This prototype T-cell vaccine contains components from multiple genes of the virus — the genes encoding the Gag, Pol, Nef and Env proteins — and is representative of diverse virus clades throughout the world. It will be tested in partnership with the Division of AIDS in NIAID and three clinical trial networks in different regions of the world. Phase II studies have begun at most of these sites and, barring unforeseen hurdles, proof-of-concept efficacy studies will begin next year. The results from recent non-human primate studies with a comparable vaccine are encouraging. When this analogous virus was used in the aggressive SIVmac239 challenge model, an increase in survival was noted in vaccinated animals[13]. More importantly, an immune correlate of survival, the memory $CD4^+$ T-cell population, was defined. Should this marker hold true in human studies, it could provide a surrogate for vaccine efficacy that would greatly accelerate future human clinical trials.

## Outlook

What does the future hold for HIV vaccine research? An HIV vaccine might not prevent infection, but could prevent the occurrence of symptoms. In the absence of a vaccine, it is likely that the pandemic will continue to spread although, with the help of other prevention and intervention strategies, the rate of increase of new infections could be reduced. If the levels of virus in the blood can be lowered sufficiently by a vaccine, we could have an opportunity to reduce transmission and eventually eliminate the disease. With a highly effective preventive vaccine, we can begin to confer protection to individuals, and rapidly bring extinction to the ever-expanding pandemic.

There are scientific questions that must be solved in order to make progress towards an effective HIV vaccine. Can we develop the types of potent immune responses at the sites of mucosal entry that are necessary to prevent the spread of the virus? Are there natural innate immune mechanisms that can contain the virus before it spreads? Are there molecular structures that we can identify and use to elicit broadly neutralizing antibodies and penetrate the shield of carbohydrate and moving structures that the virus presents to evade the immune system?

The solution will come from innovative and creative science, perhaps even from approaches not yet imagined that are fostered by groundbreaking research and progress in basic science. It is vital that the full range of intellectual resources and biomedical infrastructure be brought to bear on this problem. The collective energy and creativity of aspiring scientists will be needed to achieve the goal of a practical, cost-effective vaccine to contain and control emerging outbreaks so that AIDS becomes a disease of our past rather than our future.

*Gary J. Nabel is at the Vaccine Research Center, NIAID, National Institutes of Health, Bldg. 40, Room 4502, MSC-3005, 40 Convent Drive, Bethesda, Maryland 20892-3005, USA.*
*e-mail: gnabel@nih.gov*

doi:10.1038/nrmicro1713

1. Center for Biologics Evaluation and Research, Food and Drug Administration. *Vaccines Licensed for Immunization and Distribution in the US* [online] http://www.fda.gov/cber/vaccine/licvacc.htm (2007).
2. Korber, B. *et al.* Evolutionary and immunological implications of contemporary HIV-1 variation. *Br. Med. Bull.* **58**, 19–42 (2001).
3. Kwong, P. D. *et al.* HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. *Nature* **420**, 678–682 (2002).
4. Douek, D. C., Kwong, P. D. & Nabel, G. J. The rational design of an AIDS vaccine. *Cell* **124**, 677–681 (2006).
5. UNAIDS. *AIDS Epidemic Update: Special Report on HIV/AIDS December 2006*. [online] http://data.unaids.org/pub/EpiReport/2006/2006_EpiUpdate_en.pdf (2006).
6. Letvin, N. L. Progress and obstacles in the development of an AIDS vaccine. *Nature Rev. Immunol.* **6**, 930–939 (2006).
7. The rgp120 HIV Vaccine Study Group. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J. Infect. Dis.* **191**, 654–665 (2005).
8. Zhou, T. *et al.* Structural definition of a conserved neutralization epitope on HIV-1 gp120. *Nature* **445**, 732–737 (2007).
9. Chen, B. *et al.* Structure of an unliganded simian immunodeficiency virus gp120 core. *Nature* **433**, 834–841 (2005).
10. Rowland-Jones, S. L. *et al.* Cytotoxic T cell response to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. *J. Clin. Invest.* **102**, 1758–1765 (1998).
11. Schmitz, J. E. *et al.* Control of viremia in simian immunodeficiency virus infection by $CD8^+$ lymphocytes. *Science* **283**, 857–860 (1999).
12. Jin, X. *et al.* Dramatic rise in plasma viremia after $CD8^+$ T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.* **189**, 991–998 (1999).
13. Letvin, N. L. *et al.* Preserved $CD4^+$ central memory T cells and survival in vaccinated SIV-challenged monkeys. *Science* **312**, 1530–1533 (2006).

