

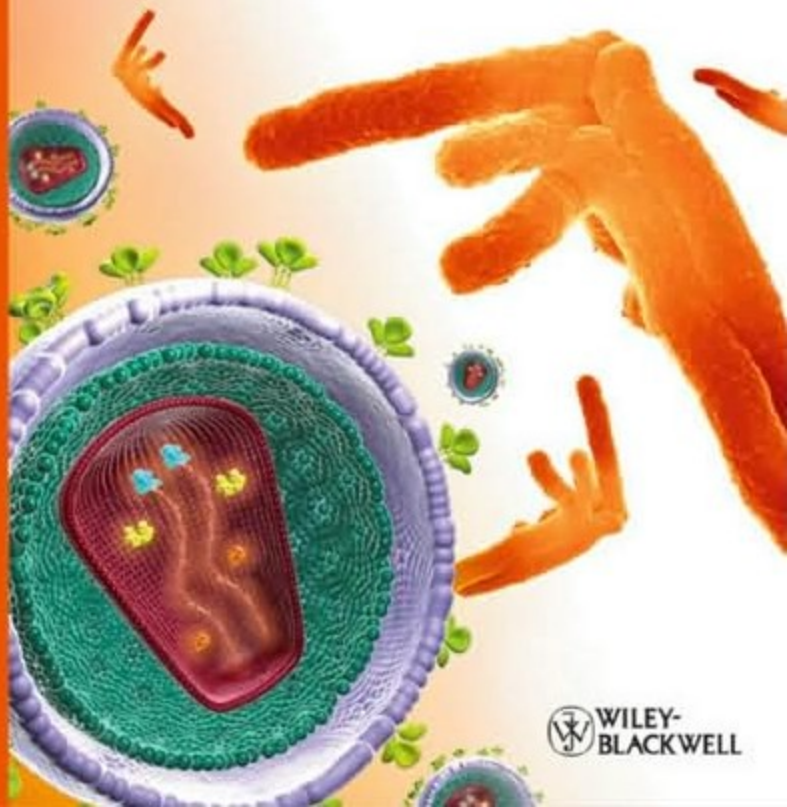


Infection Biology Handbook Series

AIDS and Tuberculosis

A Deadly Liaison

Edited by Stefan H. E. Kaufmann
and Bruce D. Walker



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*Edited by
Stefan H. E. Kaufmann and
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Preface

AIDS-TB: A Deadly Liaison

In ranking perfect storms, the intersection of the tuberculosis (TB) and acquired immunodeficiency syndrome (AIDS) pandemics is high on the list, and is occurring in places least equipped to deal with the broad health implications. Over the past two decades, the AIDS pandemic has exploded in Africa, increasing in some places from less than 1% of the population to well over 40% of certain age groups in certain regions of Southern Africa. The global burden of human immunodeficiency virus (HIV) infection is now over 33 million cases, most occurring in resource-scarce settings, and 25 million people have already died.

At the same time, and by no coincidence, the TB pandemic has also flourished. Currently, there are two billion people infected with the etiologic agent *Mycobacterium tuberculosis* (*Mtb*), and sadly the burden of the TB pandemic lies squarely in the same regions as the HIV pandemic. This is particularly obvious in Africa (Figure 1). At the center of this storm is KwaZulu Natal in South Africa, where up to 70% of persons with *Mtb* infection are dually infected with HIV, and 30% or more of persons who are HIV-infected have active TB. But other areas in Africa are similarly affected, and these two pandemics are finding each other on numerous other continents.

The reason for this deadly liaison between HIV and *Mtb* is rooted in the pathogenesis of these two infections. HIV infects cells of the immune system, gaining access to CD4 T lymphocytes and monocytes via coreceptors that bind the HIV envelope, namely the surface CD4 molecule and a chemokine coreceptor, usually CCR5. From the earliest stages of infection, there is a dramatic loss of CD4 T cells, particularly in the gut-associated lymphoid tissue, where the majority of these cells reside. This loss of the central orchestrator of effective immune responses leaves the body unable to successfully contain HIV, leading to persistent viremia and continued loss of CD4 T cells, and resulting in profound immune suppression in untreated persons.

