TIMELINE

Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology

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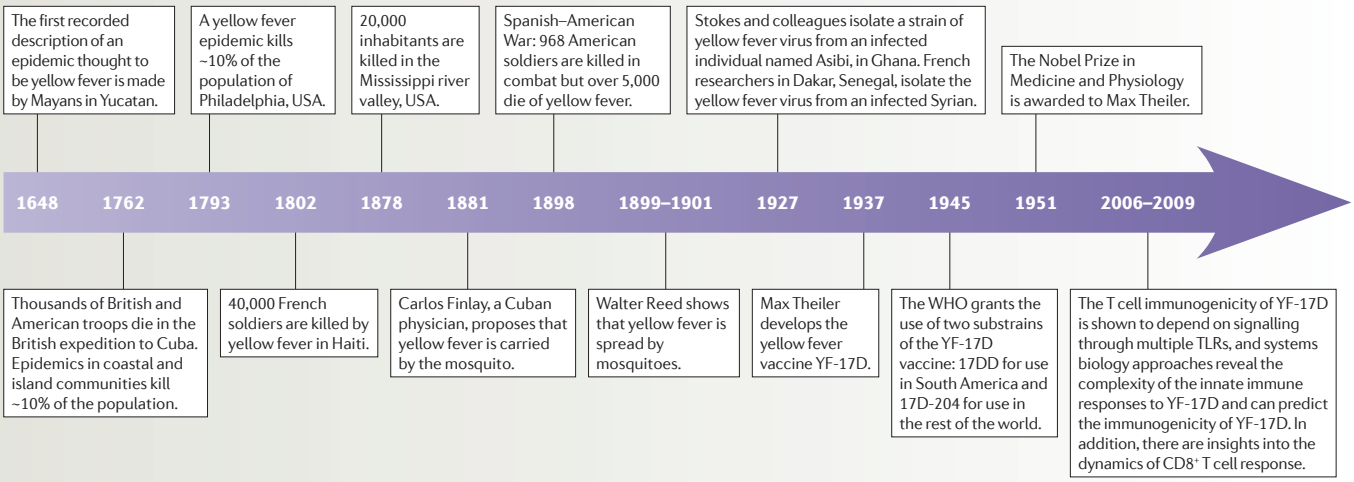
Abstract | Despite their great success, we understand little about how effective vaccines stimulate protective immune responses. Two recent developments promise to yield such understanding: the appreciation of the crucial role of the innate immune system in sensing microorganisms and tuning immune responses, and advances in systems biology. Here I review how these developments are yielding insights into the mechanism of action of the yellow fever vaccine, one of the most successful vaccines ever developed, and the broader implications for vaccinology.

Yellow fever is one of the deadliest diseases to afflict mankind[1]. It is caused by the mosquito-borne yellow fever virus and is characterized by a multi-organ failure of the liver, kidneys and myocardial tissues, resulting in haemorrhagic shock, bleeding from the eyes, nostrils, anus and other mucous membranes, black bloody vomit, and up to 50% fatality[1]. The liver is the primary target of infection and yellow bile pigments released from the damaged liver result in jaundice-like symptoms, hence the term 'yellow fever'. The disease occurs predominantly in sub-Saharan Africa and the tropical regions of South America. The early history of yellow fever is poorly documented, but the first description of a probable yellow fever epidemic was recorded in a Mayan manuscript and describes haematemesis (the vomiting of black blood or *xekik*) in Yucatan, Mexico, in 1648 (REF. 1) (TIMELINE). The virus and the mosquito vector are thought to have been introduced into Mexico during the slave trade from West Africa around this time. In the eighteenth century, there were major epidemics of yellow fever in many cities in America, with the disease killing 10% of the population of Philadelphia in 1793 (REF. 1).

Its aetiology and mode of transmission remained a mystery for many years. During the Spanish–American War of 1898, a severe yellow fever epidemic broke out among Cuban peasants and American soldiers stationed in Havana[1]. Carlos Finlay, an eminent Cuban physician, proposed that yellow fever was transmitted by mosquitoes. The US Surgeon General commissioned a team led by the army medical scientist Walter Reed to visit Cuba and identify the cause of the disease. By exposing a group of geographically isolated volunteers to mosquitoes that had feasted on patients with yellow fever, Reed showed that yellow fever was caused by a blood-borne agent and was primarily transmitted by *Aedes aegypti* mosquitoes[1]. It was more than a quarter of a century later that Stokes, Bauer and Hudson showed that rhesus macaques were susceptible to the disease following inoculation with blood isolated from an infected human. This was the starting point for the development of the live attenuated yellow fever vaccine, YF-17D[2,3].

Timeline | **Events in the development and understanding of the YF-17D vaccine**



TLR, Toll-like receptor; WHO, World Health Organization.

