Tuberculosis

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Tuberculosis is still a leading cause of death in low-income and middle-income countries, especially those of sub-Saharan Africa where tuberculosis is an epidemic because of the increased susceptibility conferred by HIV infection. The effectiveness of the Bacille Calmette Guérin (BCG) vaccine is partial, and that of treatment of latent tuberculosis is unclear in high-incidence settings. The routine diagnostic methods that are used in many parts of the world are still very similar to those used 100 years ago. Multidrug treatment, within the context of structured, directly observed therapy, is a cost-effective control strategy. Nevertheless, the duration of treatment needed reduces its effectiveness, as does the emergence of multidrug-resistant and extensively drug-resistant disease; the latter has recently become widespread. The rapid expansion of basic, clinical, and operational research, in addition to increasing knowledge of tuberculosis, is providing new diagnostic, treatment, and preventive measures. The challenge is to apply these advances to the populations most at risk. The development of a comprehensive worldwide plan to stop tuberculosis might facilitate this process by coordinating the work of health agencies. However, massive effort, political will, and resources are needed for this plan to succeed.

Introduction

Tuberculosis has troubled humankind throughout history. It has been a leading cause of death throughout the world, and still is in low-income and middle-income countries. The limitations of existing methods of prevention, diagnosis, and treatment of tuberculosis have been emphasised by the increased susceptibility of HIV-infected people to develop the disease, and by the emergence of drug-resistant strains. Overall, the worldwide burden of tuberculosis is still growing. Improvement in the control of the disease in many regions of the world is offset by the effect of HIV in the resource-poor health systems of sub-Saharan Africa. The challenge is to apply advances to the populations most at risk.

Epidemiology

The most recent estimates of the worldwide epidemic of tuberculosis are for 2004, when there were 8·9 million new cases and 1·7 million deaths.[2] The worldwide annual incidence continues to increase in Africa because of the HIV epidemic, whereas it is stable or falling in all other regions. Figure 1 shows the worldwide estimated incidence of tuberculosis.

The devastating association between HIV and tuberculosis in sub-Saharan Africa has been previously reviewed in *The Lancet*.[3] The risk of tuberculosis increases shortly after HIV seroconversion, doubling within the first year.[4] The annual incidence of tuberculosis is about 10% in HIV-infected individuals from high-burden communities in both industrialised and developing countries, with reported rates of 7·6 per 100 person years in US users of intravenous drugs who are tuberculin skin test (TST) positive,[5] and 10·4 per 100 person years in South Africans of unknown TST status.[6] This risk increases further with serious immunosuppression; an annual incidence as high as 30% has been reported in South African patients with clinically advanced HIV.[6] HIV infection predisposes to the reactivation of latent tuberculosis,[5] which is the basis for the provision of preventive therapy in HIV-infected individuals at risk.[7] HIV infection is also strongly associated with the transmission of tuberculosis between adults in sub-Saharan Africa.[8] High transmission rates of tuberculosis cause large numbers of children to be infected, which is concerning not least because of the rapid disease progression and the difficulties with diagnosis in this group.[1]

Multidrug-resistant tuberculosis is defined as resistance to rifampicin and isoniazid, with or without other drug resistance. Treatment for multidrug-resistant tuberculosis is longlasting, less effective, costly, and poorly tolerated. Estimates are that more than 4% of patients with tuberculosis worldwide are multidrug resistant, with more than 40% of these patients having been previously treated for tuberculosis.[9] Eastern Europe has the highest prevalence: multidrug-resistant tuberculosis is found in about 10% and 40% of new and previously treated patients, respectively.[9]

Extensively drug-resistant (XDR) tuberculosis is, by definition, resistant to at least rifampicin and isoniazid, in addition to any quinolone and at least one injectable second-line agent (capreomycin, amikacin, kanamycin).

Search strategy and selection criteria

We focused on tuberculosis in adults; readers are referred to a review of tuberculosis in children.[1] The search strategy was a 5 year review of PubMed (2001–2006), the Cochrane library (2001–2006), and Embase (2001–2006). We searched with the terms "tuberculosis" and "*Mycobacterium tuberculosis*". To compile table 2, we searched with the terms "tuberculosis", "human", "genetic", and "susceptibility". We mainly selected publications in the past 5 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy, and selected the ones we regarded as relevant. Review articles are cited to provide readers with more details and references than this Seminar can accommodate. Our reference list was modified on the basis of comments from peer reviewers.