This HIV-induced insult to the immune system could not be much worse for controlling *Mtb* infection, which depends on T-cell responses. *Mtb* has chosen macrophages as its preferred habitat. For many bacterial pathogens, macrophages

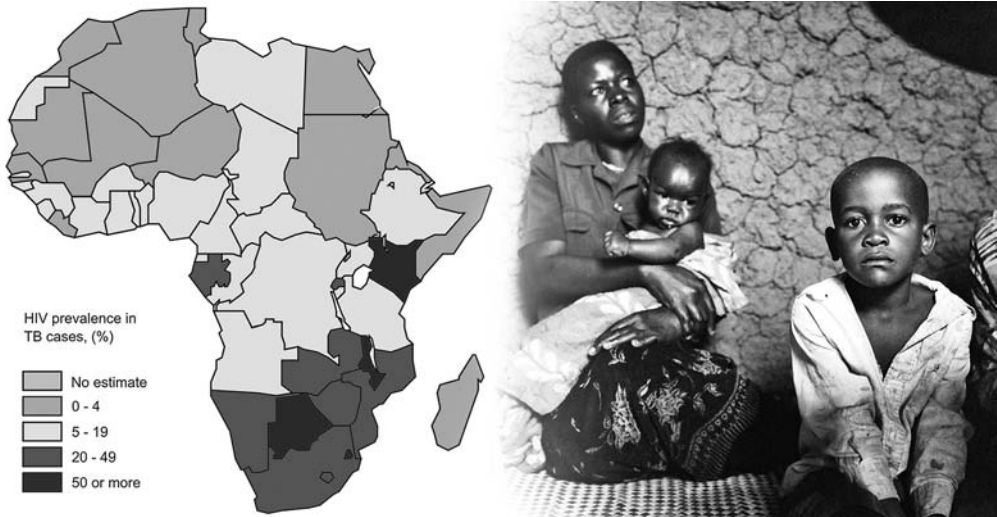


Figure 1 (left) Estimated HIV prevalence (as %) among new TB cases in Africa, 2006. Source: WHO Global tuberculosis control: surveillance, planning, financing (2008); (right) AIDS–TB patients in Uganda. Photograph courtesy of Keoki Flagg.

are a dead-end road and their engulfment results in bacterial death, at least after macrophage activation by CD4 T cells. Yet, *Mtb* has devised strategies to survive in these cells; this pathogen flourishes in resting macrophages and persists in fully activated ones. As long as CD4 T cells can fully activate macrophages, then *Mtb* persists, often without causing active disease. Once macrophage activation becomes impaired, *Mtb* multiplies and disease breaks out. This is exactly what happens in HIV–*Mtb* coinfection: impaired CD4 T cells fail to activate macrophages, which in turn fail to control *Mtb*. Although much remains to be learned, there is an expanding body of knowledge – much of it outlined in the following chapters – that indicate a particular defect in *Mtb*-specific immunity rendered by HIV, and similarly, immune dysregulation of HIV related to the pathogenesis of TB.

With the rapid expansion of these two pandemics, there is a critical need to better understand these interactions and to integrate research efforts, as the two pathogens are clearly impacting one another in ways that are yet to be fully defined. The reality is that the AIDS and TB research communities have been largely separate, similar to the treatment programs which, in most regions of the world, have yet to be effectively integrated despite overlapping infections and drug toxicities. The goal of this book is to bring together the key issues in both of these fields, as well as the key areas of overlap for which there are emerging data indicating how this deadly liaison plays out.

This book is intended to provide an overview of the key issues confronting these dual pandemics, bringing together state-of-the-art research in both fields in one volume. The book begins with an overview by Julg and Walker of the challenges in

developing an effective AIDS vaccine, together with a detailed assessment of the factors that have led to a lack of viable clinical candidate vaccines more than two decades after these efforts started.

Kaufman and Stenger then add to the initial immunologic theme, outlining the key elements in the immune response to TB, and the strategies being employed to develop an efficacious vaccination schedule. Their chapter also describes current achievements in biomarker characterization which will be instrumental for accelerating clinical trials.

Next, we move on to review the status of the one vaccine that is currently in use against TB, namely BCG, which has been administered to about four billion people worldwide since it was first introduced nearly a century ago. Hanekom and Hussey discuss the properties of this vaccine, which is poorly protective against the pulmonary form of TB in adults that accounts for most global transmissions, as well as the potential complications of this vaccine in HIV-infected infants and the need for new approaches in this age group.