Today, over 540 million doses of YF-17D have been administered, and the vaccine stands as a paradigm for a successful vaccine[2,3]. Despite its success, little was understood about the mechanisms by which it induced protective immune responses. However, the past 2 years have seen significant advances in determining the mechanism of its action and these advances are reviewed here. First, I review the development of YF-17D in a historical context, and discuss our emerging understanding of the innate and adaptive immune responses that are stimulated by this vaccine. Then, I discuss the recent insights that have been obtained by the application of systems biological approaches to studying immune responses stimulated by YF-17D. The findings show that such systems approaches could be used to identify early signatures of vaccination that predict the immunogenicity and efficacy of vaccines. Third, I consider the broader use of systems biological approaches in vaccinology. Finally, I discuss recent insights into the potential mechanisms that contribute to the development of rare, but severe, adverse reactions to this vaccine.

The yellow fever vaccine

YF-17D is one of the most effective vaccines ever made. In the 73 years that have elapsed since its development, the vaccine has been administered to over 540 million people globally[2,3]. Yellow fever virus is a member of the genus Flavivirus of the family *Flaviviridae*. It is a single-stranded RNA virus with three structural proteins (core C, membrane M and envelope E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5)[2,3]. The starting point for the development of YF-17D was the isolation, in 1927, of the Asibi strain of yellow fever virus by Stokes, Bauer and Hudson, together with the Rockefeller Foundation's West African Yellow Fever Commission laboratory. A rhesus macaque developed yellow fever-like symptoms when inoculated with the blood of a Ghanaian man (Mr. Asibi) who was showing mild symptoms of yellow fever. The blood from this monkey was subsequently passaged both directly in other monkeys and indirectly through *A. aegypti* mosquitoes to generate the Asibi strain. Simultaneously, a team of French scientists at the Institute Pasteur in Dakar, Senegal, isolated a strain of yellow fever from an infected Syrian individual with mild symptoms, which was subsequently shown to be 99.8% sequence homologous to the Asibi strain[1,2]. Shortly thereafter, Max Theiler, in searching for a less expensive animal model than rhesus macaques, showed that intracerebral inoculation of mice with this French strain of viscerotropic yellow fever virus resulted in an encephalomyelitis, different from the disease in humans and monkeys. Following Louis Pasteur, who had attenuated rabies virus by passage in rabbit brains, Theiler observed that serial neurotropic passage in mice resulted in a progressive loss of virulence in rhesus macaques that were inoculated parenterally. This suggested the possibility of developing an attenuated virus as a vaccine and therefore Theiler chose to passage the virus in various animal tissues to attenuate its virulence in humans. However, he did not use the mouse-adapted French strain of yellow fever virus, as it was found to have acquired neurotropic properties in monkeys despite its marked attenuation when inoculated in mice by the

