Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis

Stefan H E Kaufmann1 & Andrew J McMichael2

Human immunodeficiency virus (HIV) and Mycobacterium tuberculosis annually cause 3 million and 2 million deaths, respectively. Last year, 600,000 individuals, doubly infected with HIV and M. tuberculosis, died. Since World War I, approximately 150 million people have succumbed to these two infections—more total deaths than in all wars in the last 2,000 years. Although the perceived threats of new infections such as SARS, new variant Creutzfeldt-Jakob disease and anthrax are real, these outbreaks have caused less than 1,000 deaths globally, a death toll AIDS and tuberculosis exact every 2 h. In 2003, 40 million people were infected with HIV, 2 billion with M. tuberculosis, and 15 million with both. Last year, 5 million and 50 million were newly infected with HIV or M. tuberculosis, respectively, with 2 million new double infections. Better control measures are urgently needed.

The two culprits

M. tuberculosis infection does not necessarily transform into disease. Of the more than 2 billion individuals infected with M. tuberculosis, only approximately 10% will develop tuberculosis1,2. The pathogen is not eradicated, but is contained in distinct foci by the immune system. Critical for protection are CD4+ T lymphocytes. When disease develops, it generally manifests in the lung and is transmitted through the air. Hence, active tuberculosis is highly contagious and prevention of infection is nearly impossible. A vaccine against tuberculosis was developed in the early twentieth century by the French scientists Albert Calmette and Camille Guérin3, consisting of an attenuated strain of M. bovis, the etiologic agent of tuberculosis in cattle. The bacille Calmette-Guérin (BCG) vaccine protects against severe forms of childhood tuberculosis, but unfortunately, does not lead to eradication of M. tuberculosis and protective activity of the vaccine weakens during adolescence. As a consequence, BCG does not protect against the most prevalent disease form, pulmonary tuberculosis, in adults. Tuberculosis can be cured by chemotherapy, but the complex and long-lasting treatment, involving at least three drugs for 6 months, means compliance is often incomplete, resulting in a rising incidence of multidrug-resistant (MDR) strains. In several countries such as Estonia, the Russian Federation, Israel and Uzbekistan, more than 10% of all tuberculosis cases are caused by MDR strains1. In low-income countries MDR tuberculosis results in no treatment and often leads to death and further spread.

Infection with HIV, in contrast, consistently transforms into disease, and is contagious at all stages. HIV predominantly resides in CD4+ T lymphocytes. Infected and noninfected T cells are damaged, causing immunodeficiency. With new antiretroviral drugs, HIV infection can be controlled, but not eradicated. Treatment, however, is expensive and less than 5% of individuals infected with HIV in developing countries have access to effective treatment. Anti-HIV drugs target HIV reverse transcriptase, protease, integrase or the coiled coil domain of gp41. Used in combinations of three or more, they are very effective at treating HIV infection: patients with advanced AIDS have shown dramatic albeit incomplete recovery of CD4+ T cell counts and immune function, though sometimes the revitalized immune response causes severe problems by reacting to previously silent infecting pathogens. Yet even when virus loads have become undetectable for several years, cessation of drugs results in rapid rebound of virus and return to the pretreatment levels4. This implies that patients need to take these drugs for life. But because of serious side effects and expense, indefinite treatment is not possible for most of the world. Drug resistance by virus mutation is well described5 and although triple therapy reduces the risk substantially, imperfect adherence to treatment regimes may result in selection of resistant virus. As is the case for tuberculosis, this could limit treatment options in the near future. Transmission, however, can be reduced by ‘safer sex’ practices. Such behavior changes may account for the reduction in prevalence of HIV-1 infection from >20% to 8% in Uganda over 10 years6.

The dangerous liaison between AIDS and tuberculosis

In South Africa, more than 10% of the 40 million inhabitants are infected with HIV and more than 5% suffer from active tuberculosis. In this country, more than half of all individuals with tuberculosis have concurrent HIV infection. Immunodeficiency caused by HIV infection increases the risk of developing tuberculosis dramatically—10% of double-infected individuals will develop active tuberculosis within a year of coinfection, as compared to the 10% lifelong risk in individuals infected with M. tuberculosis alone. Despite the availability of effective chemotherapy to cure tuberculosis and the successful development of antiretroviral drugs that control HIV, vaccines provide the only realistic hope of effective prevention.
Host response to tuberculosis

Tuberculosis most frequently develops in the lung (Fig. 1). Once tubercle bacilli have reached the lung alveoli, they are taken up by alveolar macrophages and probably dendritic cells (DCs) in the lung interstitium (Fig. 1). M. tuberculosis is protected by a recalcitrant cell wall rich in waxes and other glycolipids, which is responsible for the unique staining characteristics of these microbes and contributes significantly to resistance against host defense. Infected macrophages and DCs, therefore, do not destroy their bacterial prey and thus serve as a mobile habitat, transporting the microbes to the lung parenchyma and eventually to the draining lymph nodes. In draining lymph nodes, these cells present mycobacterial antigens to different T cell populations. Antigen presentation probably involves cross-priming, allowing transfer of mycobacterial antigens from infected macrophages to dendritic cells. Antigen-specific CD4+ T cells, CD8+ T cells, γδ T cells and CD1-restricted T cells participate in protection. Most importantly, macrophages are activated by IFN-γ and TNF-α. In addition, T cells may kill mycobacteria present in macrophages by means of perforin and granulysin.

CD8+ T cells also become stimulated. In addition, unconventional T cells are activated, including T cells expressing a γδ T cell receptor with specificity for small phosphorylated ligands and T cells with specificity for glycolipids, which are presented by major histocompatibility complex (MHC) class I–like molecules of the CD1 family. Studies in nonhuman primates support a role for γδ T cells in protective immunity against tuberculosis. The CD1-restricted T cells presumably evolved as a result of the evolutionary cross-talk between mycobacteria and the mammalian host, as they are specific for glycolipids abundant in mycobacteria but not found in other microbial pathogens.

Antigen-specific T cells induce the formation of a granuloma around infected macrophages, primarily composed of monocyte-derived macrophages (some of which transform into multinucleated giant cells), CD4+ T cells and an outer ring of CD8+ T cells. At later stages, the granuloma is surrounded by a fibrotic wall and lymphoid follicular structures develop in the vicinity, probably orchestrating the local immune response.