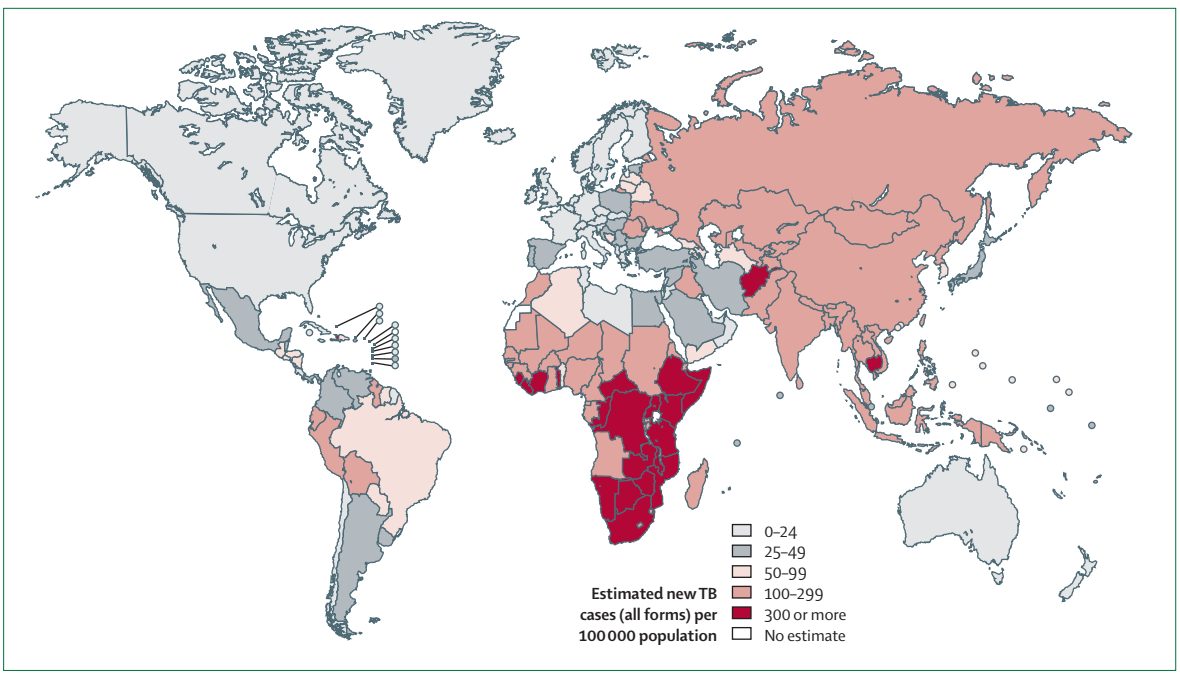

Figure 1: **Worldwide incidence of tuberculosis**
TB=tuberculosis. Source WHO.[2]

In one outbreak of tuberculosis in HIV-infected patients, XDR patients constituted 24% of all multidrug-resistant individuals.[10] This level of resistance makes tuberculosis essentially untreatable, and 52 of 53 patients with tuberculosis died after a median of only 16 days.[10] In 85% of patients with XDR tuberculosis in this study, the strain of *Mycobacterium tuberculosis* had the same genetic background, indicating recent transmission, and 67% had been admitted within the previous 2 years, raising the possibility of nosocomial transmission. This outbreak emphasises the need for new antimycobacterial drugs, increased surveillance, and caution in hospitals when nursing patients with suspected multidrug-resistant tuberculosis.[11] In the absence of satisfactory practices to ensure adherence to medication, drug resistance will continue to emerge.

Host–pathogen interaction

A complex interaction exists between host and pathogen that can last for decades. One paradox of tuberculosis is that the pathogen resides and multiplies within macrophages. The availability of genome sequences of mycobacteria,[12–15] the ability to delete and reintroduce genes into mycobacteria reliably, and the advent of microarray technology have moved *M tuberculosis* to the forefront of bacterial genomics. A mechanism by which *M tuberculosis* has evolved is via the loss or duplication of genomic segments. Clinical isolates of this organism have up to 5·5% of their genes deleted.[16] *M leprae* manifests such reductive evolution even more strikingly; less than 50% of its genes are functional.[15] Some deletions in the genes of *M tuberculosis* seem to have only happened once, and thus provide useful phylogenetic markers.[17] This information, together with the sequence of *M bovis*, has allowed us to explore the relationship between *M tuberculosis* and humankind in history. The origin of tuberculosis in human beings was thought to have taken place via the domestication of cattle, suggesting that *M bovis* was the progenitor of *M tuberculosis*. However, *M tuberculosis* and *M bovis* share a common ancestor, and thus *M bovis* did not give rise to *M tuberculosis*.[18,19] Additionally, the analysis of unique sequence deletions, in 875 strains from 80 countries has indicated that *M tuberculosis* arose and migrated together with human beings from Africa.[20] Remarkably, evidence exists that six specific lineages of *M tuberculosis* have adapted to specific populations. Thus, for example, the east African–Asian lineage appears most commonly in individuals of Indian origin, even when they migrate to the UK or USA.[21]

One lineage of tuberculosis that seems to buck the trend is the east Asian lineage, which is more commonly known as the W/Beijing family of strains; it is successful worldwide, and possibly increasing in frequency.[22] W/Beijing strains predominate in southeast Asia, but are widely distributed in the Indian subcontinent and in South Africa.[23,24] They have also been associated with several outbreaks of drug-sensitive and drug-resistant tuberculosis in the USA and Europe.[25–27] These strains have been believed to be hypermutable, as a consequence of mutations in DNA-repair genes of the *mut* (methylmalonyl coenzyme A mutase) family,[28] although this observation

has been challenged.[29] Additionally, W/Beijing strains have been found to have greater virulence than other strains, in both human beings and animal models. One mechanism that underlies the greater virulence seems to be the production of an immunosuppressive phenolic glycolipid in the presence of an intact polyketide synthase 15/1 (*pks 15/1*) gene, which is a genetic feature of W/Beijing strains.[30,31] In another lineage (east African–Indian), the deletion that characterises the lineage has been associated with an immune-subverting phenotype that potentially increases the ability of this strain to persist and cause outbreaks in populations.[32]