The most dramatic change in the HIV pandemic in terms of health care has been the introduction of highly active antiretroviral therapy, which has now been administered to more than three million people worldwide. The huge arsenal of drugs available, as well as new approaches to treatment currently being pursued, are outlined in the next chapter by Gulick, who has been intimately involved in treating HIV infection since the beginning of the treatment era in the United States. Despite issues of access, cost, infrastructure and side effects, treatment has had a huge individual benefit, although there is little evidence that it has led to changes in the kinetics of the pandemic.

Böttger and Springer next discuss the treatment of TB, including the mechanisms of drug resistance and the genetics underlying this. This treatise takes a refreshingly new view on TB drug treatment and the susceptibility testing of *Mtb*, with its direct implications for appropriate therapy. Currently, TB is treated with three to four drugs over six to nine months, and poor compliance frequently leads to drug resistance. Because of lack of attention in the final quarter of the last century, new TB drugs have not been developed. Although we now envisage the entry of a number of promising drug candidates into the pipeline, it will still take several years before they become available for broad use. New regimens based on combinations of available drugs could bridge this gap.

One of the major global challenges related to the intersection of the AIDS and TB pandemics is the need to simultaneously treat both infections, which brings forth the major problem of HIV-*Mtb* drug interactions and overlapping toxicities. Oni, Pepper, and Wilkinson, who are leaders in the area of treatment of these two diseases in one patient, provide a detailed account of the issues related to attempts to contain both infections in the same individual.

As global treatment efforts related to HIV have expanded over the past few years, so too has the experience with clinical diagnosis and management of HIV disease. Dryden-Peterson, Sunpath and Gandhi, all of whom have experience in on-site treatment in resource-scarce settings, cover the clinical issues related to diagnosis and management of HIV. The issues around the diagnosis and treatment of HIV-

Mtb coinfection are covered in chapters by Neil Schluger and by Goldfeld and Corbett, who have considerable personal experience in these areas. Both chapters focus on the challenges of diagnosis, treatment and clinical care of AIDS-associated TB, and expand on effective ways of achieving early diagnosis at the community level as an urgently required step for effective control in resource-poor settings. By using appropriate therapy schemes that consider drug interactions, AIDS and TB in one patient are treatable, and TB even curable.

Emerging as one of the most threatening consequences of the deadly liaison between HIV and *Mtb* is the development of extensively drug-resistant (XDR) TB, which is covered in the chapter by Murray and Cohen. The chapter discusses the causal role of HIV in the development of multiresistant *Mtb* and the confounding effects of HIV coinfection on diagnosis and treatment. This is followed by the final chapter by Grobusch, Menezes and John, which deals with the effect of HIV-induced treatment leading to a robust adaptive immune response to HIV that can be so vigorous as to be lethal in some individuals, namely the immune reconstitution inflammatory syndrome (IRIS). Since IRIS is an undesired consequence of AIDS treatment in HIV–*Mtb* coinfecting individuals with increasing occurrence, this chapter gains enormous importance, for both scientific and for societal reasons.

Together, we hope that these chapters provide an overview of not just the challenges being produced by the intersection of the TB and AIDS pandemics, but also the opportunities available to address critical research issues. These efforts will have a direct impact on future policies designed to contain these epidemics, and hopefully ultimately to end both of them. We thank the talented authors who have contributed to this volume, and hope that it serves to better integrate research in two fields that, through the intersection of pandemics, has forced our attention on them.

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October 2009

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Part One

Immunology and Vaccination Strategies for AIDS and TB

1

HIV Immunology and Prospects for Vaccines

Boris Julg and Bruce D. Walker

1.1

Introduction

As the HIV epidemic approaches its fourth decade, the world remains without a vaccine for a disease that has claimed more than 25 million lives, and currently infects over 33 million persons. The vast majority of these infections are in resource-scarce settings, and in most places the humanitarian crisis is enhanced because of overlap with the expanding global tuberculosis (TB) epidemic. The introduction of highly active antiretroviral therapy (HAART) in 1995–1996 resulted in a dramatic decrease in the mortality and morbidity of HIV infection in developed countries fortunate enough to have access to these life-extending medicines [1], and more recent expanded global access has resulted in more than three million persons receiving treatment in 2008. However, this still leaves an enormous gap in those who have advanced disease and are in desperate need of therapy, and in addition there are likely to be nearly 2.5 million new infections in 2009 (UNAIDS, <http://www.unaids.org>).