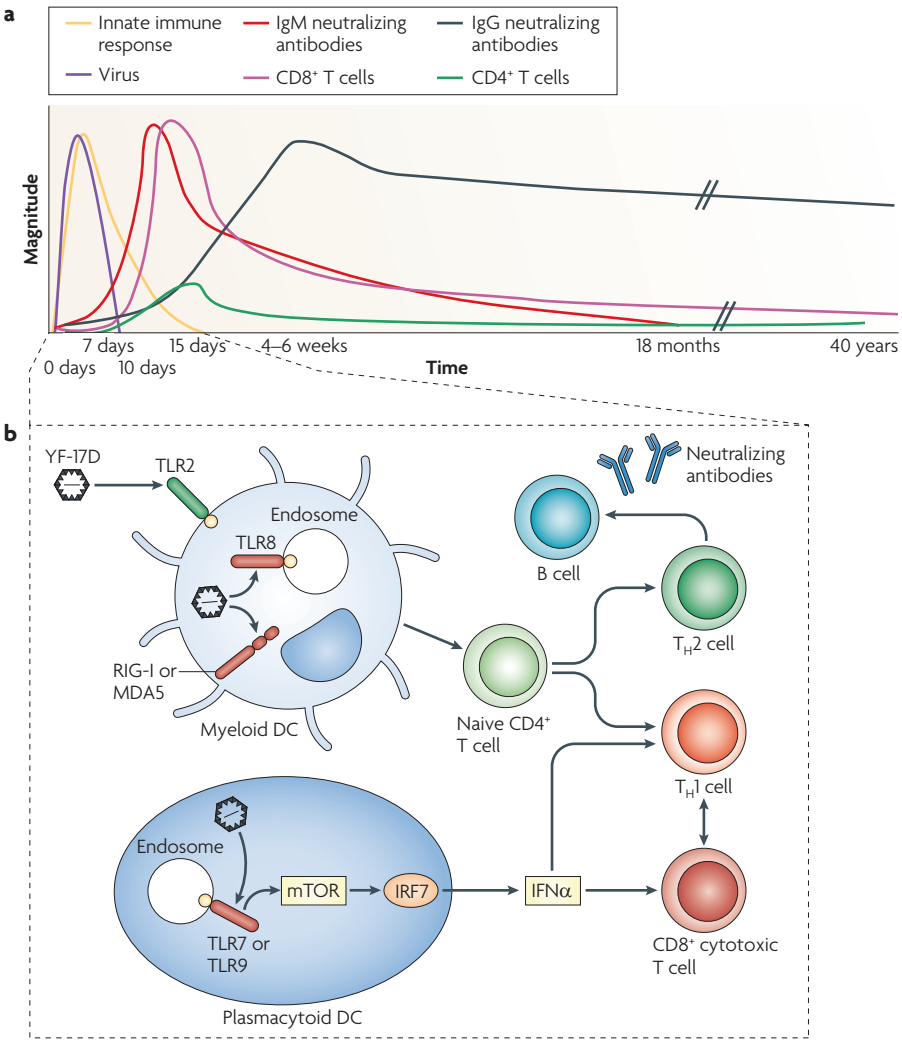


Figure 1 | **Immune responses to YF-17D. a** | YF-17D results in an acute viral infection. Viral replication peaks at days 5–7 and is undetectable by 14 days. IgM neutralizing antibodies are rapidly induced and peak at 2 weeks before declining, but they persist for at least 18 months. IgG neutralizing antibodies are produced more slowly and can persist for up to 40 years. $CD8^+$ T cell responses develop rapidly after immunization, peaking at day 15 (with roughly 15% of $CD8^+$ T cells being activated at day 15) and declining to nearer normal levels by day 30 (REF. 13). In addition, there is a brisk $CD4^+$ T cell response of a mixed T helper 1 (T_H1) and T_H2 profile[19,31,47]. The innate response occurs rapidly and seems to persist for more than 15 days[30,31,48], presumably owing to the ongoing viral replication in the blood. **b** | YF-17D signals through multiple Toll-like receptors (TLRs) — TLR2 and TLR8 on myeloid dendritic cells (DCs), TLR7 and TLR9 in plasmacytoid DCs[19], and retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5)[30]. Stimulation through multiple TLRs results in a balanced T_H1- and T_H2-type immune response[19]. TLR stimulation of plasmacytoid DCs induces activation of mammalian target of rapamycin (mTOR; also known as FRAP1) and its downstream mediators p70 ribosomal S6 protein kinase 1 (p70S6K1) and p70S6K2 (not shown), which mediate phosphorylation of interferon regulatory factor 7 (IRF7). This leads to induction of type I interferons (IFNs), such as IFNα, which activate $CD8^+$ T cells[21].

parenteral route; instead he used the Asibi strain. Various passages of the Asibi virus were also checked for the ability to produce neutralizing antibodies and protect monkeys from challenge with a virulent strain of the virus. Most strains resulted in a virus that was either still too lethal in monkeys or poorly immunogenic. The one exception was strain YF-17D, which was developed by passaging the virus 176 times first in mouse brain and mouse embryo tissue culture and then in chicken embryo and chicken embryo tissue culture with the brain and spinal cord removed to attenuate its neurotropism[4,5]. This work culminated in the 1937 *Journal of Experimental Medicine* paper[5], in which YF-17D was first used in human volunteers, including the authors, and was shown to induce neutralizing antibodies. In 1945 the World Health Organization granted the use of two substrain seed lots of this vaccine in humans: 17DD, which is used in South America, and 17D-204, which is used in most of the rest of the world[2,3]. Both substrains of the vaccine are currently manufactured in embryonated chicken eggs. Theiler was awarded the Nobel Prize in 1951 for this work, and this remains the only Nobel Prize to be awarded for the development of a viral vaccine.

The molecular basis for the attenuation of yellow fever virus virulence is not known. The YF-17D vaccine strain and the wild-type Asibi strain differ by only 68 nucleotide mutations out of over 10,860 nucleotides (~0.63%), which results in 32 amino acid differences[6]. The gene encoding E protein is the most heavily mutated region of the entire genome, with 11 nucleotide and 8 amino acid differences[6]. As the E protein has a key role in cell entry, some of these mutations must alter the virus tropism and thus affect its virulence. Interestingly, neural tissue passage of YF-17D can convert it into a neurovirulent virus by causing mutations in the E protein[7–10]. In addition to mutations in the gene encoding the E protein, mutations in the 3′ untranslated region are thought to have a role in attenuation[7–10].

Immune responses to YF-17D

Vaccination with YF-17D induces polyvalent adaptive immune responses, including the production of cytotoxic T cells, a mixed T helper 1 (T_H1) and T_H2 cell profile and robust neutralizing antibodies that can persist for up to 40 years after vaccination[3] (FIG. 1a). However, until recently little was known about the interaction of YF-17D with the innate immune system and the consequences of such interactions for stimulating the adaptive immune response.

Adaptive immune response to YF-17D. Vaccination with YF-17D results in an acute viral infection, in which there is transient viral replication that peaks at approximately 5 to 7 days and subsequently dissipates. Neutralizing antibodies are thought to be the primary correlate of protection against infection with yellow fever virus[2,3,11], and immunization is known to protect against infection in more than 90% of vaccinees[2,3]. YF-17D induces rapid antigen-specific neutralizing antibody responses of the IgM subclass within 7 days post vaccination, which peak at 2 weeks[3]. During the first 4–6 weeks the titres of IgM antibodies are actually higher than the IgG antibody titres, and they persist for at least 18 months (FIG. 1a). IgG neutralizing antibodies develop more slowly and can persist for up to 40 years. The mechanisms that stimulate such a sustained antibody response are unknown, as are the cellular and molecular mechanisms that result in the initial prolonged IgM response.