**DATABASES**
**The following term in this article is linked online to:**
**Entrez Genome:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome
HIV
**Access to this links box is available online.**

VACCINE WATCH

# Tuberculosis vaccines — an update

*Peter Andersen*

The current tuberculosis (TB) vaccine *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is the most widely used vaccine worldwide, but it does not prevent the establishment of latent TB or reactivation of pulmonary disease in adults. Peter Andersen looks at the progress of the candidates to improve or replace BCG.

Tuberculosis (TB), a disease which is both curable and preventable, still kills 2–3 million people every year. After decades of neglect, the immense public health impact of TB is now widely recognized, and the development of new tools to combat and control the epidemic has become an international priority. The current strategy for TB control is based on reducing the spread of infection through effective treatment of individuals with active disease and vaccination of children. The WHO has initiated the directly observed therapy (DOTS) campaign in many regions, but so far this programme has not been able to control the global TB epidemic or prevent the increase in multidrug resistant (MDR) strains of *Mycobacterium tuberculosis*[1].

The current TB vaccine *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) is the most widely used vaccine worldwide. BCG provides efficient protection against TB in newborns, but does not prevent the establishment of latent TB or reactivation of pulmonary disease in adults. Being a viable organism, the activity of BCG depends on its initial replication, and it therefore cannot be used as a booster in an adult population that is already sensitized by prior BCG vaccination, exposure to environmental mycobacteria or latent TB[2]. A novel, effective vaccination strategy against adult pulmonary TB is therefore a crucial goal and an active field of research, development and clinical evaluation.

## Global distribution and disease burden

In 2004, approximately 9 million people developed active TB. Although this places TB as one of the most important global health problems, active disease represents only the tip of the iceberg, as it has been estimated that one-third of the world's population is latently infected with *M. tuberculosis*. Globally, the incidence of TB is growing, mainly owing to the spread of HIV in

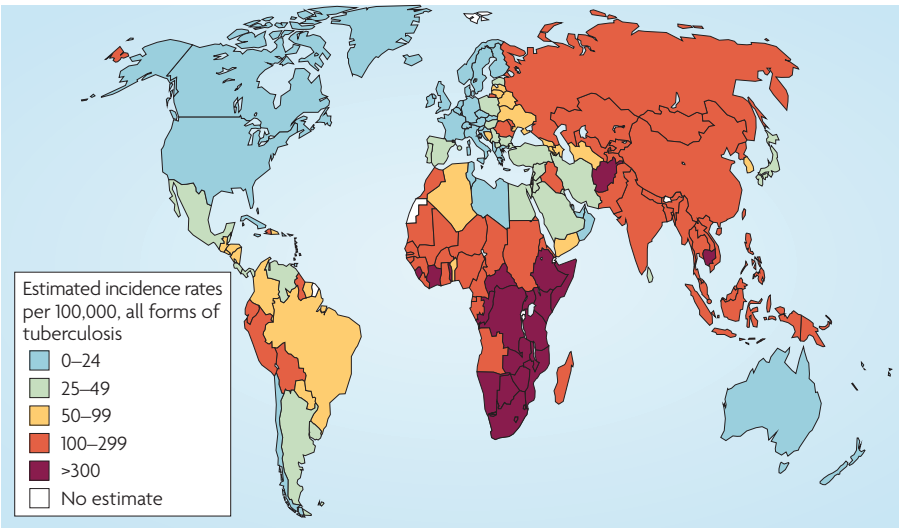


Figure 1 | **The distribution of tuberculosis in 2003.** Data taken from REF. 1.

Africa, where it has been estimated that 13% of adults with newly diagnosed TB are also co-infected with HIV[3]. However, in recent years, the increasing TB problem in Eastern European countries has contributed to the worsening global epidemic. Africa has the highest estimated incidence (356 per 100,000 population per year), but major parts of Asia also have a significant TB problem[1] (FIG. 1). In most of these regions, the incidence of TB has now reached such a magnitude that it is overwhelming the limited resources available to identify and treat active contagious pulmonary TB. Furthermore, by primarily targeting the working population, TB is a major roadblock to healthy economic development in many developing countries.