Several components of the mycobacterial cell wall have immunomodulatory activity, including phenolic glycolipid, phosphatidylinositol mannosides, lipoarabinomannan, and lipoproteins.[33] These molecules are recognised by the Toll-like receptors (TLRs) and other innate receptors on macrophages and dendritic cells, which trigger both protective and pathogenic immune responses.[34] The combination of TLR2 and TLR1 recognises phosphatidylinositol mannosides and the 19-kDa lipoprotein in the cell wall.[35–37] In combination with TLR2, TLR9 also contributes to the best possible host resistance.[38] The role of TLR4 in tuberculosis is controversial, with some studies of knock-out mice showing that TLR4 is very important in tuberculosis,[39,40] but others indicating little effect.[41,42] Ligation of TLR2 and TLR1 by mycobacterial cell wall components promotes antimycobacterial immunity, although the final effector pathway remains unclear. In mice, the key intracellular pathway seems to be activation of inducible nitric oxide synthase and p47 GTPase by the cytokine interferon γ, which is produced by T cells.[43] Whether nitric oxide contributes to the intracellular defence against mycobacteria in man is still unclear. TLR2 and TLR1 ligation in mononuclear phagocytes activates 1-α hydroxylase, which converts 25-hydroxyvitamin D3 into 1,25 dihydroxyvitamin D3.[44] Activated vitamin D has pleiotropic immune effects, including the induction of antimicrobial peptides such as the cathelicidin LL-37.[45] Deficiency of vitamin D is associated with tuberculosis in immigrants to the UK.[46] Overall, these findings suggest not only a novel mechanism of intracellular killing, but also the possibility of prevention of tuberculosis by vitamin D supplementation.

Mice have 23 genes encoding immunity-related GTPases, which are subdivided into five families (*Irga*, *Irgb*, *Irgc*, *Irgd*, and *Irgm*). Only two homologues exist in human beings, *IRGC* and *IRGM*.[47] A report suggested that the activity of IRGM is associated with autophagy,[48] which is a cellular homoeostatic mechanism whereby cytoplasmic remnants and bacteria are taken into the endoplasmic reticulum, with consequent death of intracellular mycobacteria.[49] Other novel regulatory and effector pathways of the host defence against mycobacteria include the uptake of apoptotic macrophages by dendritic cells, thereby enabling efficient T-cell recognition of tuberculosis antigens present in macrophages at the surface of dendritic cells,[50] and the uptake of apoptotic neutrophils by macrophages, after which potent antimicrobial peptides are released from the neutrophils into the macrophage vacuoles that contain the mycobacteria.[51] Thus, cells that eat other cells, or themselves, seem to be a way of dealing with this unwanted intruder.

Potent defence mechanisms need induction and regulation. Knowledge of essential protective immune regulatory cytokines has come from the analysis of rare mutations that confer susceptibility to mycobacteria, and serendipitously by postmarketing surveillance of biological therapies for autoimmune disease. In a remarkable series of studies done during the late 1990s, mutations in the interleukin-12- and interferon-γ-driven type-1 cytokine pathway proved to predispose to severe atypical mycobacterial infections (table 1).

These studies were coincident with an increased interest in the host genetic determinants of susceptibility. A moderate genetic component in susceptibility to tuberculosis exists,[58] and several studies have examined it. Three whole-genome-based approaches have yielded moderate linkage to various chromosomal regions, which have been different in each population studied.[59–61] Case–control studies are better suited to detect weak effects, and various associations have now been described. Table 2 lists some of the associations that have been replicated in more than one study, together with some studies that show novel pathways that might be implicated in pathogenesis.

The host genetic component of susceptibility seems to be distributed in many genes, and the genes implicated seem to vary between populations, which is consistent with the emerging evidence that *M tuberculosis* itself might be population specific. Integrated studies of host and pathogen genetic variability are desirable; such studies will need to be very large, and thus expensive.

The immune control of tuberculosis has also been emphasised by the association between biological therapies that neutralise tumour necrosis factor and the rapid reactivation of tuberculosis.[111,112] These observations

	Mutation	Phenotype	Reference
Interferon γ receptor 1	Point mutation at nucleotide 395, which introduces a stop codon	Severe atypical mycobacterial infection	52
Interferon γ receptor 2	Homozygous dinucleotide deletion at nucleotides 278 and 279, resulting in a premature stop codon	Infection of *M fortuitum* and *M avium*	53
Interleukin 12p40	Large homozygous deletion	BCG infection	54
Interleukin 12β1 receptor subunit	Various missense mutations and deletions	Severe mycobacterial and salmonella infections	55,56
STAT1	Point mutation at nucleotide 2116	Disseminated BCG or *M avium* infection	57

STAT1=signal transducer and activator of transcription 1. BCG=Bacille Calmette Guérin.

Table 1: **Mendelian susceptibility to mycobacteria**

have confirmed the crucial role of tumour necrosis factor in protective granuloma formation.[113] Less certainty exists about the key factors in the downregulation of the immune response, which is not only needed to restrict immunopathological changes, but also might be exploited by the pathogen to subvert the immune response.[32] Factors that might be implicated include T-cell apoptosis,[114] the action of regulatory T cells,[115] or type-2 T-helper cells opposing the effects of type-1 cells.[116] Type-2 T-helper cells produde interleukin 4, but appreciable secretion of this cytokine in response to *M tuberculosis* antigens has not frequently been shown.[117] However, interleukin 4 is highly active even in small amounts, and its antagonistic splice variant (interleukin 4 delta 2) is associated with protection in several studies.[118,119]

How does *M tuberculosis* survive for so long in such an immune barrage? Two large-scale studies that used microarray screening of mutated mycobacteria after passage in mice and cells give some insight. In the first analysis, Sassetti and Rubin[120] mutated almost every non-essential gene of *M tuberculosis*, and reported that 194 genes are needed for growth in mice. With similar genetic techniques, Stewart and colleagues[121] examined the effect of a random mutation in BCG on intracellular growth and on the ability to resist phagosomal acidification, a key aspect of early immune evasion in slow-growing mycobacteria.[122] Although the studies differed in some conclusions, several striking similarities exist. First, both studies identified membrane-associated proteins as important, especially those of the mce (mycobacterial cell entry) operon, which had previously been ascribed a role in virulence.[123] Second, small-molecule transporters—including those used in ion, aminoacid, and disaccharide transport—are important early in the infection to bacterial replication. Thus, the rapid aerobic growth of mycobacteria early in infection seems to be carbohydrate dependent. Later in infection and during latency, bacilli can switch to using lipids as a source of energy.[124] Both studies also found that kefB, a K^+ efflux channel, protects bacilli against electrophile toxic effects by lowering intracellular pH. These studies, therefore, not only shed light on pathogenesis, but also on potential new drug targets.