T cells are also thought to have an important role[2,3,11], however, only a few studies have investigated T cell responses to YF-17D. Human $CD8^+$ T cells that respond to YF-17D have been found to recognize epitopes from E, NS1, NS2b and NS3 proteins[12]. A recent study in humans has confirmed the increasing population of effector $CD8^+$ T cells after immunization with live attenuated YF-17D vaccine, by monitoring the expression of the activation markers CD38, HLA-DR and Ki67 and the downregulation of intracellular B cell lymphoma 2 (BCL-2) in T cells in the blood[13]. Downregulation of intracellular BCL-2 is known to be a characteristic of activated effector $CD8^+$ T cells[13]. By analysing HLA-DR- and CD38-expressing $CD8^+$ T cells at multiple time points, the magnitude and kinetics of the T cell responses after immunization could be assessed. The peak of the $CD8^+$ T cell response was observed at day 15 post immunization, when 4–13% of peripheral $CD8^+$ T cells co-expressed CD38 and HLA-DR. Thus, immunization with YF-17D induces a massive expansion of the population of activated $CD8^+$ T cells[13]. The number of activated $CD8^+$ T cells decreased after day 15, returning to normal levels by day 30 post immunization (FIG. 1a).

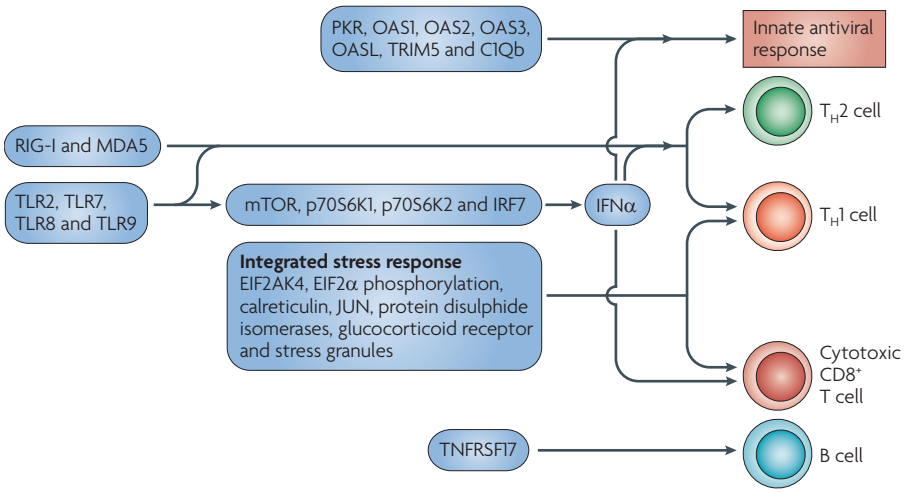


Figure 2 | **Innate correlates of YF-17D immunogenicity identified by systems biological approaches.** YF-17D stimulates polyvalent functional modules of innate immune activation[30,31]. For example, the virus is sensed by innate immune receptors, including retinoic acid-inducible gene I (RIG-I; also known as DDX58), melanoma differentiation-associated gene 5 (MDA5; also known as IFIH1), Toll-like receptor 2 (TLR2), TLR7, TLR8 and TLR9; signalling through TLRs stimulates a mixed T helper 1 (T_H1) and T_H2 cell profile. Furthermore, the virus also induces a robust antiviral response, including interferon-induced, double-stranded RNA-activated protein kinase (PKR), 2′, 5′-oligoadenylate synthetase 1 (OAS1), OAS2, OAS3, OASL, tripartite motif-containing protein 5 (TRIM5) and complement cascade components such as C1Qb. In addition, eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4), a protein encoded by a gene known to be central to the integrated stress response, is present in many of the signatures that predict the magnitude of $CD8^+$ T cell responses. Furthermore, the expression of other proteins that have a role in the integrated stress response (for example, calreticulin and protein disulphide isomerases) also correlates with the magnitude of the $CD8^+$ T cell response. Consistent with the induction of a stress response, YF-17D induces phosphorylation of elongation initiation factor 2α (EIF2α) and the formation of stress granules. Finally, YF-17D induces TNF receptor superfamily, receptor 17 (TNFRSF17), a receptor for BAFF (also known as TNFSF13B) that is known to regulate B cell responses. IRF7, interferon regulatory factor 7; mTOR, mammalian target of rapamycin; p70S6K, p70 ribosomal S6 protein kinase.