## Immunity to *M. tuberculosis*

*M. tuberculosis* infection remains latent with no overt clinical symptoms throughout life in more than 90% of infected individuals. Progressive mycobacterial infection in patients with deficient interferon-γ (IFN-γ) and tumour necrosis factor (TNF) signalling provides convincing evidence for the importance of these cytokines in the control of TB. The major source of these cytokines are $CD4^+$ T cells, the most important lymphocyte population in the protective immune response and the main target for most vaccination strategies[4]. The role of $CD8^+$ T cells is less clear. They are induced during natural *M. tuberculosis* infection, and although they do not seem to have a major role in the initial control of the infection, they might be more involved in the later, chronic stages of the disease[5]. To target this lymphocyte subset, some of the new vaccines are delivered through live carriers such as viral vectors or genetically modified strains of BCG. In the 5–10% of latently infected individuals who go on to develop active TB, the balance between the natural immunity of the host and the pathogen is thought to change, for example, following an immunosuppressive event, resulting in massive bacterial replication and reactivation of the disease.

All of the new TB vaccine candidates that are under clinical evaluation (TABLE 1) are designed as pre-exposure vaccines and, hence, are aimed at stimulating an immune response that controls subsequent infection more efficaciously than the immune response that is stimulated during natural infection, thereby delaying reactivation. It is not known whether post-exposure administration of these vaccines to already latently infected individuals would prolong host containment of latent TB and prevent reactivation, or whether this would require specially designed post-exposure vaccines based on antigens that are expressed by the bacteria during latency, as recently discussed elsewhere[6].

## Vaccine concepts and clinical trials

Current attempts to develop improved TB vaccination strategies can be divided into two approaches — replacing or boosting BCG. The first strategy aims to replace BCG with a more effective vaccine. This is generally believed to demand an improved, attenuated mycobacterial vaccine strain, obtained either through the generation of gene-deletion mutants of *M. tuberculosis*, or by re-introducing important antigens or other factors into the existing BCG vaccine strain. Viable, attenuated mycobacterial vaccines obviously present a broad variety of antigens and will potentially cover a combination of different T-cell populations, but such vaccines must be not only more potent than BCG, but also at least as safe, in order to be considered as candidates for clinical trials[7].

The second strategy involves the development of a booster vaccine that takes advantage of BCG priming vaccination in childhood, and is given to increase the immune response and prolong immunity to also cover the adult population. It is generally agreed that such a vaccination strategy can be best accomplished with a subunit vaccine. Subunit vaccines are based on a restricted number of antigens and hence on a highly focused immune response for protection. In several of the leading vaccine candidates, the individual antigens are fused into polyproteins, something that both

Table 1 | **The leading tuberculosis vaccine candidates in clinical trials**

| Vaccine name | Vaccine type | Development stage | Institution or company |
|---|---|---|---|
| rBCG30 | Live, recombinant BCG | Clinical Phase I completed in 2004 | UCLA School of Medicine/ Aeras |
| rBCG Δ*ureC*: Hly | Live, recombinant BCG | Clinical Phase I planned for 2007 | Max Planck Institute for Infection Biology/Vakzine Projekt Management |
| MVA-85A | Modified vaccinia virus | Completed and ongoing clinical Phase I | University of Oxford |
| H1/IC31 | Adjuvanted subunit | Completed and ongoing clinical Phase I | Statens Serum Institut/ Intercell |
| Mtb72f/ AS02A | Adjuvanted subunit | Completed and ongoing clinical Phase I | GlaxoSmithKline |
| H1/LTK63 | Adjuvanted subunit | Clinical Phase I ongoing | Statens Serum Institut/ Novartis |
| HyVac4 /IC31 | Adjuvanted subunit | Clinical Phase I planned for mid-2007 | Statens Serum Institut/ Intercell/Aeras |

BCG, *Mycobacterium bovis* bacillus Calmette–Guérin

increases the immunogenicity of the individual antigens and has obvious advantages from a manufacturing point of view. The success of the booster strategy is underpinned by recent advances in adjuvant development. Until recently, the only adjuvants appropriate for use in TB vaccines were either ineffective at stimulating T-cell responses or were too toxic for human use. This situation has rapidly changed in recent years, and a number of novel, promising T-cell adjuvants such as the IC31 adjuvant, cationic liposomes, the AS2 formulation and LTK63 (for mucosal delivery) are now under late-preclinical or clinical development in TB vaccines (TABLE 1).