Figure 1 Different outcomes of M. tuberculosis infection and underlying immune mechanisms. M. tuberculosis enters the host within inhaled droplets. Three outcomes are possible. (i) Immediate eradication of M. tuberculosis by the pulmonary immune system. This alternative is rare to absent. (ii) Infection transforms into tuberculosis. This frequently occurs in immunodeficient individuals, with the notable example of HIV infection increasing the risk of developing tuberculosis 800-fold. (iii) Infection does not transform into disease because M. tuberculosis is contained inside granulomas. In the diseased individual, M. tuberculosis is no longer contained because caseation of the lesion results in dissemination and transmission of M. tuberculosis. After inhalation, M. tuberculosis is engulfed by alveolar macrophages and DCs. In draining lymph nodes, these cells present mycobacterial antigens to different T cell populations. Antigen presentation probably involves cross-priming, allowing transfer of mycobacterial antigens from infected macrophages to dendritic cells. Antigen-specific CD4+ T cells, CD8+ T cells, γδ T cells and CD1-restricted T cells participate in protection. Most importantly, macrophages are activated by IFN-γ and TNF-α. In addition, T cells may kill mycobacteria present in macrophages by means of perforin and granulysin.

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In the granulomatous lesion, macrophages are activated by T lymphocytes through production of type I cytokines, notably interferon (IFN)-γ and tumor necrosis factor (TNF)-α. IFN-γ activates the capacity to control M. tuberculosis in macrophages. Moreover, IFN-γ and TNF-α are instrumental in walling off M. tuberculosis inside granulomatous lesions. The importance of these cytokines in the containment of mycobacteria is well illustrated by the observation that individuals with deficient IFN-γ signaling suffer from rapid onset of mycobacterial diseases, and that treatment with antibodies that neutralize TNF-α in individuals with rheumatoid arthritis reactivates tuberculosis in those with latent infection.

The granuloma persists for years and efficiently contains tubercle bacilli as long as the individual remains immunocompetent. The granuloma deprives the arrested mycobacteria of oxygen and nutrients and as a direct consequence, the microbes survive, probably in a state of dormancy. The gene expression profile of M. tuberculosis is altered in response to the restricted growth conditions in the granulomatous lesion. It is highly probable that some of these dormancy-related gene products serve as the major antigens of dormant M. tuberculosis and hence represent promising candidate antigens for a postexposure vaccine aimed at preventing reactivation tuberculosis. The best-known dormancy antigen is the α-crystallin, a small heat-shock protein (Hsp) also termed HspX.

Host resistance, latency and tuberculosis outbreak

The mechanisms that determine latency and disease outbreak in tuberculosis remain elusive. Although the risk of reactivation is understood to be determined by both host and microbial factors in addition to environmental components, virtually nothing is known about the responsible genes. Increasing epidemiologic evidence that strains of the M. tuberculosis Beijing/W genotype family, many of which are of the MDR type, are spreading throughout the world suggests that this family of M. tuberculosis evolved against the evolutionary pressure of BCG vaccination and chemotherapy.

A careful histologic analysis of lung autopsy material performed from 284 cadavers more than 100 years ago in Zürich showed almost 100% M. tuberculosis infection in individuals over 21 years of age, who had died of unrelated reasons. We can assume similarly high incidences nowadays for individuals living in tuberculosis ‘hot spots’ (e.g., prisons in some countries) and infection rates between 10% and 30% in areas with high M. tuberculosis incidences.

How and why disease outbreak occurs after a period of latency are unclear. Increased risk of tuberculosis as a result of unique environmental and behavioral factors such as alcohol abuse or dietary iron overload emphasizes the importance of these components, but again provides little explanation of specific control mechanisms. A considerable proportion of individuals with tuberculosis develop disease within the first year after infection. Moreover, superinfection as well as reinfection after successful tuberculosis chemotherapy have been described. It is probable that these individuals develop an insufficient immune response against natural infection with M. tuberculosis because of genetic host susceptibility, immune suppression by the pathogen or both. Elucidation of the genetic mechanisms underlying susceptibility and protective immune mechanisms in resistant individuals that prevent disease outbreak in face of ongoing infection, as well as identification of promising vaccine candidates, will be key to containing the global tuberculosis epidemic.

| Vaccine candidate | Comment | Examples |
|-------------------|---------|----------|
| Subunit vaccine: Antigen in adjuvant | Mild side effects, skewed antigen profile, potent adjuvant needed | Methylated Hbha (ref. 123) |
| Naked DNA | CD4+ and CD8+ T cells stimulated, skewed antigen profile, potent packaging system needed, safety concerns | Ag85 (ref. 35) |
| Recombinant carrier expressing antigen | CD4+ and/or CD8+ T cells stimulated, skewed antigen profile, safety concerns | r-MVA expressing Ag85 (refs. 40, 41) |
| Viable mycobacterial vaccine: M. tuberculosis deletion mutant | CD4+, CD8+ and unconventional T cells stimulated, safety concerns | Icl - M. tuberculosis |
| Auxotrophic mutants | Improved safety (BCG), reduced immunogenicity, safety concerns M. tuberculosis | Met, leu, ilv - BCG |
| Recombinant BCG expressing cytolysin | CD4+ and CD8+ T cells and unconventional T cells stimulated, devoid of tuberculosis-specific antigens, safety concerns | Δ urease r-BCG expressing listeriolysin |
| Recombinant BCG overexpressing antigen | Improved antigenicity, primarily CD4+ T cells stimulated, safety concerns | r-BCG-Ag85 (ref. 53) |
| Prime-boost vaccination: based on BCG prime | Improved immunogenicity, builds on BCG prime, safety concerns | BCG/protein (Ag85) |

Table 1 Selected promising tuberculosis vaccine candidates

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*Adapted from ref. 16. Hbha, heparin binding hemagglutinin adhesion molecule; Icl, isocitrate lyase; Pdim, phthiocerol dimycocerosate; PhoP/phoQ, two-component system that regulates phosphatase activity; Cim, counterimmune; Met, methionine; Leu, leucine; Ilv, isoleucine, leucine or valine; MetB, methionine; proC, proline; trpD, tryptophan.
of the pathogen genes that promote transformation of latent infection into active tuberculosis, will facilitate rational design of a postexposure vaccine.\textsuperscript{27,28,31}