Innate immune response to YF-17D. The possibility that the early innate immune response to YF-17D may be important was raised by a study in 1945 in which it was shown that monkeys given YF-17D and then challenged with virulent virus 1–3 days later (before the appearance of antibodies) were partially protected[14]. Furthermore, infection with YF-17D simultaneously with or shortly before inoculation with dengue virus was shown to delay the onset of dengue fever[15]. It is now clear that the innate immune system has a key role in determining the strength and quality of the adaptive immune response[16–18]. Dendritic cells (DCs) are pivotal in sensing microbial stimuli and initiating and fine-tuning the adaptive immune response. DCs sense microbial stimuli through pathogen recognition receptors (PRRs) such as the Toll-like receptors (TLRs)[17,18] or through other families of receptors such as retinoic acid-inducible gene I (RIG-I; also known as DDX58)-like receptors, C-type lectins and the cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors[17,18].

Despite our increasing understanding of the role of the innate immune system in modulating adaptive immune responses, it was not known until recently whether successful empirically derived vaccines mediated their immunogenicity by stimulating TLRs. A few years ago it was shown that YF-17D activates several DC subsets (such as myeloid DCs and plasmacytoid DCs) through TLR2, TLR7, TLR8 and TLR9, resulting in the production of potent pro-inflammatory cytokines, including interferon-α (IFNα), by plasmacytoid DCs[19] (FIG. 1b). Although the precise viral components that trigger these TLRs are unknown, it is likely that TLR7 and TLR8 are triggered by viral nucleic acids[16]. Intriguingly, it was shown that robust induction of IFNα production by plasmacytoid DCs, following stimulation with YF-17D, required TLR-mediated activation of the mammalian target of rapamycin (mTOR; also known as FRAP1) pathway[20] — which regulates cell growth and metabolism, as well as cytokine and growth factor expression, in response to environmental cues[20] — and its downstream mediators p70 ribosomal S6 protein kinase 1 (p70S6K1) and p70S6K2 (REF. 21). Thus, targeting rapamycin-encapsulated microparticles to antigen-presenting cells *in vivo* (to inhibit mTOR activation in these cells) resulted in lower levels of type I interferons and impairment of antigen-specific CD8$^+$ T cell responses following immunization with YF-17D[21].

YF-17D infects human DCs but replicates minimally in these cells[19,22,23]. However, this minimal replication seems to be sufficient for the presentation to T cells of endogenous epitopes and those generated from proteins encoded by foreign genes inserted into the YF-17D vector by recombinant DNA technology[19,22,23]. One immunological consequence of engaging multiple TLRs is the stimulation of a mixed T_H1- and T_H2-type cytokine profile[19]. Thus, although mice deficient for the TLR adaptor molecule MYD88 (myeloid differentiation primary-response gene 88) show an impairment of T_H1 cell cytokines, TLR2-deficient mice show greatly enhanced T_H1 and cytotoxic CD8$^+$ T cell responses to YF-17D[19]. These results show that successful empirically derived vaccines such as YF-17D mediate their immunogenicity, in part, by signalling through multiple TLRs that are expressed on distinct subsets of DCs[17,18], and raise the possibility that synthetic vaccines that activate the appropriate combination of TLRs may recapitulate the immunogenicity of YF-17D. The question of whether engagement of such TLRs on subsets of human DCs by YF-17D also results in a mixed T_H1 and T_H2 cell profile remains unanswered. Thus the highly successful YF-17D vaccine is a model vaccine, which can be used to decipher the immunological rules that induce robust and persistent protective immune responses. Such insights will be valuable in designing new vaccines against global pandemics and emerging infections. Recently, we and others have begun to apply the tools of systems biology to further explore the mechanisms by which YF-17D induces protective immunity.