Diagnosis

Bacteriological diagnosis of tuberculosis continues to rely on the detection of acid fast bacilli on microscopic examination and on culture. In tuberculous meningitis, where conventional smear diagnosis has a low yield, a study[125] that is more than 50 years old showed that yields approaching 50% can be obtained by centrifugation of 5–10 mL of cerebrospinal fluid followed by longlasting microscopic examination. This simple technique is seldom used. Fluorescent microscopy is faster and more sensitive than are conventional carbolfuchsin methods,[126] but is not sensitive or widely available in resource-limited settings. Liquid culture in automated systems has considerably shortened the time and labour required for positive culture; however, this technique is expensive. An affordable, rapid diagnostic test that has better sensitivity than smear examination is highly desirable, but remains elusive. Nevertheless, substantial advances have been made, notably in the diagnosis of latent infection and the rapid diagnosis of drug resistance.

Many studies have assessed the value of in-house nucleic-acid amplification tests for the diagnosis of tuberculosis, but the absence of reproducibility makes the assessment of these tests difficult. However, several commercial nucleic-acid amplification tests for tuberculosis are available. These tests generally have high specificity. Sensitivity is high in smear-positive sputum, where tests have little value other than confirming that the acid-fast bacilli are *M tuberculosis*.[127] However, their sensitivity in sputum that is smear negative or in extrapulmonary specimens is moderate (table 3).[128–130] Moreover, a Vietnamese study reported that smear examination with the technique from the study cited above[125] was at least as sensitive as the nucleic-acid amplification test in cerebrospinal fluid.[131] Thus, the diagnostic role of

	Effect (location of study)
HLA DR2	Associated with pulmonary disease (India and Indonesia);[62-66] no association (India, Mexico, Hong Kong)[67-69]
IL-1 complex (*IL-1Rα A2* and *IL-1β(+3953) A1*); *IL-1β(+3953) A1*	Increased DTH and pleural disease;[70] Protection[71]
IL-10 (promoter variants)	Susceptibility (Hong Kong, Korea, Cambodia)[72-74]
MCP1 (AG/GG promoter genotype)	Susceptibility (Mexico)[75]
Interferon γ (+874 AA genotype)	Increased susceptibility to tuberculosis (South Africa, Spain, Croatia, Hong Kong)[72,76-78]
Interferon γ receptor 1; various; −56T/C	No association (Gambia, Croatia);[79,80] Susceptibility (West Africa)[81]
DC-SIGN (−871G and −336A promoter variants)	Increased susceptibility[82]
IL-12B1 (various)	Increased susceptibility (Japan, Morocco, Hong Kong);[83,84] no association (China, USA)[85,86]
Vitamin D receptor; vitamin D receptor tt or Tt genotype; ff genotype	Resistance; decreased time to sputum clearance;[87,88] resistance[46,89]
Toll-like receptor 2 (various)	Overall susceptibility (Turkey, Tunisia)[90,91]
TIRAP (C558T)	Susceptibility to tuberculous meningitis (Vietnam)[37]
CR1 (Q1022H)	Susceptibility (Malawi)[92]
Haptoglobin (2-2)	Increased severity of pulmonary tuberculosis[93-95]
MBL (mutations associated with low serum)	Protective (Spain, South Africa, USA, Denmark);[96-99] no association (Gambia)[100]
SLC11A1 (various)	Susceptibility (Gambia, China, Cambodia, Denmark, Guinea Bissau, USA, South Africa, Korea, Malawi)[73,89,92,101-107]
SP110 (various SNPs)	Susceptibility (west Africa)[108]
PTPN22 (R620W)	Susceptibility (Spain)[109]
Surfactant protein A (various)	Susceptibility (Ethiopia)[110]

Studies of less than 100 patients and controls were omitted. Negative associations are not shown except in selected instances. IL=interleukin. IL-1Ra=interleukin-1 receptor antagonist. DTH=delayed type hypersensitivity. MCP1=monocyte chemotactic protein-1. DC-SIGN=dendritic cell-specific ICAM-grabbing non-integrin. TIRAP=toll-interleukin 1 receptor (TIR) domain containing adaptor protein. CR1=complement component (3b/4b) receptor 1. MBL=mannose-binding lectin. SLC11A1=solute carrier family 11, member 1. SP110=SP110 nuclear body protein. SNPs=single nucleotide polymorphisms. PTPN22=protein tyrosine phosphatase, non-receptor type 22.

Table 2: **Polymorphic genes associated with tuberculosis**

	Sensitivity	Specificity	DOR	Reference
Sputum	66%	82%	16·6 (8·4–32·6)	128
CSF*	56% (46–66)	98% (97–99)	96·4 (42·8–217·3)	129
Pleural fluid*	62% (43–77)	98% (96–98)	80·9 (34·4–190·4)	130

Figures in parentheses are 95% CI. CSF=cerebrospinal fluid. DOR=diagnostic odds ratio. *Only commercial assays were tested.