Of equal importance will be the careful analysis of the efficacy of BCG vaccination. BCG was found to protect against adult tuberculosis in the UK and against leprosy in Malawi, but did not provide any protection in the World Health Organization trial in south India.\textsuperscript{32} Overall, a 50\% protective efficacy has been estimated for BCG.\textsuperscript{33} Although environmental factors, notably frequent infection with atypical mycobacteria, probably have a role, contribution of genetic host factors should be assessed more carefully. It is possible that a small proportion of BCG-vaccinated individuals develops a long-lasting immune response against tuberculosis, whereas the majority does not. Because any new vaccine has to protect individuals who do not mount an appropriate immune response to natural infection with \textit{M. tuberculosis} or to vaccination with BCG, the susceptible and resistant target populations and the criteria that distinguish them from each other need to be defined carefully.

**Vaccination strategies against tuberculosis**

Subunit vaccines given alone or in addition to BCG. New subunit vaccines against \textit{M. tuberculosis} comprise antigen-adjuvant formulations, naked DNA vaccine constructs or recombinant carriers expressing antigen (Table 1).\textsuperscript{34} They are mainly based on protein antigens. Unfortunately, reliable rules for defining protective antigens for T cells do not exist. Most vaccine antigens investigated thus far are early produced secreted antigens such as Ag85, a shared antigen with BCG, and Esat-6, which is an antigen unique to \textit{M. tuberculosis} (Fig. 2).\textsuperscript{35,36} Such antigens are probably adequate for pre-exposure vaccination. For individuals latently infected with \textit{M. tuberculosis}, a postexposure vaccine composed of dormancy-related antigens such as HspX seems more suitable (Fig. 2).\textsuperscript{21–23}

A recent screen has assessed the protective efficacy in mice of more than 30 antigens that are differentially expressed in the proteomes of \textit{M. tuberculosis} versus BCG.\textsuperscript{37} From these, only one antigen was identified that induced a robust protection similar to that of BCG, arguing against a unique role in protection of proteins exclusively present in the proteome of \textit{M. tuberculosis}.

Although proteins will constitute the major antigens of subunit vaccines, glycolipid antigens can be included in these vaccine formulations. Such glycolipids could serve both as antigen in the context of CD1 and as adjuvant for Toll-like receptors (TLRs).\textsuperscript{31} Generally, subunit vaccines crucially depend on appropriate adjuvants that stimulate T helper type 1 (TH1) immune responses by the different T cell populations required for protection against tuberculosis.

Naked DNA constructs can stimulate CD8\(^+\) and CD8\(^+\) T cells. Although they have proven their high immunogenicity in small-rodent animal models, human studies are thus far mostly disappointing. But new carrier systems such as polylactide glycolyde microparticles may facilitate application of naked DNA vaccines in humans.\textsuperscript{39} Recombinant bacterial and recombinant viral carriers expressing defined mycobacterial antigens have been constructed and their vaccine efficacy against tuberculosis have been determined in experimental animal models. These vectors induce potent CD8\(^+\) and CD4\(^+\) TH1 responses. The most advanced vaccine of this type is recombinant modified vaccinia virus Ankara (r-MVA) expressing Ag85, which has already entered phase 1 clinical trials.\textsuperscript{40,41}

Vaccination with Mtb 72F protein in AS02A adjuvant or in the form of naked DNA induces protective immunity in both mice and guinea pigs.\textsuperscript{42} Mtb 72F is a recombinant fusion protein composed of Rv0125 and Rv1196 with a predicted size of 70 kDa. The adjuvant AS02A is composed of a nontoxic lipopolysaccharide derivative and QS21 from quillaja saponaria (a triterpene glycoside) as an oil-in-water emulsion. The Mtb

![Figure 2](https://example.com/figure2.png)

**Figure 2** Differential antigen requirements of pre- and postexposure vaccines. (**a**, **b**) A pre-exposure vaccine should comprise the set of antigens that are rapidly secreted early after infection, but would be ineffective in latent infection because its antigenic composition does not match the dormancy-induced antigenic repertoire. (**c**) A postexposure vaccine composed of dormancy-induced proteins should match the antigenic repertoire of dormant \textit{M. tuberculosis}. (**d**) Ideally, a combination of both types of antigens would protect against both early childhood tuberculosis and adult reactivation tuberculosis because the antigenic repertoire would reflect the different stages of infection. Red line, nonvaccinated; blue line, vaccinated.
72F–AS02A vaccine formulation has now entered a phase 1 clinical trial in healthy *M. tuberculosis*-uninfected volunteers (Table 2). Another protein-based vaccine comprising a fusion protein of Esat-6 and Ag85 in a mixture of oligodeoxynucleotides and a polycationic peptide (IC31) as an adjuvant has shown promising results in experimental animals and hence is aimed at entering a phase 1 clinical trial later in 2005 (Table 2).36

Generally heterologous prime-boost schemes are composed of a relatively simple first component (e.g., DNA) that primes a focused immune response, and a second boost vaccine that induces an inflammatory response (e.g., using a recombinant virus) to magnify the initial response. To achieve a proper prime-boost effect, shared antigens are required for the subunit boost vaccination. In tuberculosis, however, heterologous prime-boost vaccination schemes starting with a BCG prime followed by a subunit boost are considered most promising, not only for scientific reasons but also because BCG vaccination of newborns cannot be given up prematurely. The recently discovered antigen Rv3407 is encoded in the BCG genome but is undetectable in its proteome.37 The increased protection seen in BCG–primed mice after boost vaccination with naked DNA encoding Rv3407 indicates that this protein is an important antigen of *M. tuberculosis* that is suboptimally expressed in BCG. Similarly, boost with Mtb 72F in AS02A adjuvant increased protection induced by BCG prime.38 The r-MVA expressing Ag85 induced substantial protection both when given alone or as booster vaccination on top of BCG prime.39 A recent clinical phase 1 trial showed that r-MVA–Ag85 induced Ag85–specific IFN-γ-secreting T cells (Table 2).40 More profound effects were observed in the BCG prime–r-MVA–Ag85 boost group, underlining the importance of heterologous prime-boost vaccination in the rational delineation of future tuberculosis vaccination strategies.