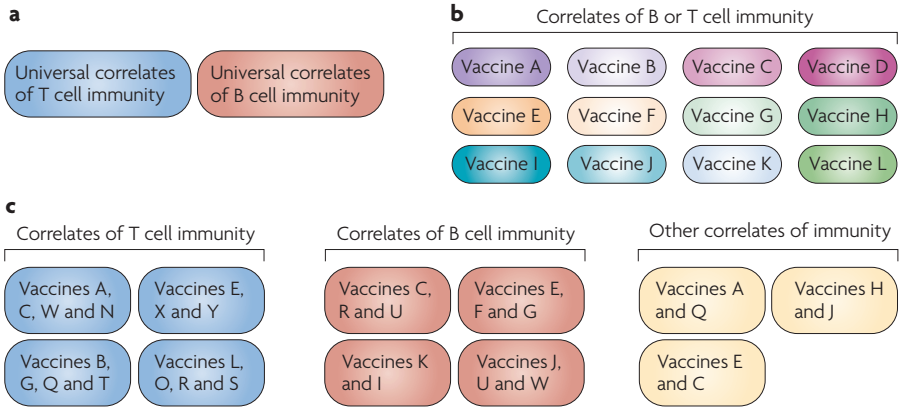


Figure 3 | **Predictive signatures of gene expression for other vaccines.** Systems biology allows the identification of the gene expression patterns, or molecular signatures, that are induced after vaccination, which might be used to predict the later development of protective immune responses. **a** | One extreme scenario is that of T or B cell-mediated immunity having a single archetypal molecular signature. This would allow prediction of the protective immune response for all vaccines. **b** | Another extreme scenario is that molecular signatures might be highly vaccine specific, with each vaccine having a unique predictive signature. **c** | The most likely scenario is that there will be clusters of correlates, or 'meta-signatures', that predict the immunogenicity or protective capacity of clusters of vaccines that work through similar mechanisms. For example, the hypothetical vaccines A, C, W and N may mediate protection by stimulating the production of high numbers of polyfunctional CD8$^+$ T cells, and these may have a common signature. By contrast, vaccines L, O, R and S may mediate protection by stimulating T cells that migrate to the lung, and these may have a different common signature. By clustering all such signatures related to T cell-mediated mechanisms of protection, one could have a meta-signature that predicts the immunogenicity or protective capacity of virtually all T cell-based vaccines. Similarly, one could have a meta-signature for B cell-based vaccines — for example vaccines that protect through neutralizing antibodies would have a common signature, whereas those that protect through opsonizing antibodies would have a separate signature. Meta-signatures may also exist for vaccines that mediate protection by other mechanisms.

Systems vaccinology of YF-17D

Hypothesis building and testing are the bedrock of the modern scientific method[24]. The advancement of high-throughput technologies has facilitated large-scale biological measurements, thus enabling the formulation of new hypotheses. These systems biological approaches are likely to be of value in identifying the gene expression patterns, or molecular signatures, that are induced rapidly after vaccination and that correlate with, and predict, the later development of protective immune responses. Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of untested vaccines, or in identifying individuals who are likely to respond suboptimally to vaccination. Furthermore, the predictive signatures

would highlight new correlates of protective immunity and stimulate the formulation and validation of new hypotheses on the biological mechanisms by which such molecular signatures modulate vaccine-induced immunity and protection. Despite the increasing use of such approaches in prognosis and therapy response prediction in oncology[25–27] and their promise in identifying molecular signatures of autoimmunity and infections[28,29], they have only recently been applied to vaccinology.

We and others have carried out two independent studies to identify early molecular signatures that are induced by YF-17D[30,31]. Both studies analysed peripheral-blood mononuclear cells (PBMCs) from humans (who had not been previously vaccinated with YF-17D) at various time points following vaccination. Interestingly, there was remarkable variation in the magnitude of the antigen-specific CD8$^+$ T cell responses and in the neutralizing antibody titres that were measured at day 15 or 60 post vaccination[30]. We sought to determine whether YF-17D-induced gene expression signatures of early innate immune activation could predict the ensuing adaptive immune responses. Vaccination with YF-17D induced the activation of DCs and monocytes, as well as the production of the pro-inflammatory mediators CXC-chemokine ligand 10 (CXCL10) and interleukin-1α (IL-1α), in most vaccinees[30], which was consistent with similar results observed during other flavivirus infections, such as dengue virus and West Nile virus infections, and during tick-borne encephalitis[32,33]. However, these variables did not correlate with the magnitudes of the later CD8$^+$ T cell responses or neutralizing antibody titres.

Microarray analysis of total PBMCs showed a molecular signature comprised of genes involved in innate sensing of viruses and antiviral immunity in most of the vaccinees. Of note, both studies showed that there was robust induction of a network of genes encoding innate sensing receptors such as TLR7, RIG-I and melanoma differentiation-associated gene 5 (MDA5; also known as IFIH1), the cytoplasmic receptors for members of the 2′, 5′-oligoadenylate synthetase family, as well as transcription factors that regulate the expression of type I IFNs, IFN regulatory factor 7 (IRF7) and signal transducer and activator of transcription 1 (STAT1)[30,31]. Consistent with this, YF-17D was also shown to signal through RIG-I and MDA5 (REF. 30). Furthermore, there was induction of the gene encoding RIG-I-like RNA helicase, *LGP2* (also known as *DHX58*)[30,31], which is a negative regulator of RIG-I- and MDA5-mediated responses[34].