Table 3: **Meta-analyses of nucleic acid amplification tests for diagnosing sputum smear negative and extrapulmonary tuberculosis**

nucleic-acid amplification tests in smear-negative sputum or extrapulmonary disease is limited by their moderate sensitivity—so that cultures usually still need to be done. Furthermore, the cost of nucleic-acid amplification tests is too high for routine use in developing countries.

The attenuating deletion that defines BCG (designated region of difference 1, *RD1*) contains two highly antigenic proteins, the 6-kDa early secretory antigenic target (ESAT-6) and culture filtrate protein-10 (CFP-10).[132–134] The fact that these antigens are largely restricted to the *M tuberculosis* complex, and their ability to stimulate T cells, form the basis for novel assays that assess the presence of tuberculosis infection, by detection of the release of interferon γ by T cells in response to these antigens in vitro.[135] The limitations of the TST are well recognised; false positives occur because the purified protein derivative contains many antigens that are present in BCG and non-pathogenic mycobacteria, and false negatives occur in immunocompromised patients, early in primary tuberculosis, and in disseminated tuberculosis.

In three studies, Lalvani and colleagues[136–138] showed that ESAT-6-based and CFP-10-based enzyme-linked immunospot (ELISpot analysis) is about 90% sensitive for active tuberculosis and more specific than the TST in BCG-vaccinated individuals, correlates better than the TST with exposure to a point source of infection, and seems less compromised than the TST by the presence of HIV infection.[138] Although the test cannot differentiate active from latent infection, in some clinical circumstances (for instance in children, or when cells from pleural fluid are assayed) knowledge that *M tuberculosis* infection is present can aid the diagnosis of active disease.[138,139] This knowledge has led to the development and marketing of the commercially available T-SPOT.TB test (Oxford Immunotec, UK) for tuberculosis infection. Two other commercial tests based on the same principle exist: QuantiFERON TB Gold (QFG) and QuantiFERON TB Gold in tube (QFGIT, both made by Cellestis, Carnegie, Australia). QFG has been extensively assessed in immunocompetent adults,[135] in whom sensitivity and specificity seem similar to those of the T-SPOT.TB test. Preliminary reports showing that the sensitivity of interferon γ release assay is impaired by immunosuppression[140,141] are not in agreement with a recent analysis suggesting that these tests might have an important role in the identification of HIV-infected people at risk of developing active tuberculosis.[142] The QFGIT test has the advantage of in-tube incubation of whole blood without the need for a CO_2 incubator, but studies are so far too few to draw definitive conclusions. More detailed assessment of all these tests in high-incidence settings, people infected with HIV, and children is needed. Also, whether these tests are more accurate than the TST at predicting the risk of subsequent tuberculosis needs to be established; if this is true, these tests would allow more accurate prescription of preventive therapy against tuberculosis.

The diagnosis of drug resistance by conventional methods takes 6–8 weeks, or even longer if solid media are used. However, the microscopic examination of growth in wells that are filled with liquid culture medium, with or without the addition of drugs, enables the rapid (within 10 days) detection of drug resistance.[143,144] This technique is potentially applicable in resource-limited settings, but is labour intensive.

Resistance to rifampicin is almost invariably a marker for multidrug resistance, and several techniques are available to detect it rapidly. Rapid detection of rifampicin resistance by molecular methods (line probe assay) is sensitive and specific on culture-positive isolates.[145] Although sensitivity is lower in clinical specimens,[145] line probe assays are useful for the early detection of multidrug-resistant tuberculosis, enabling early initiation of therapy and appropriate infection control measures. Bacteriophage assays, in which the uptake of bacteriophages into mycobacteria is used as an index of mycobacterial growth, have similar performance characteristics, but specificity is variable.[146]

When tuberculosis and HIV are comorbid, tuberculosis is often sputum-smear negative. Resource-poor settings have restricted access to mycobacterial culture and almost no access to nucleic-acid amplification tests. Therefore, clinical diagnoses, supported by radiology, are commonly made in developing countries. WHO has issued guidelines for the diagnosis of smear-negative and extrapulmonary tuberculosis, in settings with high prevalence of HIV.[147] Additionally, international standards for tuberculosis care have recently been drawn up.[148] Case definitions for smear-negative pulmonary tuberculosis have been developed, with mixed results in African studies.[149] Expanded case definitions, including extrapulmonary tuberculosis, performed well with positive predictive values of around 90% for most of the case definitions in an HIV-infected population.[150] However, clinical case definitions can never be completely accurate; therefore, they should be coupled with an objective assessment of response to treatment.[150] Patients who fail to respond should be referred for further investigation.