**Attenuated gene deletion mutants of *M. tuberculosis* as vaccine candidates.** Vaccine efficacy data of mutant *M. tuberculosis* are still limited. During the early stage of infection, *M. tuberculosis* replicates actively. Accordingly, *M. tuberculosis* knockout mutants, in which synthesis of essential amino acids is prohibited, will not grow in lungs of experimentally infected mice. Typically, these are auxotrophic mutants that die of starvation in the absence of the required nutrients (Table 1).44,45 A second group of *M. tuberculosis* knockout mutants show reduced growth in lungs of experimentally infected mice, but persist at a lower level than wild-type microorganisms. These mutations include deficient regulatory proteins, such as the phoP/phoQ two-component regulatory system and a variety of mutants with deficient lipid metabolism or transport.46,47 Although genes of this group include virulence factors, it is also possible that the gene products are needed for persistence rather than harming the host directly. *M. tuberculosis* knockout mutants deficient in genes expressed during dormancy replicate in the lungs of experimental mice in an unconstrained manner early after infection, but cease to grow at later stages because of their impaired dormancy gene program. One of the best described genes of this group is that which encodes isocitrate lyase.48 *M. tuberculosis* knockout mutants, which grow as well as wild-type microorganisms but induce weaker pathology, lack true virulence factors. Some knockout mutants behave like wild-type *M. tuberculosis* in the lung but do not disseminate to other organs. The heparin-binding hemagglutinin adhesion molecule seems to participate in the dissemination of *M. tuberculosis* to extrapulmonary sites and hence qualifies as a member of this group.49 Factors that facilitate dissemination to other tissues should be deleted in a viable vaccine in order to focus the response on the lymph nodes. A recently defined group of *M. tuberculosis* knockout mutants comprises strains that grow as well as wild-type microorganisms in normal mice, but do not persist in mouse knockout mutants with defined immunodeficiency.50

Vaccine candidates based on *M. tuberculosis* knockout mutants will probably face strong objections against use in human clinical trials largely because of the genetic stability of the mutants. Reversion is best prevented by having two independent chromosomal gene deletions. The advantage of *M. tuberculosis* knockout mutants is their profound stimulation of all T cell populations relevant to protective immunity. On the other hand, *M. tuberculosis* is known to impair the host immune response. Therefore, it is probably not sufficient to reduce the virulence of *M. tuberculosis* knockout mutants. Additional genetic modifications improving the immune response and reducing pathology will be required. Although these vaccines will have a long way to go, a rationally designed *M. tuberculosis* mutant lacking a defined set of virulence genes in the long run could have great potential as a tuberculosis vaccine.

**Improved recombinant BCG as vaccine candidates.** As compared to *M. tuberculosis*, the current vaccine BCG lacks approximately 130 genes that are clustered in 16 regions of difference (RD) and are at least in part involved in pathogenicity and persistence. Reintroduction of selected genes may increase immunogenicity, antigenicity or both of BCG without reverting it to a pathogen (Table 1). Improved immunogenicity results in the stimulation of a profound immune response. Antigenicity can be improved by introduction of additional antigens or by overexpression of antigens expressed at suboptimal level.

Pym et al. have introduced the entire RD1 region of *M. tuberculosis* into BCG, comprising at least 11 genes.52 Although it remains to be clarified whether the recombinant BCG (r-BCG) expressing RD1 genes is endowed with improved immunogenicity, antigenicity or both, it was claimed that this vaccine candidate provided slightly better protection than parental BCG in mice. Not unexpectedly, however, this r-BCG candidate showed higher virulence in immunocompromised mice as compared to wild-type BCG.

Reintroduction of genes missing in BCG that do not confer virulence might increase vaccine efficacy of BCG without decreasing its safety. Horwitz et al. introduced the gene encoding Ag85 into BCG, an abundant secreted protein in *M. tuberculosis*.53 Although the gene is also expressed in BCG, it was assumed that overexpression of this immunodominant antigen would increase protective immune responses. Indeed, in guinea pigs, r-BCG expressing Ag85 induced substantially higher protection than parental BCG against tuberculosis. This vaccine was at least as safe as BCG and has recently entered a phase 1 clinical trial (Table 2).

| Candidate | Type | Status |
|-----------|------|--------|
| Mtb 72F in AS02A | Subunit (protein/adjuvant) | Clinical phase 1 trial in healthy uninfected volunteers ongoing |
| Ag85-Esat-6 fusion protein in IC31 | Subunit (protein/adjuvant) | GMP production and clinical phase 1 trial planned for 2005 |
| r-MVA-Ag85 | Subunit (recombinant virus), prime-boost | Chemical phase 1 trial in BCG-vaccinated and unvaccinated healthy uninfected volunteers completed |
| r-BCG-Ag85 | Viable | Clinical phase 1 trial in healthy uninfected volunteers ongoing |
| r-BCG-Hly | Viable | GMP production initiated, clinical phase 1 trial planned for 2005 |
The r-BCG vaccine candidates with higher immunogenicity comprise strains that express human cytokines such as IFN-γ, IFN-α, interleukin (IL)-2, IL-12 and granulocyte macrophage colony stimulating factor (GM-CSF)54–56. However, these cytokine-secreting r-BCG have yet to prove superior protection in animal models against tuberculosis, as they have not yet been compared to parental strains.

Another approach to improving immunogenicity of BCG takes advantage of the biological activity of listeriolysin (Hly)57. In its natural host, Listeria monocytogenes, this cytolysin forms pores in the phagosomal membrane, promoting listerial egression into the cytosol58. In the cytosol, listerial antigens are readily introduced into the MHC class I pathway, causing preferential stimulation of CD8+ T cells. Compelling evidence suggests that BCG is insufficiently equipped for stimulating CD8+ T cells, whereas CD4+ T cell stimulation is satisfactory59, the gene encoding Hly was introduced into BCG to improve CD8+ T cell stimulation. Indeed, such vaccine constructs induced better protection than parental BCG in mice (Grode, L. et al; unpublished data). Production of this vaccine under good manufacturing practices (GMP) conditions has been initiated and this candidate is aimed at entering a phase 1 clinical trial in 2005 (Table 2).