Eventually, the ultimate vaccine strategy could be based on a combination of both approaches, that is, a prime–boost vaccination regime that comprises priming with the best possible viable vaccine candidate and boosting with the best possible subunit vaccine candidate[4].

## BCG replacement vaccines

***rBCG30.*** rBCG30 is a recombinant BCG vaccine in which the well-known and well-characterized antigen 85B (Ag85B) is overexpressed. This 30 kDa enzyme, which is involved in outer cell-wall synthesis, is a key component in several TB vaccines, and although Ag85B is already abundantly secreted by BCG, overexpression appears to increase responses to this important antigen[8]. rBCG30 has been tested in a Phase I trial in humans and was well tolerated.

***rBCG ΔureC:Hly.*** To amplify the $CD8^+$ T-cell response induced by BCG, a recombinant BCG mutant has been constructed that expresses listeriolysin (Hly), which can perforate the phagosome membrane. The gene (*ureC*) encoding the urease enzyme that increases the pH of the phagosome containing BCG was additionally deleted to avoid neutralizing the phagosome, as this would reduce the activity of Hly[9]. Surprisingly, apoptosis of infected macrophages and cross-priming of dendritic cells seems to be the major mechanisms responsible for the increased activity of this vaccine[10]. A clinical Phase I trial is planned to commence by the end of 2007.

## BCG booster vaccines

***Ag85B–ESAT6/TB10.4 fusion molecules.*** The Ag85B–ESAT6 fusion molecule (H1) is made up of the two secreted antigens Ag85B and ESAT6. These individual antigens both have an impressive track record of studies confirming their antigenicity in humans and their vaccine potential. H1 has shown promise both for parenteral (in IC31 or cationic liposomes) and mucosal (in LTK63) delivery[11,12]. In addition to being a valuable vaccine component, ESAT6 (the component of H1 localized in the region that was deleted during the original attenuation of BCG, and which is therefore absent from all BCG vaccine strains) is a key component in a new generation of diagnostic tests for *M. tuberculosis* infection[13]. An alternative fusion construct, called H4, has been engineered and consists of Ag85B and the TB10.4 antigen, which is also from the ESAT family of small secreted antigens[14]. TB10.4 has similar immunological properties to ESAT6, but it is highly expressed and immunodominant in BCG. H4 is a powerful booster vaccine for BCG, whereas the H1 vaccine for comparison, in addition to boosting Ag85B responses, will supplement the BCG antigen repertoire with the important ESAT6 antigen component.

H1 is currently in clinical trials administered both parenterally and through the mucosal route. The first clinical trial in Leiden, Holland (Dissel and Ottenhoff, unpublished data) evaluated the vaccine in a conventional parenteral vaccination strategy, using the IC31 adjuvant. This trial was conducted in purified protein derivative (PPD)-negative individuals and the vaccine was shown to be both safe and strongly immunogenic. The H1/IC31 vaccine is currently being evaluated in PPD-positive BCG-vaccinated individuals at the same site. Another trial that has recently started will test the nasal administration of the H1 antigen, using the LTK63 adjuvant. The H4/IC31 vaccine will commence clinical trials in mid-2007 in Sweden.

***MTB72f.*** The MTB72f vaccine is a fusion molecule consisting of two antigens that are strong targets for T helper 1 ($T_H1$) cells in PPD-positive individuals. Rv1196 (MTB32) is inserted into the middle of the serine protease Rv0125 (MTB39), which is thus present as two fragments[15]. MTB72F in the AS02A adjuvant formulation has recently completed two Phase I trials in healthy PPD-negative adults in the United States and Belgium. The vaccine was well tolerated and safe, and could induce both antigen-specific humoral and cell-mediated immune responses.

***MVA85A.*** MVA85A is a modified vaccine virus Ankara (MVA) strain expressing antigen 85A, another member of the Ag85 family of protective antigens. In Phase I studies in humans, MVA85A was found to be safe and well tolerated, and this vaccine has induced strong immune responses, particularly in previously BCG-vaccinated individuals[16].