Treatment

Conventional short-course therapy has remained unchanged for decades. The most frequently recommended and effective combination is isoniazid, rifampicin,

pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months.[151] This regimen is very effective for treatment of patients with tuberculosis, including patients with HIV infection.[152] The internationally recommended tuberculosis control strategy is directly observed treatment short course (DOTS). DOTS is based on five elements: political will, case detection, standardised observed therapy, effective drug supply, and monitoring and evaluation. The intervention is based on achieving the maximum benefit of existing methods for diagnosis and treatment, and is a highly cost-effective intervention in developing countries.[153] An extended version of the strategy, DOTS plus, is used to treat drug-resistant tuberculosis. However, the evidence base for the implementation of policy for multidrug-resistant tuberculosis is far from ideal.[154]

Although the DOTS strategy has contributed to the falling incidence of tuberculosis in many parts of the world, as recognised in the recently published plan for global tuberculosis control, DOTS should be augmented by a combined approach, including new diagnostics, new drugs, new vaccines, reduction of incidence of HIV, and advocacy.[155,156] Under programme conditions, completion rates of therapy are variable, partly because many patients discontinue treatment when their symptoms improve. Failure to complete therapy is associated with longlasting infectious status, relapse, and drug resistance.[9] With every dose supervised by clinic or community healthcare workers, direct observed therapy (DOT) attempts to improve treatment completion. A recent meta-analysis has shown that DOT did not improve the key outcomes of cure and treatment completion.[157] Provision of DOT at home results in similar,[158] or slightly better[157] outcomes than in the clinic or community. Programmes that support home DOT, either via family or community workers, should be encouraged, and new interventions to improve adherence should only be implemented with good evidence. Very high adherence rates have been achieved in antiretroviral roll-out programmes in the developing world with a patient-centred approach, which encourages the belief that empowerment of patients can promote adherence.

Rifapentine is a long-acting rifamycin suitable for administration once a week during the continuation phase.[159] Unfortunately, acquired rifamycin resistance has arisen in HIV-infected individuals and in those with widespread disease.[160] Studies in progress are attempting to improve outcomes with higher doses of rifapentine, and with longer-acting companion drugs.

Two African pharmacokinetic studies reported low concentrations of rifampicin in a strikingly high proportion of patients with pulmonary tuberculosis treated with standard doses.[161,162] Higher doses of rifampicin are associated with improvements in outcomes[163] and in early bactericidal activity.[164] More data are needed to recommend a change in practice, and higher doses are being included in future research of newer regimens.

New antitubercular drugs are needed to improve the treatment of patients with multidrug-resistant tuberculosis, and might enable the duration of treatment to be shortened. Some new compounds with antimycobacterial activity exist,[165] and some licensed antimicrobials have good activity. However, the chances of the generation of a successful new compound by 2010 have been estimated to be slim, and would need a commitment of up to US$400 million.[166]

Among antimicrobial agents already licensed, some of the newer fluoroquinolones, notably moxifloxacin and gatifloxacin, have good in-vitro activity against *M tuberculosis*. The substitution of isoniazid by moxifloxacin reduces the time to bacillary clearance and cure in the mouse model of tuberculosis.[167,168] Furthermore, intermittent therapy with rifapentine, moxifloxacin, and pyrazinamide is more potent than it is with rifampicin, isoniazid, and pyrazinamide in the same animal model.[169] Moxifloxacin reduces the time to sputum culture conversion compared with ethambutol when added to conventional therapy.[170] Earlier sputum culture conversion might enable the duration of treatment to be shortened, but this remains to be confirmed in clinical trials. One concern is that resistance to fluoroquinolones can develop rapidly, especially when these drugs are used as monotherapy to treat suspected bacterial infections of the lower respiratory tract before tuberculosis has been diagnosed.[171,172] Moreover, some fluoroquinolones, including gatifloxacin, with promising antimycobacterial activity have been withdrawn postmarketing because of toxic effects.

The nitroimidazopyran PA-824 and the diarylquinoline R207910 are promising new antimycobacterial drugs.[173,174] Both have novel mechanisms of action, and have activity against isolates that are resistant to other drugs. Both agents have begun early clinical trials.

Tuberculosis is characterised by immunopathological changes; indeed, a competent immune response is needed to produce pulmonary cavitation, which is a feature of adult tuberculosis but seldom seen in advanced HIV infection. The course of treatment can also be complicated by paradoxical deterioration,[175] which has recently received great attention because of its frequency and severity in HIV-infected patients treated with antiretroviral therapy. Although adjuvant corticosteroid therapy predisposes to tuberculosis, it is sometimes also used to suppress inflammation in tuberculosis and in paradoxical reactions. However, evidence to support this therapy only exists for tuberculous meningitis, and possibly pericarditis.[176,177] No clear evidence that steroids improve the outcome of tuberculous pleural effusions exists.[178]

In HIV-infected patients, steroids have been shown to be beneficial in tuberculous meningitis, although the overall prognosis is still extremely poor.[176] Steroid use in pleural tuberculosis was associated with a higher incidence of Kaposi's sarcoma.[179] An additional concern is

that steroid therapy of HIV-associated pulmonary tuberculosis is associated with a transient increase in HIV viral load.[180]

Particularly in view of the advent of XDR tuberculosis, investigators are asking whether the immune response to tuberculosis can be modified or augmented to assist clearance of bacilli. In addition to the possible antibacterial effects of vitamin D outlined above, preliminary clinical studies of inhaled interferon γ in patients with multidrug-resistant tuberculosis are being followed up.[181] The most widely tested immunomodulatory agent is immunisation with killed *M vaccae*, which showed some promise in early studies.[182] However, two clinical trials subsequently showed little evidence of additional efficacy above DOTS, although one study showed a little more rapid sputum clearance in the group treated with *M vaccae* than in the control group.[183–185] Further research into the relation between the immune response and the successful treatment of both active and latent tuberculosis may lead the way to more targeted interventions.[186,187]