Pros and cons of subunit and viable vaccines

The use of subunit vaccines is based on the assumption that one or a few protective antigens and T cell clones will suffice for efficacious protection59. In contrast, attenuated viable vaccines utilize the wide spectrum of expressed antigens to stimulate an optimal combination of T cell subtypes. Genetically engineered viable vaccines are therefore best suited as replacements for BCG. Despite previous reluctance, a recent T cell subtypes. Genetically engineered viable vaccines are therefore best suited as replacements for BCG. Although subunit vaccines are probably not affected prematurely eradicate an improved r-BCG or attenuated recombinant M. tuberculosis and thereby impair efficacy of genetically engineered viable vaccines. Although subunit vaccines are probably not affected by the preexisting cross-reactive immunity to environmental bacteria, generally immunity induced by subunit vaccines is short-lived. Given the complementary strength of both types of new vaccine candidates, a heterologous prime-boost regimen comprising a prime with a viable vaccine candidate superior to BCG and a boost with a subunit vaccine candidate will probably be the most promising combination to ensure long-lasting efficacy.

AIDS: infection, pathogenesis, immune response and disease

HIV is a member of the lentivirus subgroup of the retrovirus family, so named because of a long period of clinical latency after infection. Its envelope is derived from cell membrane and expresses the glycoproteins gp120 (responsible for virus attachment to cells) and gp41 (responsible for membrane fusion). Internally, matrix (gag p17) and capsid (gag p24) proteins enclose the viral RNA. Other viral proteins include the reverse transcriptase, protease, integrase and regulatory proteins nef, tat, rev, vif, vpr and vpu. The virus infects by attaching gp120 to the CD4 molecule and either the chemokine receptor CCR5 or CXCR4 present on T cells61. Gp41 mediates fusion and in the cytosol the RNA is reverse transcribed and the preintegration complex moves to the nucleus, where the cDNA is integrated as provirus into host DNA in activated cells. Gene expression is normally immediate; first regulatory and then structural proteins are made, regulated by tat and rev proteins. New virus particles bud from the surface of infected cells about 24 h after infection. Virus is shed for a further day or so before the cell dies62.

Pathogenesis, immune response and disease. AIDS is insidious. HIV-1 infection often causes a transient acute febrile illness shortly after infection, when virus levels in the blood peak, but after that, infection is silent. A degree of immune control by CD8+ T cells is achieved, and neutralizing antibodies eventually appear, although too late to prevent infection, and are easily evaded by viral mutation. T cell immunity seems to control the infection over several years but with a dynamic process of immune selection and escape occurring63. CD4+ T cells are both deleted and functionally impaired early64, with HIV-specific T cells preferentially infected and depleted65. However, in some cells (e.g., long-lived memory T cells and macrophages), gene expression is delayed and the infection becomes latent. The progressive loss of CD4+ T cells leads to clinical immunodeficiency, which is manifested by opportunistic infections and, often, reactivation of tuberculosis. These infections rapidly cause death if untreated.

HIV infects CD4+ T cells and monocytes and macrophages. The virus is cytopathic in activated T cells, but less so in macrophages, and not at all in latently infected cells with integrated provirus. These different forms of infection present problems for vaccines. Those that stimulate and maintain high levels of neutralizing antibodies could prevent initial infection, but once that has occurred, it is almost impossible to eliminate HIV. Under prolonged antiretroviral drug therapy, virus is eliminated from the activated T cells that express the enzymes targeted by the drugs, but not from cells in which virus turns over slowly or is latent66. Thus if an HIV vaccine does not prevent infection, it is unlikely to eliminate the virus but may be able to control the ongoing infection and prevent disease.

There is no known protective immunity against HIV, in the sense that virus is never eradicated from those infected so that it is impossible to find people protected by previous infection, as is the case for many other viruses, such as measles, mumps and influenza. This poses serious problems for vaccine development, although it is not unique (Epstein-Barr virus is another such example). Adult monkeys infected with attenuated SIV (e.g., with deletions in nef) do not become sick, and are protected from challenge with pathogenic SIV67. This protection is probably, but not certainly, immunological68. Some highly HIV-exposed individuals resist HIV infection; others develop T cell responses to HIV69 but not serum antibodies, and their immunity depends on continuing exposure to the virus70. This may represent immune resistance, although it is hard to prove. Another clue may come from the rarity of superinfection in people infected with HIV who are repeatedly exposed to further infection. Although there are elegant studies of superinfection71, it is suspected that it is rare; if so, its rarity probably reflects immune protection.

The spread of HIV-1 infection around the world in 20 years has been alarming. The worst epidemics are in sub-Saharan Africa, where more than 50% of young adults are infected. Mortality from HIV infection without drug treatment is close to 100%, although infected people usually survive from 5 to 10 years before developing AIDS. The introduction of effective antiretroviral drugs in high-income countries has reduced mortality without reducing infection.

Virus variability. HIV is the most variable virus known. There are six major subtypes, A, BC, D, E, F and G, which are ‘ancient’ (in the HIV context of 30–50 years) and have distinct geographical distribution (Fig. 3)72. The complexity is even greater because of recombinations between subtypes and within subtypes. Each subtype differs by >20% in amino-acid sequence, so that T cells specific for one subtype are unlikely to cross-react substantially with the others; each epitope of 9–15
amino acids will probably have two or more mutations, and this level of variability is known to adversely affect T cell recognition. Although there are a few cross-reactive T cell epitopes, as well as conserved segments of HIV, it may be necessary to match any virus subtype with that of the circulating viruses in the population to be vaccinated. It is less certain that subtype-specific vaccines will be necessary for stimulating neutralizing antibodies, although sequence variability is known to be a major problem.