There is no doubt that the development of a safe and effective HIV-1 vaccine will be the best solution for the ultimate control of the worldwide AIDS pandemic [2], and this will likely also impact the TB epidemic. However, all attempts to achieve this have failed so far, reinforcing the fact that an AIDS vaccine is unlikely to be available in the near future [3]. As the TB and HIV epidemics intersect across the globe, the need for a vaccine to prevent the immunodeficiency induced by HIV that is accelerating expansion of the TB epidemic is even more acute [4]. In this chapter we will discuss the current challenges to the development of an effective AIDS vaccine, and address the progress made and persisting gaps in our quest for an effective method to prevent new infections.

1.2

Challenges for HIV Vaccine Design

The history of successful immunization dates back to the time of Jenner, whose success with a smallpox vaccine in 1796 was achieved with little understanding of the

actual mechanisms of protection that were being induced. By mimicking infection with smallpox by inducing a benign cowpox infection, Jenner laid the foundation for modern vaccinology. Most vaccines currently in use, if not all, do not actually prevent infection, but rather attenuate disease caused by the pathogen. In fact, most mimic something that happens naturally – namely that some fraction of people who become infected clear their infections [5].

The situation with HIV is quite different as HIV is an infection in which, to our knowledge, spontaneous clearance never occurs. The natural history of HIV infection is one of progressive viremia, in which the targets of the virus are cells of the immune system itself, particularly CD4 + T-lymphocytes. Following infection, there is a gradual decline in CD4 + cell number and an increase in viral load, typically resulting in AIDS within 8–10 years, which is defined by a CD4 + cell count of less than 200 or specific AIDS-defining illnesses. HIV is actually an infection of the immune system, with CD4 + T-lymphocytes being a key target of the virus, which enters these cells through its coreceptors CCR5 (or occasionally other chemokine coreceptors such as CXCR4) and CD4.

There are five main properties of HIV that render the development of an HIV vaccine an unprecedented challenge.

1. **Massive infection of immune cells:** HIV uses its envelope protein to gain access to cells bearing its coreceptors, CD4 and the chemokine receptor CCR5 or CXCR4. The major target of the infection are CD4 + T-cells, and because activated cells are preferentially infected by HIV, the infection preferentially appears to deplete HIV-specific CD4 + cells. The infection of CD4 + cells is massive at the acute stage of infection, when up to 60% of CD4 + T-cells in the gut-associated lymphoid tissue (GALT) are depleted [6].
2. **Integration into the host chromosome:** HIV is a retrovirus, and following viral entry the viral reverse transcriptase initiates the production of a double-stranded proviral DNA that can remain as free circular DNA and undergo processes of transcription and translation to make new virion particles. Alternatively, it can use the viral integrase protein to create a nick in the host chromosome, and integrate. Once integration occurs – which all indications suggest happens very early after acute infection [7] – the virus can remain in an immunologically latent state. This is possible because the lack of transcription and translation of viral proteins means that the normal immune mechanisms, which rely on the detection of foreign viral protein within cells to induce immune attack, do not occur.
3. **Viral diversity:** HIV is a retrovirus, and viral replication is dependent on an error-prone viral reverse transcriptase that has a poor proofreading function. As a result, with each replication cycle there is likely to be at least one nucleotide misincorporation. At least some of this diversity is driven by immune selection pressure, which has been shown to be progressively deleting some key epitopes of the virus at a population level [8]. Globally there are three main groups of HIV – M, N, and O – with group M (the largest) being further divided into nine distinct clades and additional circulating recombinant forms. Viruses within a clade may

differ by up to 20% in the highly variable Env protein, which is the target for neutralizing antibodies, and by up to 38% between clades. Even within a single individual HIV mutates such that individuals carry unique strains. Developing a vaccine to target all of these viruses simultaneously is an enormous task.