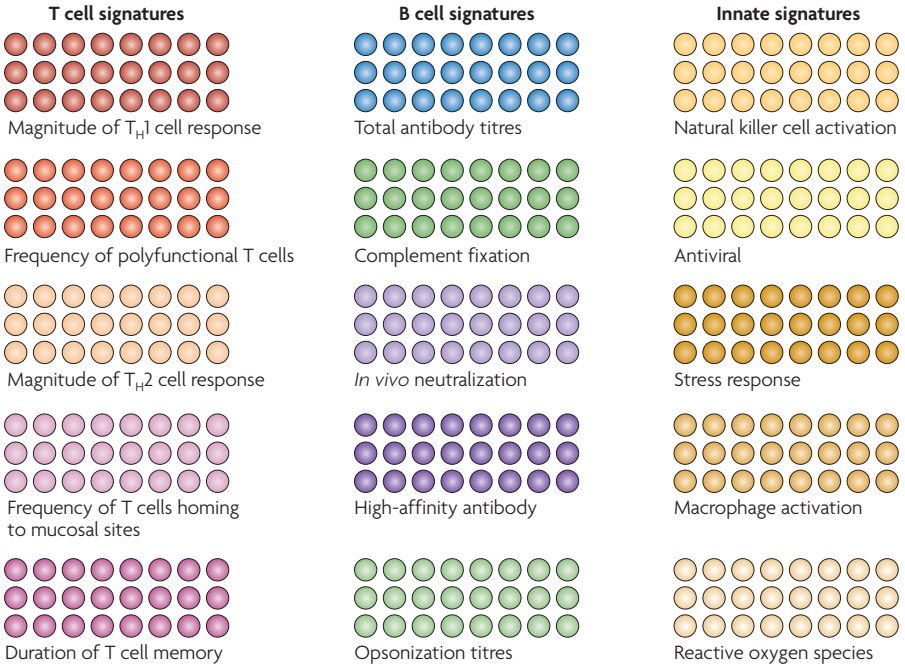


Figure 4 | **The vaccine chip.** The identification of molecular signatures that predict the immunogenicity and/or protective capacity of many vaccines would enable the development of a vaccine chip. This chip would consist of perhaps 200–1,000 genes, organized in clusters. Each cluster of genes would predict a particular facet of the innate or adaptive immune response that is known to mediate protection against a disease (for example, magnitude of effector CD8$^+$ T cell response, frequency of polyfunctional T cells, balance of T helper 1 (T_H1), T_H2 and T_H17 cells, high-affinity antibody titres and so on). This would allow the rapid evaluation of vaccinees for the strength, type, duration and quality of protective immune responses stimulated by the vaccine. Thus, the vaccine chip is a device that could be used to predict immunogenicity and protective capacity of virtually any vaccine in the future.

Furthermore, genes encoding proteins in the complement pathway (for example, *C1QB*) and the inflammasome were induced. By visualizing these gene networks, a group of transcription factors, including IRF7, STAT1 and ETS2, were identified as key regulators of the early innate immune response to the YF-17D vaccine[30,31]. Importantly, there was a persistent upregulation of this antiviral molecular signature for more than 2 weeks post vaccination[30], presumably reflecting the ongoing stimulation of innate immune cells in response to viral replication, which peaks at 7 days[2,3]. This molecular signature reflects the fact that vaccination with YF-17D results in a live viral infection, and it is likely that other viruses that stimulate potent immune responses induce a similar signature. However, the question of whether the pathogenic Asibi strain also induces a similar signature remains to be determined. Thus, it is unclear whether this molecular signature simply occurs during viral infection or actually influences the efficacy of the adaptive immune response. Indeed, there was no correlation between the induction of these genes and the magnitude of the CD8$^+$ T cell or neutralizing antibody response.

Using additional bioinformatics approaches we identified gene signatures that did correlate with the magnitude of antigen-specific CD8$^+$ T cell responses and antibody titres[30]. To evaluate the actual predictive ability of these signatures, we determined whether they could predict the magnitude of the CD8$^+$ T cell or B cell response in individuals from a second YF-17D vaccine trial. We observed that several signatures for CD8$^+$ T cell responses from the first vaccine trial were predictive with up to 90% accuracy in the second vaccine trial and vice versa. Interestingly, two genes — solute carrier family 2, member 6 (*SLC2A6*) and eukaryotic translation initiation factor 2 alpha kinase 4 (*EIF2AK4*) — were present in the predictive signatures identified using two independent bioinformatics prediction models. *SLC2A6* belongs to a family of membrane proteins that regulate glucose transport and glycolysis in mammalian cells[35]. *EIF2AK4* has an important role in the integrated stress response[36–40] and regulates protein synthesis in response to environmental stresses by phosphorylating elongation initiation factor 2α (EIF2α)[36]. The translation of constitutively expressed proteins is terminated by

redirection of their mRNAs from polysomes to discrete cytoplasmic foci, known as stress granules, for transient storage[37]. Consistent with this, YF-17D induced the phosphorylation of EIF2α and the formation of stress granules[30]. Moreover, several other genes involved in the stress response pathway, including calreticulin, protein disulphide isomerase, glucocorticoid receptor and *JUN*[37–40], correlated with the $CD8^+$ T cell response[30]. Recent work has shown an antiviral effect of EIF2AK4 against RNA viruses[40], but the effect of this factor on adaptive immune responses is not known. It is thus tempting to speculate that the induction of the integrated stress response in innate immune cells might regulate the adaptive immune response to YF-17D and perhaps other vaccines or microbial stimuli. With respect to antibody responses, TNF receptor superfamily, receptor 17 (TNFRSF17), which is a receptor for B cell-activating factor (BAFF; also known as TNFSF13B)[41], was shown to be a key gene in the predictive signatures. BAFF is thought to optimize B cell responses to B cell receptor- and TLR-dependent signalling[41]. Thus, taken together these studies provide a global description of the innate and adaptive immune responses that are induced by YF-17D vaccination and highlight the complexity of the innate immune response that is required for the induction of long-lasting immune protection (FIG. 2).