A further complication is the variability within each subtype and within each individual. It is clear that both antibodies and CD8+ T cells are very effective at selecting virus escape mutants. As human leukocyte antigen (HLA) type controls the epitopes recognized by T cells, it is not surprising that HLA type influences virus sequence in individuals as a consequence of T cell–driven escape. However, the mutant virus may be less fit and the escape can offer advantages to the host. The constraints on the virus probably account for the overall conservation of virus sequence in primary infection, before the immune response evolves. Therefore, vaccines that focus on conserved consensus sequences may have the best chance, at minimum selecting slightly less fit viruses during primary infection.

A T cell vaccine that permits infection and induces selection of escape mutants could undermine vaccine effectiveness, as has been seen in macaques. But selection of less fit virus has also been observed in macaques in whom SIV infection remains controlled, so this could favor the host.

**Vaccination strategies against AIDS**

**Challenges.** For HIV there are two major challenges: to find (i) immunogens that can stimulate broadly cross-reacting neutralizing antibodies and (ii) immunogens that stimulate high levels of persisting CD8+ and CD4+ T cells. Both humoral and cellular immunity may be needed, but they require different types of immunogens; eventually vaccines could be mixed to achieve both (Fig. 4).

A prophylactic vaccine will be given months or years before exposure and, hopefully, prevent or ameliorate infection. An antibody-inducing vaccine could prevent infection, but as indicated above, a vaccine that stimulates T cell immunity cannot stop virus infecting cells. The latter vaccination might enable the host to suppress the infection for long periods and prevent disease. Thus, macaques vaccinated to stimulate T cell immunity against SIV became infected when challenged with virus, but had very low virus loads and experienced little effect on their health as compared to unvaccinated control. Because it is almost impossible to eliminate HIV infection, this may be the best that can be achieved. However, many chronic virus infections as well as latent M. tuberculosis infection are controlled effectively in the long term by cellular immunity without causing disease. HIV-2 does not cause disease in more than 80% of those infected in West Africa, suggesting that the immune response and the virus are in balance in the infected but healthy individual.

**Antibody-stimulating vaccines.** At present there is no realistic candidate HIV vaccine. It was thought that gp120 preparations would stimulate neutralizing antibodies, and they do against tissue culture–adapted HIV strains. However, all such vaccines have failed to neutralize primary virus isolates and one tested in two large efficacy trials failed to protect (NAM, http://www.aidsmap.com/). Now the aim is to design HIV envelope protein immunogens that will stimulate protective antibodies. Unfortunately the dice are loaded against this: (i) the envelope is heavily glycosylated, making it largely nonimmunogenic; (ii) it is conformationally variable, so that the most sensitive site, the chemokine receptor binding site, is not exposed unless CD4 has bound; (iii) the CD4+ binding site is deeply recessed and hard to access by antibodies; (iv) besides the carbohydrates, crucial parts of the gp120 surface are protected by hypervariable loops, which can and do vary by mutation at no cost to the virus, thereby escaping neutralizing antibodies. Real novelty in immunogen design is needed to get around these problems.

**T cell–stimulating vaccines.** As an alternative, much effort is being made to create vaccines that stimulate T cell immunity, particularly CD8+ T cells. In mice, several approaches comprising plasmid DNA and various recombinant viruses and recombine bacteria have given promising results. Also palturate proteins and peptide-pulsed DCs can efficiently stimulate CD8+ T cells. But these findings are not easily transferable to primates. DNA vaccines stimulate only weak responses in humans and recombinant viruses give mixed results. The poxviruses (canary pox, the attenuated vaccinia virus (NYVAC) and MVA) induce weak primary responses, possibly because the recombinant immunogen is swamped by more than 200 poxvirus proteins. Also, these vaccines may be too attenuated, with little or no proliferation after infection. However, they may be better at boosting T cell responses that have already been primed (e.g., BCG; Table 1). The most promising results to date come from recombinant adeno virus 5, which has stimulated strong and sustained T cell responses, but its applicability is constrained by the high frequency of antibodies to this virus in most human populations. Alternate candidates in trial include other strains of adenovirus, adeno-associated virus, alphaviruses and r-BCG as vectors for HIV.

As for tuberculosis, heterologous prime-boost regimes offer enhanced T cell immune responses and have been observed in macaques and humans. It seems that in humans, recombinant adenoviruses are good at both priming and boosting, whereas recombinant poxviruses such as MVA are better at boosting than priming (McMichael, A.J, Goonetilleke, N., Guimaeres-Walker, A., Dorrell, L., Yan, H., Hanke, T., unpublished data).

Vaccine-induced T cell responses should include both CD4+ and CD8+ T cells, even though the former are targets for the virus. CD4+ T cell help is important for optimal CD8+ T cell responses—an issue of particular importance to HIV vaccine development because natural infection results in impaired CD4+ T cell immunity.

Protein immunogens typically elicit antibody responses and T helper cell responses, but the latter may be of the Th2 type, especially when alum is used as an adjuvant; Th2 responses have little or no antiviral activity. Live intracellular infections generally elicit Th1 responses and CD8+ T cell responses, hence the use of live attenuated vectors to mimic a virus infection. These deliver the required immunogen into the cytosol, from which it can enter the MHC class I antigen presentation pathway directly, or indirectly when the cell dies and virus protein is taken up by DCs for ‘cross-priming’ of CD8+ T cells.

People who are homozygous for the δ32 variant of CCR5 do not express an intact chemokine receptor and are resistant to HIV infection. Immune resistance is suspected for people who are highly exposed, yet uninfected. Many generate CD8+ and/or CD4+ T cell responses, but without any serum antibody. It is not certain that the T cell responses are responsible for resistance. If they are, it is encouraging because the levels of T cell response seen in current vaccine trials could be sufficient.

**HIV vaccines and mucosal immunity**

HIV enters the host through (usually genital) mucosa. HIV infects, or attaches to, mucosal Langerhans cells first and then migrates to local lymphoid sites to set up systemic infection with a predominance of early virus in intestinal tract CD4+ T cells. Mucosal IgA and T cells may have an important role in control of this infection.
An HIV vaccine should stimulate B and T cell as well as mucosal and innate immunity. Live attenuated HIV probably provokes all of these, but because it will set up a persistent infection, there are insoluble safety issues that render its use unlikely in humans. Therefore, it may be necessary to build a set of vaccines to stimulate each component separately and administer them in combination.