4. **Envelope glycosylation:** The HIV envelope is heavily glycosylated, and also very flexible, in that it allows for a high degree of random mutations to be stably incorporated. This combination of Env variability, together with heavy glycosylation that renders key epitopes poorly exposed to antibody-mediated immune attack, has been a major challenge for any vaccine to provide broad cross-neutralizing protective antibody responses (for a review, see Ref. [5]). Indeed, at the current time this is such a challenge that many in the field have focused not on a preventive HIV vaccine – which would require the induction of broadly cross-reactive neutralizing antibodies – but rather on a T-cell-based vaccine which would be intended to provide a durable reduction in viral load, and thereby retard disease progression and reduce the likelihood of transmission to others [9].
5. **Immune evasion:** The HIV accessory protein Nef interacts indirectly with the cytoplasmic tail of HLA A and B alleles, leading to endocytosis and a down-regulation of class I expression on infected cells [10]. This impairs the ability of cytotoxic T lymphocytes to recognize infected cells, and has been shown to have functional significance on the ability to contain HIV replication [11]. Neutralizing antibodies are unable to recognize the variants that arise *in vivo* [7, 12, 13], so that the humoral immune response is always playing “catch-up.” In addition, mutations arising within targeted CD8 + T-cell epitopes also lead to either a loss of recognition by the T-cell receptor (TCR) of established responses, or to a loss of binding of the epitope to HLA class I, allowing immune escape.

1.3

What Immune Responses will be Required for an Effective AIDS Vaccine?

A fully preventive HIV vaccine would almost certainly require the induction of broadly cross-reactive and highly potent neutralizing antibodies, which would have to prevent the infection of cells and the establishment of latent infection. There is widespread agreement that this is not likely to occur, for the reasons outlined below. Indeed, most – if not all – vaccines currently in use do not achieve this level of protection. This reality has directed the field toward vaccine strategies that would prevent disease progression rather than prevent infection – which, at least in theory, would cause the epidemic to contract – if the viral load could be kept low enough to limit both disease progression and transmission.

The challenges to this direction for vaccine development are compounded by the fact that we still lack an understanding of the correlates of immune protection, despite an intricate understanding of the molecular biology of the virus (Figure 1.1). Despite marked differences in disease outcome following infection, we lack a fundamental understanding of the mechanisms that account for these differences.

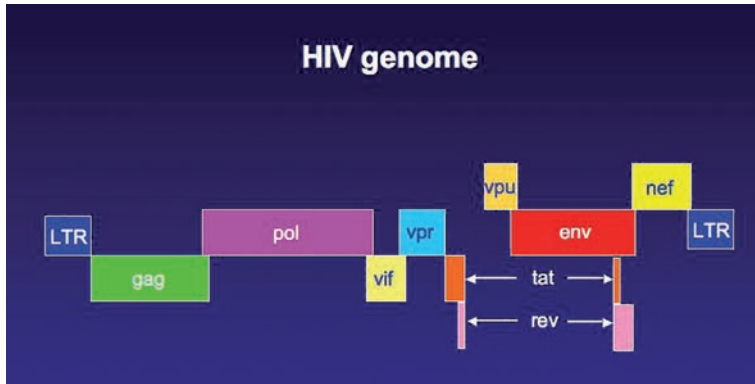


Figure 1.1 The HIV-1 genome. There are nine coding regions, in three different reading frames. *gag* is the main structural protein, *pol* encodes the replicative functions, and *env* encodes the heavily glycosylated outer envelope. The regulatory proteins include *vif*, *vpr*, *vpr*, *rev*, *tat*, and *nef*. LTR, long terminal repeat.

There is a growing body of data indicating that adaptive host immune responses play a role, but the key elements of protective immunity that would have to be induced by a vaccine are not known. What is known is that some persons are able to maintain successful control of HIV viremia for 30 years or more without therapy. This, in turn, provides some level of optimism that a vaccine might be able to result in a similar equilibrium with durable control of HIV, even if a totally preventive vaccine is not possible [14]. In contrast, others progress from acute infection to AIDS within six months [15]. Whilst the factors that account for these dramatic differences in outcome remain elusive, a growing body of data is beginning to shed light on the rational induction of specific arms of the immune response for HIV vaccine design (Figure 1.2).

