36. Kool M, Pettitt V, De Smedt T, Rolaz A, Hammad H, van Nimwegen M, Bergen IM, Castillo R, Lambrecht BN, Tschopp J: Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. J Immunol 2008, 181:3755-3759.

37. Franchi L, Nunez G: The Nlrp3 inflammasome is critical for aluminium hydroxide-mediated IL-1beta secretion but dispensable for adjuvant activity. Eur J Immunol 2008, 38:2085-2089.

38. Nolle MA, Leibundgut-Landmann S, Joffre O, Reis e Sousa C: Dendritic cell quiescence during systemic inflammation driven by LPS stimulation of radioresistant cells in vivo. J Exp Med 2007, 204:1487-1501.
72. Brookes RH, Hill PC, Owiafe PK, Ibanga HB, Jeffries DJ, Donkor SA, Fletcher HA, Williams A, Goonetilleke NP, McShane H, Clark SO, Hatch G, Gilbert SC, Hill AV. Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in healthy adults in South Africa. J Infect Dis 2008; 199:544-552.
73. Danah PA, Patel DT, De Luca PM, Lindsay RW, Davey DF, Flynn BJ, Hoff ST, Andersen P, Reed SG, Morris SL, Roederer M, Seder RA. Multifunctional T cells define a correlate of vaccine-mediated protection against Leishmania major. Nat Med 2007; 13:843-850.
74. Kagi D, Ledermann B, Burki K, Seiler P, Odermatt B, Olsen KJ, Podack ER, Zinkernagel RM, Hengartner H. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature 1994, 369:31-37.
75. Kagi D, Seiler P, Pavlovic J, Ledermann B, Burki K, Zinkernagel RM, Hengartner H. The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses. Eur J Immunol 1995, 25:3256-3262.
76. Topham DJ, Tripp RA, Doherty PC. CD8+ T cells clear influenza virus by perforin or Fas-dependent processes. J Immunol 1997, 159:5197-5200.
77. Walsh CM, Matloubian M, Liu CC, Ueda R, Nakahara CG, Christensen JL, Huang MT, Young JD, Ahmed R, Clark WR. Immune function in mice lacking the perforin gene. Proc Natl Acad Sci USA 1994, 91:10854-10858.
78. McMichael AJ, Gotch FM, Noble GR, Beare PA. Cytotoxic T-cell immunity to influenza. N Engl J Med 1983, 309:13-17.
79. Malik A, Egan JE, Houghten RA, Sadow JF, Hoffman SL. Human cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein. Proc Nat Acad Sci USA 1991, 88:3300-3304.
80. McElhaney JE, Ewen C, Zhou X, Kane KP, Xie D, Hager WD, Barry MB, Klepping A, Wang Y, Bealecky RC. Granzyme B: Correlates with protection and enhanced CTL response to influenza vaccination in older adults. Vaccine 2009, 27:2418-2425.
81. Smith RE, Donachie AM, Grdic D, Lycke N, Mowat AM. Protective immunity against Plasmodium falciparum malaria: correlates of vaccine-induced protective immunity. Immunity 1999, 162:5536-5546.
82. Smith RE, Donachie AM, Grdic D, Lycke N, Mowat AM. Protective immunity against Plasmodium falciparum malaria: correlates of vaccine-induced protective immunity. Immunity 1999, 162:5536-5546.
83. Andrian UH, Ahmed R. Cytotoxic T cell fates via the graded expression of T-bet transcription factor. Immunity 2007, 28:1-10.
84. Sweet RS, Glodelew JW, Albott S, Masopust D, Murali-Krishna K, Mayer P, Edupuganti S, Lalor S, Germon S, Del Rio C, Mulligan MJ, Stavrans SI, Altmann JD, Feinberg MB, Ahmed R. Human effector T cells producing IFN-γ and memory CD8+ T cells producing IFN-γ-α produce IFN-γ-α. Immunity 2003, 176:6333-6339.
85. Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity 1996, 4:25-36.
86. Sun P, Schwenk R, White K, Stroue JA, Cohen J, Ballou WR, Voss G, Kester KE, Heppner DG, Krycz J. Protective immunity induced with malaria vaccine, RTS,S, is linked to Plasmodium falciparum circumsporozoite protein-specific CD4+ and CD8+ T cells producing IFN-α. J Immunol 2003, 171:6961-6967.
87. Richie T. High road, low road? Choices and challenges on the pathway to a malaria vaccine. Parasitology 2006, 133 Suppl:S113-144.
approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol* 2009, **10**:116-125.

93. Theiler M, Smith HH: The use of Yellow Fever virus modified by in vitro cultivation for human immunization. *J Exp Med* 1937, **65**:787-800.

94. Clements CJ, Griffiths E: The global impact of vaccines containing aluminum adjuvants. *Vaccine* 2002, **20** Suppl 3:S24-S33.

95. Ott G, Barchfeld GL, Van Nest G: Enhancement of humoral response against human influenza virus with the simple submicron oil/water emulsion adjuvant MF59. *Vaccine* 1995, **13**:1557-1562.