Degree of protection

If control equivalent to that of chronic Epstein-Barr virus or cytomegalovirus or latent M. tuberculosis infection could be achieved for HIV, with reduced or negligible further transmission, it would represent a considerable advance. Measuring the efficacy of the vaccine would be problematic, being dependent on reduced virus load at set point, the time at around 6 months after primary infection when virus level stabilizes. The set-point level is inversely correlated with time of survival. Only neutralizing antibodies, stimulated by vaccines, offer any real chance of sterile immunity and such vaccines are a long way off.

A prophylactic vaccine for HIV is badly needed. Unless current trials of vaginal microbicides are unexpectedly successful, there is no other way of controlling the infection and both antibody- and T cell–inducing vaccines need a total rethink. The most advanced of these vaccines is the Merck recombinant adenovirus 5 (Ad5) vaccine that is about to enter clinical trials. But even if successful, a different vector will be needed to deal with the problem of pre-existing antibody immunity to Ad5. The Merck trial may, however, indicate whether vaccines that stimulate T cell immunity offer any benefit, and that type of news is to be welcomed. Could AIDS and tuberculosis vaccines be given together? Probably, but this may be decades away. In parts of Africa, BCG is given at birth. Combining BCG with an AIDS vaccine, with a boost at puberty, may be feasible in the distant future.

From animal models to clinical trials

Preclinical studies: tuberculosis and AIDS vaccine testing. Most tuberculosis vaccine candidates have been tested in mice or guinea pigs. Studies in mice take advantage of the in-depth knowledge of the mouse immune system, whereas guinea pigs have the advantage of developing a pathology that highly resembles human tuberculosis. These experimental animal studies form the starting point of the tedious pipeline leading to a new vaccine candidate. The US National Institutes of Health, through their preclinical tuberculosis screening program, and the European Union, through its TBVac integrated project in the Framework Program 6, sponsor vaccine testing in experimental animals under standardized conditions. Thus far, the two programs utilize different screening procedures, which need to be harmonized in order to achieve comparability of different vaccine candidates. However animal models cannot unequivocally predict the efficacy of a vaccine candidate against tuberculosis or HIV and immunogenicity results in mice often do not predict responses in humans. Although non-human primate studies can be bypassed before phase 1 and phase 2 clinical trials, they may become necessary before embarking on phase 3 trials.

Phase 1 and phase 2 clinical trials should also be exploited for determination of immunological correlates of protection (i.e., markers that unequivocally predict protective efficacy of a vaccine candidate). Currently, IFN-γ produced by T cells with specificity for defined antigens is probably the best marker of protection for tuberculosis, but this is much less clear for HIV. In the most impressive vaccine protection studies, using attenuated SIV as a vaccine it is not known what confers protection and the IFN-γ ELISPOT assay for T cells does not correlate with protection in vaccine-protected macaques. Additional markers are required.

The SIV challenge model in macaques has been very useful for HIV vaccine development, but there are limitations. In these experiments, the virus challenge dose is always large, compared to repeated low-dose exposures in humans. In addition, the vaccine is nearly always homologous to the challenge virus, giving good chances of success that are unrealistic in humans. Attempts are being made to introduce more representative challenges. Finally the SIV or SIV-HIV hybrid (SHIV) challenges may be slightly misleading; it seems easier to protect against the aggressive hybrid virus SHIV 89.6P compared to the less virulent SIV mac 239. It is unclear which will be closer to an HIV exposure in humans. Despite these problems, the existing data suggest vaccine-induced protection is possible.

Therapeutic vaccination for HIV is rarely discussed, but must be considered as recent studies in macaques and humans give some credibility. In humans, therapeutic vaccination would probably be used to stimulate T cell responses in HIV-infected people whose virus was well controlled by antiretroviral drugs, with the aim of terminating antiretroviral therapy (ART) once the T cell levels were boosted. The rationale is that T cells, particularly CD8+ T cells, are known to be effective in long-term control of the virus in the absence of drug treatment, but T cell responses decline in patients on effective ART. When ART is stopped, virus rebounds to pretreatment levels. If the T cell levels were already boosted, this might lead to lower virus levels at the new set point. At its best, this treatment could enable withdrawal of ART for prolonged periods and offer new options in low-income countries in particular. Patients whose virus was controlled in this way might also be less likely to transmit virus.

Clinical trials. The earliest vaccine trials are small and are concerned with vaccine safety and immunogenicity. Then immunogenicity is optimized by finding the best vaccine dose and route of delivery. Finally, the vaccine efficacy trials involve several thousand volunteers who are at high risk for infection. Translation of vaccine candidates from bench science into clinical trials is relatively straightforward scientifically but complicated logistically and extremely expensive (Box 1).

Phase 1 trials can be done in the country of origin of the vaccine, but increasingly good sites are being established in developing countries and several countries have HIV vaccine trial experience. In contrast, experience with tuberculosis vaccine trials is marginal and currently only a few phase 1 trials have been initiated. Two phase 3 trials of Vaxgen gp120 in 10,000 people showed clearly that the AIDS vaccine candidate offered no protection. A phase 2b trial has now been proposed before
of vaccines against tuberculosis and AIDS will be most successful as joint public-private enterprise with support from governmental and nongovernmental funding organizations and philanthropic foundations. Such a consortium would be best equipped to bring forward vaccine development by a combination of ‘push’ and ‘pull’ programs. Although support for preclinical research will be best directed by classical push programs, incentives should be made available for alternative approaches. The removal of roadblocks serves as a good example for a support strategy emphasizing innovation and not restrictive in the experimental approach. Unconventional research areas, such as therapeutic HIV-1 and tuberculosis vaccination, could be further stimulated by pull programs rewarding a vaccine candidate that proves successful.