Such approaches are likely to be of broad value in vaccinology in at least two different ways. First, the identification of molecular signatures of vaccine efficacy could have a public health use in identifying vaccinees who are unlikely to respond well to a vaccine, or in identifying individuals with suboptimal responses among high-risk populations, such as infants or the elderly. In this context, whether the signatures identified with YF-17D can also predict the immunogenicity of other vaccines remains to be determined. In principle, one could envision a universal 'archetypal' signature that predicts the T cell immunogenicity of all vaccines, and another archetypal signature that predicts the B cell immunogenicity of all vaccines (FIG. 3a). However, our preliminary data with influenza virus vaccines suggest that this is unlikely to be the case. At the other extreme, one could envision a scenario in which each vaccine had a unique signature (FIG. 3b). However, I think that the most likely scenario is that there will be classes of vaccines that induce similar signatures of immunogenicity. For example, vaccines that stimulate protective T_H2-type responses against helminths may have a common innate immune signature, whereas other vaccines that mediate protection through, for example, polyfunctional $CD8^+$ T cells that produce multiple cytokines may have a different innate immune signature that correlates with such T cell responses (FIG. 3c). Thus, one could imagine a cluster of signatures ('meta-signatures') or correlates that predict various aspects of T cell immunogenicity. Like for the antibody response, those vaccines that stimulate long-lived plasma cells producing high-affinity antibodies may have a common innate immune signature. Other vaccines that rely on opsonizing antibodies for protection, such as the meningococcal or pneumococcal vaccines, may have a different innate immune signature (FIG. 3c). Thus, a cluster of correlates would predict various aspects of B cell immunogenicity. Similarly, a different cluster of correlates could exist that predicts protective immunity that is not mediated by B or T cell-dependent mechanisms but involves other mechanisms mediated perhaps by natural killer cells or the activation of stress responses or reactive oxygen species (FIG. 3c). The elucidation of such meta-signatures will facilitate not only the rapid screening of vaccines but also the formulation of new hypotheses on how vaccines mediate protective immune responses. The realization of these challenges could ultimately lead to the development of a 'vaccine chip' (FIG. 4), which would consist of a few hundred gene probe sets that can identify predictive signatures for all of the correlates of immunogenicity and protection.

Box 1 | Dark side of the yellow fever vaccine: serious adverse events

Despite the excellent safety record of YF-17D, there are rare (1 in 250,000) cases of serious adverse events, which are often fatal[3,42–46]. Vaccine-associated neurotropic disease caused by neuroinvasion of YF-17D may include post-vaccinal encephalitis, Guillain–Barré syndrome and autoimmune disease with central or peripheral nervous system involvement.

Vaccine-associated viscerotropic disease is characterized by the failure of multiple organ systems, characteristic of pathogenic yellow fever virus infection[3,42–46]. Within 2–5 days of vaccination, patients develop high fever, malaise and myalgia, followed by jaundice, oliguria, cardiovascular instability, haemorrhage, and renal and respiratory failure. The fatality rate is over 50% and high levels of yellow fever virus antigen can be found in the liver, heart and other organs, primarily in tissue-associated macrophages[3,42–46]. The mechanisms underlying such events remain unknown, but the speed of disease onset suggests a possible role for innate immune responses. Genetic mutations in the YF-17D do not seem to be the cause, because YF-17D isolated from patients with serious adverse events has had the same nucleotide sequence as the original vaccine strain[43]. A major obstacle to mechanistic studies is the rarity of cases and samples. Elevated cytokines and reduced platelet counts have been described[44]. A recent study[45] showed enhanced and persistent viraemia (which was characteristic of pathogenic yellow fever virus infection), enhanced and prolonged antigen-specific $CD8^+$ T cell and antibody responses (suggesting that persistence of the virus was not due to suboptimal adaptive immunity), constitutively high (20–200-fold) levels of $CD14^+CD16^{hi}$ inflammatory monocytes, elevated levels of interleukin-1α (IL-1α), IL-6, CXC-chemokine ligand 10, CC-chemokine ligand 2 (CCL2) and CCL5, and genetic polymorphisms in CC-chemokine receptor 5 and its ligand, CCL5 (which influence the migration of effector T cells and $CD14^+CD16^{hi}$ monocytes to tissues). However, such mutations were not noted in a separate study of a different vaccine-associated viscerotropic disease case[46]. These data suggest an involvement of the innate immune response in serious adverse events of YF-17D.

Conclusion

Vaccination is said to be the most cost-effective public health tool in history. However, most vaccines have been developed empirically and, despite their success, we understand little about their methods of operation. The past 3 years have witnessed significant progress in our understanding of YF-17D, one of the most successful vaccines ever made, enabled largely by advances in our understanding of innate immunity and the emergence of systems biology. Systems biological approaches not only permit the observation of a global picture of vaccine-induced innate immune responses but also can be used to predict the immunogenicity of a vaccine and uncover new correlates of vaccine efficacy. Further application of such approaches could facilitate vaccine development in several ways: by identifying vaccine responders versus non-responders, by identifying new correlates of protection or mechanisms of vaccine action and by predicting vaccine-induced adverse reactions, such as the infrequent, but often fatal, viscerotropic and neurotropic diseases that are associated with YF-17D vaccination[42–46] (BOX 1).

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

DATABASES
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FURTHER INFORMATION
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