Treatment of latent tuberculosis infection

Preventive therapy reduces tuberculosis incidence in HIV-positive and HIV-negative individuals.[7,188] It is a successful component of tuberculosis control in Europe and North America, where special attention is also given to the prevention of tuberculosis in patients who receive immunosuppressive therapies.[189] Difficulty in the identification of those at risk, uncertainty about effectiveness in higher-transmission settings, and concerns about cost-effectiveness and acquired drug resistance have limited the implementation of preventive therapy in resource-poor countries. Notwithstanding the theoretical and operational deficiencies of TST, the greatest benefit is in patients who are positive to the skin test. The best-studied regimen is 6–12 months of isoniazid. However, isoniazid resistance can occur if tuberculosis arises despite preventive therapy—and is particularly likely to develop when preventive therapy with isoniazid is inadvertently given to patients with subclinical or unrecognised tuberculosis. A systematic review reported a non-significant trend (relative risk 1·45, 95% CI 0·85–2·47) of increased resistance to isoniazid when tuberculosis arose despite preventive therapy.[190]

The well recognised hepatotoxicity of antitubercular drugs is a very important consideration in the treatment of latent infection, because it increases the risk–benefit ratio. Several randomised controlled trials have shown that short courses (2 or 3 months) of rifampicin and pyrazinamide were well tolerated by HIV-positive people, and as effective as standard courses of isoniazid (6 or 12 months) for the prevention of tuberculosis.[191] However, two subsequent trials in HIV-negative people showed that severe derangement of liver function occurred five to ten times more often with rifampicin and pyrazinamide than it did with isoniazid;[192,193] many clinicians used rifampicin and pyrazinamide to prevent tuberculosis in people without HIV infection before the trials were done, resulting in several cases of severe hepatotoxicity.[194] Indeed, in HIV-negative people, preventive treatment with rifampicin and pyrazinamide is reported to cause more hepatotoxicity than treatment of tuberculosis with rifampicin, pyrazinamide and isoniazid.[191] This finding shows that data obtained in clinical trials cannot be extrapolated to different populations. Investigators are seeking to understand better the biology of latent infection, with the long-term aim of developing new drugs for latent tuberculosis.[187,195]

Vaccination

Meta-analyses of BCG vaccinations have not been entirely uniform in their conclusions,[196] but consensus exists that BCG provides some protection, especially against severe tuberculosis in children. The duration of protection seems to be variable. BCG does not seem to reduce the transmission of tuberculosis, which is a serious shortcoming. A more effective vaccine might greatly improve tuberculosis control.[197]

BCG confers some protection against tuberculosis in animal models.[198] During the past 10 years, vaccinologists have developed various novel vaccines with equal or greater efficacy than BCG in animals (table 4). Some of these candidates are now in phase I or II clinical trials,

	Type	Evidence of effectiveness	Developmental stage	Notes	Reference
Modified vaccinia Ankara 85A	Live attenuated vector	Mice, guineapigs	Phase II		199,200
72f fusion protein	Subunit	Guineapigs	Phase I	Delivered in AS02 adjuvant	201
rBCG30	Recombinant BCG	Guineapigs	Phase I	A recombinant BCG that overexpresses antigen 85B	202
ESAT-6-85B fusion	Subunit	Guineapigs, macaques	Phase I	Delivered in IC31 adjuvant	200,203
ESAT-6–TB10.4 fusion	Subunit	Mice	Preclinical	Delivered in IC31 adjuvant	204
ΔureC hly⁺ rBCG	Attenuated recombinant BCG	Mice	Preclinical	A recombinant BCG without the urease C gene and expressing listeriolysin	205

Table 4: **Novel vaccines against tuberculosis**

and the STOP-TB partnership intends to bring one such candidate into clinical use by 2015.[156] Because childhood BCG vaccination has some effectiveness, a pragmatic consensus has emerged that novel vaccines should be assessed by how much more immunity they confer than does BCG. Vaccination after infection might reduce reactivation, and is being considered as a possibility.

However, two serious bottlenecks exist in tuberculosis vaccine research. First, efficacy trials need to enrol many individuals and to follow them up for a long time, because disease manifests only in a minority of people infected with *M tuberculosis*, and can do so many years after a vaccine is given. Second, convincing in-vitro correlates of a protective immune response are needed. Despite considerable advances in the understanding of immunity to mycobacteria, and in particular of the role of T cells that produce interferon γ, enumeration of these cells has not emerged in studies as a definitive single marker of immunity. A recent report suggests that the absence of interferon-γ-secreting T cells that respond specifically to antigens from RD1 might be a marker of resistance to primary tuberculosis induced by BCG vaccination.[206] Reliable correlates of protection might be established only during the course of a vaccine trial, by comparing the responses after vaccination in those who remained disease-free with those who develop the disease.

Control of HIV-associated tuberculosis

While we await new drugs and vaccines, a pressing need to address a tuberculosis catastrophe exists: the HIV-driven epidemic in sub-Saharan Africa. The ways to cope with this epidemic are restricted, and poor health infrastructure limits their implementation. Antiretroviral treatment has been shown to reduce the incidence of tuberculosis,[207–210] but the risk of developing tuberculosis is still much higher than in HIV-negative individuals. Furthermore, the net gain in life expectancy provides more years in which the patient can contract tuberculosis. A recent model has shown that antiretroviral therapy and treatment of latent tuberculosis infection had a very modest effect on tuberculosis incidence;[211] the only preventive strategy that substantially affected incidence of tuberculosis was reduced HIV incidence. The detection and cure of active tuberculosis were the most effective interventions.[211] Other interventions that might lower incidence include secondary preventive therapy, and preventive therapy combined with antiretroviral therapy. In locations where the tuberculosis epidemic is driven by HIV, access to mycobacterial culture or nucleic-acid amplification tests would, in a mathematical model, greatly reduce tuberculosis prevalence and mortality, with a more modest reduction in tuberculosis incidence.[212] A combined approach with all the available interventions is reasonable because of the scale of the epidemic, but precious resources should be directed towards those interventions with the greatest effect.