**BOX 1 REGULATORY ISSUES FOR VACCINE TRIALS**

- Intellectual property. For regulatory and manufacturing reasons, vaccines that go forward into trials must be protected as intellectual property. This ensures that sponsors, indemnifiers, GMP producers, etc. know what they are dealing with and that others will not be working unlicensed with the same product.
- GMP production. Preparation of a vaccine to ‘Good Manufacturing Practice’ quality is essential for the regulators and ensures that each batch is identical and can be tracked. This is expensive (e.g., $100,000–200,000 for MVA) and requires outsourcing. The manufacturer provides detailed documentation of processes and purity.
- Toxicity testing. This is required before submission to the US Food and Drug Administration (FDA) or the European Medicines Agency (EMEA). Normally this requires acute toxicity and studies of distribution and persistence of vaccine at distant and critical sites after immunization. Dose schedules should be the same as used for the trial, even though the animals are smaller and a detailed report is needed.
- Regulatory submission to the national or international authority (FDA, EMEA, Medicines and Healthcare Products Regulatory Agency, etc.). These follow standard procedures but require very detailed information including full protocols, standard operating procedures of assays, details of safety monitoring, definition of endpoints, statistical calculations, details of data handling.
- Ethical approval. This connects to regulatory submission. Besides all protocols, details of informed consent, explanation to volunteers, details of volunteer recruiting and letters to family doctor explaining the trials are required.
- Other approvals may be needed from the Gene Therapy Advisory Committee (in the UK) and local and national safety committees for handling recombinant organisms.

**Figure 4** The major components of the immune response can be stimulated by different types of vaccine.
in preclinical studies. At the transition from the preclinical to the clinical phase, pull programs providing incentives for the market-ready final product would be most appropriate\(^1\). These include tax reduction for vaccine production, secured future markets in developing countries, a tiered price system, and reduced interest rates or debt release by the World Bank for vaccine purchase and distribution. In the absence of such incentives, even the best vaccine candidate may fail to reach the desired goal of unrestricted distribution in countries affected most by AIDS and tuberculosis.

Lack of financial incentive may provide some unique opportunities for vaccine development against AIDS and tuberculosis. Notably, low-to-absent competition for financial profits can promote comparative clinical trials under the umbrella of governmental, nongovernmental or philanthropic organizations either alone or together with the aim first to select the most promising candidates, and second to proceed by "learning by doing" (i.e., continuous vaccine improvement of candidates in iterative clinical trial processes). Analysis of the immune responses of vaccine trial participants may provide new information that can be further explored in experimental animal models and help to define the next clinical trial step. Strong efforts are required to harmonize vaccine trials and accompanying vaccine efficacy testing. It is rewarding that several major organizations including the US National Institutes of Health though their intramural and extramural programs, the Bill and Melinda Gates Foundation through Aeras for tuberculosis and their Vaccine Enterprise for AIDS as well as the European Union through their European & Developing Countries Clinical Trials Partnerships program have committed themselves to bring vaccines against AIDS and tuberculosis from the bench to the field.

Vaccination strategies against these two diseases need to be integrated as soon as possible, considering the intimate interdependence of the two deadly pathogens and the consequences of their liaison. Obviously, numerous hurdles need to be overcome. Yet, even if only partially protective vaccines can be developed, return on investment will be enormous. This is not only true for the most impoverished countries, for which a rapid solution is vital, but also for industrialized countries, which may suffer significantly from global economic and social regressions and further weakening of unstable states.

ACKNOWLEDGMENTS
S.H.E. Kaufmann acknowledges financial support for his work on tuberculosis vaccination from: the Fonds Chemie, EU FP6 TB-VA, EU FP6 MUVAPRED, Bundesministerium für Bildung und Forschung Competence Network "Structural Genomics of M. tuberculosis", BBME joint project "Genomics of M. tuberculosis", EU project "X-TB", Deutsche Forschungsgemeinschaft Priority Program "Novel Vaccination from: the Fonds Chemie, EU FP6 TB-VA, EU FP6 MUVAPRED, S.H.E. Kaufmann acknowledges financial support for his work on tuberculosis vaccination from: the Fonds Chemie, EU FP6 TB-VA, EU FP6 MUVAPRED, Bundesministerium für Bildung und Forschung Competence Network "Structural Genomics of M. tuberculosis", BBME joint project "Genomics of M. tuberculosis", EU project "X-TB", Deutsche Forschungsgemeinschaft Priority Program "Novel Vaccination Strategies". A.J. McMichael acknowledges grant support from the UK Medical Research Council and the International AIDS Vaccine Initiative. We thank A. von Gabain, J. Cohen, H. McShane, P. Anderssen, T. Ottenhoff, M. Klein, T. Hanke and L. Castello-Branco for discussions, T. Walker and A. Ozin for reading this manuscript, D. Schad for graphical work and S. Sibaei for secretarial help.
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CORRIGENDUM: Annulling a dangerous liaison: vaccination strategies against AIDS and tuberculosis
Stefan H E Kaufmann & Andrew J McMichael

Nat. Med. 11 Suppl 4, S33–S44

The sentence “In South Africa, more than 10% of the 40 million inhabitants are infected with HIV and more than 5% suffer from active tuberculosis” should read “more than 0.5% suffer from active tuberculosis.”

In Table 2, line 3 should read “Clinical phase 1 trial in BCG-vaccinated and unvaccinated healthy uninfected volunteers completed”.

CORRIGENDUM: Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia
Takuya Matsunaga, Naofumi Takemoto, Tsutomu Sato, Rishu Takimoto, Ikuta Tanaka, Akihito Fujimi, Takehide Akiyama, Hiroyuki Kuroda, Yutaka Kawano, Masayoshi Kobune, Junji Kato, Yasuo Hirayama, Sumio Sakamaki, Kyuhei Kohda, Kensuke Miyake & Yoshiro Niitsu

Nat. Med. 9, 1158–1165 (2003)

Two sentences in this article appeared incorrectly. On page 1164, in the eighth paragraph, the third sentence should read “To the U937-transplanted SCID mice, 1 mg of either SG/17, YN907 or vehicle was injected intravenously, and 20 mg of either AraC or normal saline was injected intraperitoneally on day 7 of transplantation.” On page 1164, in the eighth paragraph, the fourth sentence should read “To NOD-SCID mice transplanted with patients’ leukemic cells, 2 mg of either SG/17 or YN907 was injected intravenously, and 40 mg of either AraC or normal saline was injected intraperitoneally on days 3 and 4 of transplantation.”