96. Durando P, Fenoglio D, Boschi D, Toschi L, Chirico M, Gori A, Dainelli R, Podda A, Del Giudice G, Frigapane E, Indiveri F, Coviari P, Gasparini R: Safety and immunogenicity of two influenza virus subunit vaccines, with or without MF59 adjuvant, administered to human immunodeficiency virus type 1-seropositive and -seronegative adults. *Clin Vaccine Immunol* 2008, **15**:253-259.

97. Coler RN, Carter D, Friede M, Reed SG: Adjuvants for malaria vaccines. *Parasite Immunol* 2009, **31**(5):252-528.

98. Heineman TC, Clements-Mann ML, Poland GA, Jacobson RM, Izu AE, Coler RN, Barchfeld GL, Van Nest G, Hu HH: A randomized, controlled study in adults of the immunogenicity of a novel hepatitis B vaccine containing MF59 adjuvant. *Vaccine* 1999, **17**:2769-2778.

99. McFarland EJ, Borkowsky W, Fenton T, Wara D, McNamara J, Samson P, Kang M, Mofenson L, Cunningham C, Salanga F, Spector SA, Jimenez E, Bryan Y, Burchett S, Frenkel LM, Yogev R, Gigliotti F, Luzuriaga K, Livingston RA; AIDS Clinical Trials Group 230 Collaborators: Human immunodeficiency virus type 1 (HIV-1) gp120-specific antibodies in neonates receiving an HIV-1 recombinant gp120 vaccine. *J Infect Dis* 2001, **184**(13):1331-1335.

100. Vélez ID, Gilchrist K, Martinez S, Ramirez-Pineda JR, Ashman JA, Alves FP, Coler RN, Bogatitzky LV, Kahn SJ, Beckmann AM, Cowgill KD, Reed SG, Piazza FM: Safety and immunogenicity of a defined vaccine for the prevention of cutaneous leishmaniasis. *Vaccine* 2009, **28**:329-337.

101. Sun HX, Xiao Y, Ye YP: ISCOMs and ISCOMATRIX. *Vaccine* 2009, **27**:4388-4401.

102. Ennis FA, Cruz J, Jameson J, Klein M, Burt D, Thippawong J: Augmentation of human influenza A virus-specific cytotoxic T lymphocyte memory by influenza vaccine and adjuvanted carriers (ISCOMs). *Virology* 1999, **259**:256-261.

103. Lell B, Agnandji S, von Glasenapp I, Haertle S, Oyakhiromen S, Issifou S, Vekemans J, Leach A, Lefevres M, Dubois MC, Dernotte MA, Carter T, Villafana T, Ballou WR, Cohen J, Kremsner PG: A randomized trial assessing the safety and immunogenicity of AS01 and AS02 adjuvanted RTS,S malaria vaccine candidates in children in Gabon. *PLoS One* 2009; **4**(7):e7611.

104. Boland G, Beran J, Lieveyn M, Sasadeusz J, Denteico P, Nothdurft H, Zuckerman JN, Genton B, Steffen R, Loutan L, Van Hattum J, Stoffel M: Safety and immunogenicity profile of an experimental hepatitis B vaccine adjuvanted with AS04. *Vaccine* 2004, **22**:3116-3120.

105. Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Desly F, Fourneau MA, Colau B, Szuich J, Losonsky G, Martin MT, Dubin G, Wetendorff MA: Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. *Vaccine* 2006, **24**:5937-5949.

106. Morefield GL, Sokolovska A, Jiang D, HogenEsch H, Robinson JP, Hern SL: Role of aluminum-containing adjuvants in antigen internalization by dendritic cells in vitro. *Vaccine* 2005, **23**:1588-1595.

107. Rimaniol AC, Gras G, Verdiere F, Capel F, Grigoriev VB, Porcheray F, Sauzeat E, Fournier JG, Clayette P, Siegrist CA, Dormont D: Aluminum hydroxide adjuvant induces macrophage differentiation towards a specialized antigen-presenting cell type. *Vaccine* 2004, **22**:3127-3135.

108. Del Giudice G, Hilbert AK, Bugarini R, Minutello A, Popova O, Toneatto D, Schoendorf I, Borkowski A, Rappuoli R, Podda A: An MF59-adjuvanted inactivated influenza vaccine containing A/Panama/1999 (H3N2) induced broader serological protection against heterovariant influenza virus strain A/Fujian/2002 than a subunit and a split influenza vaccine. *Vaccine* 2006, **24**:3063-3065.

109. Atmar RL, Keitel WA, Patel SM, Katz JM, She D, El Sahly H, Pompey J, Cate TR, Couch RB: Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H1N2 vaccine preparations. *Clin Infect Dis* 2006, **43**:1133-1142.

110. Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, Zambron MC, Katz JM: Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N1) vaccine: a potential priming strategy. *J Infect Dis* 2005, **191**:1210-1215.

111. Lahiri A, Das P, Chakravortty D: Engagement of TLR signaling as adjuvant: towards smarter vaccine and beyond. *Vaccine* 2008, **26**:6777-6783.