1.3.1

Cytotoxic T Lymphocytes

Following acute HIV-1 infection, the resolution of acute-phase plasma viremia to a semi steady-state level, or set-point, coincides with the activation and expansion of HIV-1- specific cytotoxic T lymphocytes (CTL), suggesting that virus-specific CD8 + T-cells may be responsible for reducing the levels of virus at this stage of infection [16–18]. Direct evidence for the role of CD8 + T-cells in mediating the decline in viremia during acute HIV infection has come from studies of the simian immunodeficiency virus (SIV)-macaque model. Here, the administration of CD8-specific monoclonal antibodies (MAbs) resulted in a transient depletion of CD8 + cells in both the peripheral blood and lymphoid tissues. When administered during primary chimeric simian/HIV infections, the CD8 MAb caused marked elevations of plasma and cell-associated virus levels in both the peripheral blood and lymphoid tissues, and led to a prolonged depletion of CD4 + cells. Eliminating CD8 + lymphocytes from monkeys during chronic SIV infection resulted in a rapid and marked increase in viremia that was again suppressed coincident with the reappearance of SIV-specific

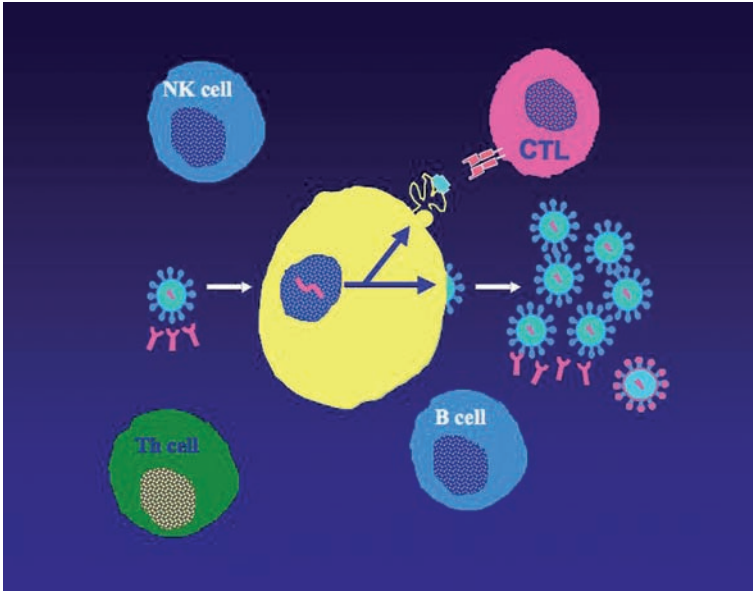


Figure 1.2 Immune responses to HIV. The B cells produce neutralizing antibodies, which are highly type-specific and poorly recognize diverse isolates, even those that arise within a single person due to reverse-transcriptase-induced errors in replication. The cytotoxic T cells (CTL) target virus-infected cells through recognition of viral proteins presented at the cell surface associated with HLA class I molecules, and deliver a lethal hit to the infected cell, ideally before progeny viruses are produced. Despite

these responses, progression ensues in most persons. T helper (Th) cells, which express CD4 and CCR5, are the central orchestrator of effective cellular immunity, but are infected in large numbers in acute infection and never fully recover; they progressively decline over time until a CD4 count of 200 is reached, which defines AIDS. Natural killer (NK) cells target virus-infected cells without requiring prior exposure; emerging data suggest that these may be important in HIV control.

CD8 + T-cells [19–21]. These results confirm the importance of cell-mediated immunity in controlling AIDS virus infection, and support the exploration of vaccination approaches for preventing infection that will elicit these immune responses.

An emerging body of data suggests that it is not just the magnitude but rather the specificity of the CTL response that may be critical for immune containment. Numerous population studies have determined that neither the total breadth nor the total magnitude of HIV-specific CD8 + T-cell responses correlate with the ability of an individual to control HIV-1 [22–24], which suggests that selected epitope-specific CD8 + T-cell responses play a relevant role. Large population studies conducted in South Africa have defined that a preferential targeting of Gag is associated with a lower viral load [25], while more recent data have indicated that the breadth of the Gag-specific response is negatively correlated with the viral load in persons with chronic infection [26]. In contrast, broad Env-specific CD8 + T-cell responses are associated with a high viral load [26]. To some extent this may reflect differences in the quality of these responses, or in the relative efficacy of different responses to recognize and kill infected cells before progeny viruses are

produced [27]. The limited ability of these responses to provide durable containment may also be due to escape mutations emerging within targeted CD8 + T-cell epitopes, which arise during primary [28–31] and chronic [32, 33] HIV-1 and SIV infection, and demonstrates significant CD8 + T-cell pressure on these regions of the virus and impacts temporally on disease progression [33, 34]. In addition, functional impairment or exhaustion of these responses over time in the setting of chronic viral stimulation may play a role. The inhibitory receptor programmed death 1 (PD-1; also known as PDCD1), a negative regulator of activated T cells, is markedly upregulated on the surface of HIV-specific CD8 + T-cells, the expression correlating with impaired HIV-specific CD8 + T-cell function as well as with predictors of disease progression – positively with plasma viral load, and inversely with the CD4 + T-cell count [35]. In contrast, the inhibitory immunoregulatory receptor CTLA-4 is selectively upregulated in HIV-specific CD4 + T-cells, but not CD8 + T-cells, in all categories of HIV-infected subjects, except for a rare subset of individuals who are able to control viremia in the absence of antiretroviral therapy [36].