Co-administration of antiretroviral and antitubercular therapy is not straightforward for three reasons: drug interactions between antiretroviral drugs and rifamycins; shared toxicities; and the immune reconstitution inflammatory syndrome.[213] Available data suggest that patients who have been given antiretroviral and antitubercular therapy are at increased risk of adverse drug reactions, but the studies are retrospective and do not enable accurate attribution of the risks conferred by HIV infection, antiretroviral therapy, or other concomitant medications.[213]

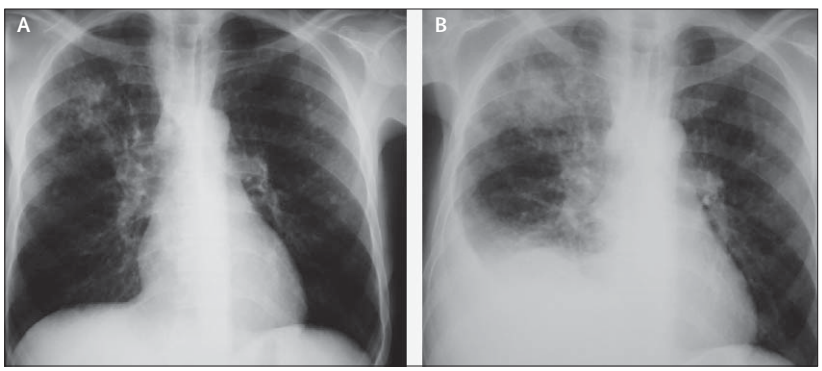

Figure 2: **Typical features of the immune reconstitution inflammatory syndrome**
An HIV-infected man (CD4 count 29 cells/μL) with pulmonary tuberculosis (A) had symptomatic improvement after 1 month of antitubercular therapy. Antiretroviral therapy was commenced. 2 weeks later he presented very unwell with marked radiographic deterioration (B).

Rifampicin is a potent inducer of cytochrome P450 enzymes, and the drug efflux pump P-glycoprotein. Two classes of antiretroviral drugs, protease inhibitors, and non-nucleoside reverse transcriptase inhibitors are substrates of cytochrome P450, and their metabolism is enhanced when rifampicin is taken. Additionally, protease inhibitors are substrates of P-glycoprotein, induction of which reduces absorption of protease inhibitors from the intestine, and enhances first-pass metabolism. These interactions can result in subtherapeutic concentrations of antiretroviral drugs, increasing the risks of disease progression and drug resistance. The reductions in protease-inhibitor concentrations are substantial when rifampicin is administered, and co-administration is possible only with high doses of the poorly tolerated protease inhibitor ritonavir, an inhibitor of P-glycoprotein and cytochrome P450.[214] An alternative strategy is to replace rifampicin with rifabutin, a weak inducer of cytochrome P450, that does not greatly alter most protease-inhibitor concentrations. However, rifabutin is at present unaffordable in developing countries. The concentrations of the non-nucleoside reverse transcriptase inhibitors are less affected by rifampicin, and co-administration is possible, especially with efavirenz. Some authorities recommend increasing the dose of efavirenz, but this measure does not seem necessary.[215,216] The non-nucleoside reverse transcriptase inhibitor nevirapine is widely used in developing countries, but few data exist on co-administration with rifampicin, and an increased dose of nevirapine might be necessary.[217]

Patients with tuberculosis commonly develop the immune reconstitution inflammatory syndrome after starting antiretroviral therapy (figure 2). This syndrome is characterised by aberrant immunopathological immunity to tuberculosis. Tuberculosis that was improving under treatment can worsen; non-apparent tuberculosis can be unmasked. Diagnosis is difficult, because immune reconstitution inflammatory syndrome in patients with tuberculosis has protean manifestations. The differential diagnosis includes tuberculosis that is deteriorating because of incomplete adherence or drug resistance, other opportunistic diseases, and drug hypersensitivity. Little is known about the cause or management of immune reconstitution inflammatory syndrome in patients with tuberculosis;[218] however, in a preliminary report, it has been associated with large expansions of T cells that produce interferon γ.[219] Improvement of severe immune reconstitution inflammatory syndrome with corticosteroids has been shown, but these drugs are not without risks in HIV-infected individuals. Immune reconstitution inflammatory syndrome in patients with tuberculosis is common in those with the most serious depletion of $CD4^+$ T cells when they start antiretroviral therapy, and when the interval between the beginning of antituberculosis therapy and that of antiretroviral therapy is short (<2 months).[220–223] Some recommend that antiretroviral therapy is deferred until the intensive phase of antituberculosis therapy is complete; however, a risk exists of increased morbidity and mortality in patients with advanced HIV disease. Clinical trials are underway to establish the optimum timing of initiation of antiretroviral therapy in patients with tuberculosis.

Conclusions

Tuberculosis, especially when combined with HIV, remains a formidable problem. Increase in drug resistance threatens the advances that have been made by wider implemetation of rational multidrug therapy through the DOTS strategy. Nevertheless, basic and applied research activity is more intense than ever, and clear progress towards better preventive measures, diagnostic tests, and drug treatment options exists. Increased political will within the international health community is essential to tackle this disease head on, which is shown by the creation of a comprehensive worldwide plan. The political will to deal with this initiative is essential, because the global control plan needs massive effort and resource to decrease incidence in all regions of the world.

Conflict of interest statement

We declare that we have no conflict of interest.

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