## Conclusions

With increasing investment from public funds such as the European Union, National Institutes of Health and the Bill & Melinda Gates Foundation in recent years, TB vaccine research, development and evaluation has become an active area, with several vaccines in various stages of early clinical development. Most of this work is conducted by public research organizations and public–private partnerships, but a recent re-analysis and demonstration of the significant commercial value of a novel TB vaccine[17] will probably result in a larger investment from private industry. This will promote streamlined development and the eventual global distribution of a novel vaccine. Although a new, improved vaccination strategy against TB is finally on the horizon, its eventual success will still depend on continued close integration with information from basic research. The identification of reliable correlates of protection, as well as the answers to more basic immunological questions relating to immunological memory and the relative importance of different T-cell subsets, will be important for the potential modification of the leading TB vaccines, the generation of second-generation products and the selection of which vaccines to move forward into expensive efficacy trials (BOX 1). It will furthermore be a high priority for the clinical development programmes to evaluate whether the current vaccines, all of which have been designed for pre-infection administration, will also prevent reactivation of TB if administered post-exposure to the large proportion of the global population already latently infected with TB.

Box 1 | **Key areas for tuberculosis (TB) vaccine development**

- Determine the correlates of vaccine induced protective immunity
- Determine the requirements for post-exposure TB vaccines
- Understand vaccine-induced T-cell memory to *Mycobacterium tuberculosis*
- Determine the role of $CD8^+$ T cells in *M. tuberculosis* infection and immunity

*Peter Andersen is at the Department of Infectious Disease Immunology, Statens Serum Institute, Copenhagen, Denmark.*
*e-mail: pa@ssi.dk*

doi:10.1038/nrmicro1703

1. WHO. Global tuberculosis control — surveillance, planning, financing. [online] http://www.who.int/tb/publications/global_report/en (2004).
2. Andersen, P. & Doherty, T. M. The success and failure of BCG — implications for a novel tuberculosis vaccine. *Nature Rev. Microbiol.* **3**, 656–662 (2005).
3. Dye, C. Global epidemiology of tuberculosis. *Lancet* **367**, 938–940 (2006).
4. Kaufmann, S. H. Recent findings in immunology give tuberculosis vaccines a new boost. *Trends Immunol.* **26**, 660–667 (2005).
5. Woodworth, J. S. & Behar, S. M. *Mycobacterium tuberculosis*-specific $CD8^+$ T cells and their role in immunity. *Crit. Rev. Immunol.* **26**, 317–352 (2006).
6. Andersen, P. Vaccine strategies against latent tuberculosis infection. *Trends Microbiol.* **15**, 7–13 (2007).
7. Kamath, A. T. *et al.* New live mycobacterial vaccines: the Geneva consensus on essential steps towards clinical development. *Vaccine* **23**, 3753–3761 (2005).
8. Horwitz, M. A. & Harth, G. A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis. *Infect. Immun.* **71**, 1672–1679 (2003).
9. Grode, L. *et al.* Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette–Guerin mutants that secrete listeriolysin. *J. Clin. Invest.* **115**, 2472–2479 (2005).
10. Winau, F. *et al.* Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* **24**, 105–117 (2006).
11. Agger, E. M. *et al.* Protective immunity to tuberculosis with Ag85B-ESAT-6 in a synthetic cationic adjuvant system IC31. *Vaccine* **24**, 5452–5460 (2006).
12. Dietrich, J. *et al.* Mucosal administration of Ag85B-ESAT-6 protects against infection with *Mycobacterium tuberculosis* and boosts prior Bacillus Calmette–Guerin immunity. *J. Immunol.* **177**, 6353–6360 (2006).
13. Pai, M., Riley, L. W. & Colford, J. M. Jr. Interferon-γ assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect. Dis.* **4**, 761–776 (2004).
14. Dietrich, J. *et al.* Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis subunit vaccine: efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. *J. Immunol.* **174**, 6332–6339 (2005).
15. Skeiky, Y. A. *et al.* Differential immune responses and protective efficacy induced by components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. *J. Immunol.* **172**, 7618–7628 (2004).
16. McShane, H. *et al.* Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nature Med.* **10**, 1240–1244 (2004).
17. BIO Ventures for Global Health. Tuberculosis vaccines: The case for investment [online] http://www.bvgh.org/resources/market/TBBC.asp (2006).