One of the strongest arguments in favor of a role for CTLs in the outcome of HIV infection is the association between certain HLA class I alleles and improved outcome [37]. Among these are the so-called protective alleles, the strongest of which include B*5701, B*5801, B51, and B*2705. These B alleles have in common that they are associated with strong immune responses to the Gag protein, and in some cases are associated with mutations that impair viral fitness [38]. Other HLA alleles, such as HLA B35, are associated with a worse outcome [39], although an understanding of the mechanism of this association remains obscure. One concern raised by these observations is that there may be genetic limitations to the efficacy of a particular vaccine candidate, in that it may be more immunogenic in certain HLA backgrounds, and may have limited immunogenicity in others. However, this concern remains unsubstantiated.

1.3.2

Neutralizing Antibodies

Following the identification of HIV as the causative agent of AIDS, it was predicted that a vaccine inducing neutralizing antibodies and thereby preventing infection would rapidly be available. Yet, a quarter of a century later an effective preventive HIV vaccine still eludes us. Neutralizing antibodies are induced by HIV, but fail to control viremia. Despite a pronounced antibody response to the viral envelope proteins, only a small fraction of these antibodies have neutralizing activity. This is partly due to the fact that the HIV-1 Env glycoprotein is a trimer on the virion surface with extensive N-linked glycosylation that effectively shields many conserved epitopes from antibody recognition [40]. Key conserved regions, such as the binding site of the chemokine coreceptor, are only formed after Env binds its cellular receptor CD4 and undergoes an extensive conformational change. The broadly reactive MAb b12 binds to the CD4-binding site, suggesting that this region of Env may represent a critical point of vulnerability that is potentially amenable to neutralization, although the CD4-binding site is recessed and only partially accessible to antibody binding. The membrane-

proximal external region (MPER) of gp41 is another conserved region, which represents the target of the broadly reactive MAbs 2F5 and 4E10 [41, 42]. However, MPER-specific neutralizing antibodies may be difficult to elicit by vaccination for multiple reasons, including tolerance control and immunoregulation, sequestration of the epitopes in the lipid membrane, exposure of the epitopes only transiently during viral entry, or possibly a combination of multiple factors.

HIV infection induces neutralizing antibodies directed against three major determinants: (i) the highly variable V3 loop; (ii) the CD4 binding domain; and (iii) the more conserved gp41 transmembrane protein. So far, most of the evidence [43, 44] suggests that these responses play only a minor role in immune containment in chronic infection as the antibody responses to autologous virus are typically weak. This applies also for persons who are able to control HIV infection without antiviral therapy [45, 46]. Furthermore, neutralization escape has been observed even in persons who persistently control viremia [47, 48]. The presumably minor role of antibodies in viral control is supported by a study in which B cells were depleted with anti-CD20 antibody in an acute infection primate model, and showed little impact on viral control. This intervention led to the delayed emergence of neutralizing antibodies and no change in early viral kinetics [49]. Despite the lack of protection, longitudinal studies of autologous neutralizing antibody responses indicate that the viral inhibitory capacity of these responses can be of sufficient magnitude to completely replace circulating neutralization-sensitive virus with successive populations of neutralization-resistant virus [12, 13]. It has even been shown that neutralizing antibody escape can exceed the rate of change observed with potent anti-HIV-1 drug selection pressure. Nevertheless, despite a gradual broadening of the neutralizing antibody response, it does not become sufficiently broad to neutralize the next population of virus to arise. Different means by which the virus evades antibody pressure have been proposed, including an evolving glycan shield and resultant steric hindrance [12]. Even so, these studies provide evidence that the neutralizing antibody responses are strong enough to drive immune escape, and also demonstrate how quickly immune escape from neutralizing antibodies can occur.