**Acknowledgements**

Vaccine research in the Andersen laboratory is supported by EU-FP6 TBVAC, EU-FP6 MUVAPRED, grants from the Danish Research Council and the Bill & Melinda Gates Foundation, Grand Challenges in Global Health (GC6, GC12) and the AERAS Global Vaccine Foundation.


**FURTHER INFORMATION**

**Bill & Melinda Gates Foundation:** http://www.gatesfoundation.org/default.htm
**WHO Stop TB:** http://www.who.int/tb/en
**Access to this links box is available online.**

VACCINE WATCH

# Malaria vaccines: the stage we are at

*Stephen M. Todryk and Adrian V. S. Hill*

With over 1 million deaths annually attributed to malaria, an effective vaccine is an urgently needed intervention. However, the various stages of the malaria parasite lifecycle have differing protective immune mechanisms and clinical endpoints, and usually different, often polymorphic, antigens. Trials using an increasing variety of vaccine platforms and antigens are under way in an attempt to achieve this long-awaited goal.

There are currently no vaccines available for parasite pathogens that infect humans, despite extensive efforts. This is partly due to the fact that a successful parasite must co-exist with the host immune response, allowing the lifecycle of the parasite to proceed without killing the host. Also, the host response to the parasite must not induce fatal pathology. This is a fine line that must be negotiated by both organisms.

## Global distribution and disease burden

The global burden of malaria is unparalleled for a parasitic disease. Up to 500 million malaria-attributable clinical cases and more than 1 million deaths occur annually, with most of the deaths being children under 5 years of age in sub-Saharan Africa[1]. Countries such as Uganda, Tanzania, Malawi and Mozambique are among the most affected of the tropical and sub-tropical countries[2] (FIG. 1), and they thus shoulder enormous economic burdens.

## The malaria lifecycle

Malaria is caused by plasmodial protozoan parasites that are transmitted by *Anopheles* mosquitoes. *Plasmodium falciparum* is responsible for most malaria mortality, and thus receives most attention in vaccine research. The continued emergence of drug-resistant parasites and insecticide-resistant mosquitoes is an obstacle to containing the disease. New drugs are often not affordable in poor countries, and this could also be an issue for complex, multi-component vaccines.

The complex lifecycle of the malaria parasite contributes to the complexity of generating a malaria vaccine. When an infected *Anopheles* mosquito takes a blood meal, it injects sporozoites into the bloodstream of the human host. Within hours, these reach the liver and infect hepatocytes. This liver stage of the life cycle is symptomless. The sporozoites reproduce asexually, giving rise to many thousands of merozoites, and the hepatocyte ruptures after approximately 7 days. The merozoites enter the bloodstream and infect red blood cells, where they continue to multiply. During this erythrocytic stage, symptoms can occur, such as fever, anaemia, loss of renal function and coma. Loss of renal function and coma are caused by the attachment of infected red blood cells to blood vessels. A proportion of the merozoites develop into male and female gametocytes, which are taken up by a feeding mosquito, in which they combine and develop into new infective sporozoites.

The different stages of the parasite lifecycle present different opportunities for vaccines, and the parasite expresses differing protein antigens that are potential vaccine targets. However, the vaccine platform and composition must ensure that an appropriate immune response is generated.

## Vaccine concepts and clinical trials

Most licensed vaccines generate antibodies against extracellular pathogens, which can be accurately measured and often correlate with protection. Such vaccines comprise whole inactivated microorganisms or, increasingly, parts (or subunits) of microorganisms with appropriate adjuvants.

The idea that it might be possible to generate a vaccine against malaria stems from several observations. Immunity to malaria can develop naturally following frequent exposure, and it is generally thought to comprise mainly antibodies against blood-stage antigens. Protective immunity can also be generated by immunization with irradiated sporozoites, by allowing infected and irradiated mosquitoes to take blood meals on human volunteers. T cells against liver-stage antigens are considered the main immune effectors in this case. However, the precise nature of the immune responses (effector mechanisms, antigen specificity and magnitude) that directly reduce or prevent malaria are unknown, and are often poorly modelled in animals. This makes the search for a malaria vaccine all the more difficult. Furthermore, despite the *P. falciparum* genome sequence being known and many stage-specific antigens having been identified,

**Andersen (1703)**

**Online Links**
Bill & Melinda Gates Foundation:
http://www.gatesfoundation.org/default.htm

WHO Stop TB:
http://www.who.int/tb/en