1.3.3

CD4 + T Helper Cells

One of the central immunological defects in most individuals with HIV-1 infection is a weak to absent HIV-1-specific CD4 + T-helper cell proliferative response [50], although when present, HIV-1-specific T-helper cell responses have been correlated with a decreased virus load [51]. Indeed, HIV appears to preferentially infect HIV-specific CD4 + T-cells [52]. It is likely that the mechanism behind this association between CD4 + help and disease outcome is due to the effect of these cells on CTL function. This has been well established in murine models of chronic viral infections, in which durable control by CTL is dependent upon the persistence of virus-specific T helper cells [53]. Several detailed studies have demonstrated that while the primary expansion of antiviral CD8 + T-cells can occur independently of CD4 + T-cell help, memory CD8 + T-cell numbers and secondary responses to bacterial

or viral challenge are decreased over time in CD4 + T-cell-deficient animal models [54, 55]. It has been shown that CD4 + help is particularly required for the long-term survival of memory CD8 + T-cells [56]. In the absence of CD4 + T-cells, memory CD8 + T-cells become functionally impaired and decrease in quantity over time.

1.3.4

Natural Killer Cells

Although natural killer (NK) cells have traditionally not been considered as a component of a vaccine approach, emerging data suggest that these cells may be critical. On the one hand, NK cells respond to Toll-like receptor (TLR) ligands and help to create the proper milieu for immune induction, whereas on the other hand, recent data suggest that at least some NK cell subsets can be endowed with memory properties, allowing for a more rapid expansion on subsequent encounters [57]. This recent discovery will no doubt influence future research directions in the HIV field.

1.4

Models of Successful Vaccination?

Because of challenges to the development of a fully preventive vaccine, which would require the induction of potent and broadly directed neutralizing antibodies, the field has in part focused on development of T-cell-based vaccines. These would be intended not to prevent infection, but rather to prevent disease progression when a person becomes infected, by limiting the production of progeny virions from infected cells (Figure 1.3). Enthusiasm for such an approach comes from the observation that a small fraction of persons who become HIV infected are able spontaneously to control HIV replication and maintain normal CD4 + cell counts without medications – some now for more than 30 years after the initial infection (for a review, see Ref. [14]). This group of persons has been termed “HIV controllers,”

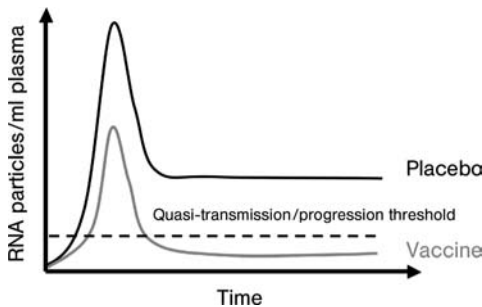


Figure 1.3 The theory behind T-cell vaccination. T-cell vaccines would be expected not to prevent infection, but rather to modulate the viral load after infection, reducing it to a level at which the likelihood of disease progression and transmission would be markedly reduced. This level is thought to be around 1000–2000 RNA copies per ml plasma.

and consists of both “elite controllers,” who maintain plasma viremia less than 50 RNA copies ml^{-1} , as well as “viremic controllers” who maintain viral loads between 50 and 2000 copies, a level at which the likelihood of progression and of transmission are markedly reduced [14, 58]. Most studies suggest that durable HIV control occurs in less than 1% of infected individuals [59–61], and may be as low as one in 300. So far, no epidemiologic factors have been associated with complete or near-complete HIV control *in vivo*. Gender does not seem to determine the ability to contain the infection, as both male and female HIV controllers are defined [62]. Controllers have been identified within multiple ethnicities, infected with different virus subtypes, and via different routes of HIV acquisition [63]. This leads to the assumption that race, geographic location, and/or viral subtype independently are not impacting immunologic and virologic outcomes [63, 64].

Although the mechanisms by which elite controllers are able to contain viral replication are still being defined, there are emerging data which indicate that it is immunologically mediated. There is an overrepresentation of certain HLA class I alleles in these individuals, particularly HLA B57 and B27, and recent studies have shown that circulating CD8^+ T-cells from these individuals are able to potently suppress viral replication in an *in vitro* assay [27, 65]. Most information is available for the subset of elite controllers who express HLA B*5701, in whom it has been shown that CTL responses select for mutations unique to those with elite control, which markedly impair viral fitness, while at the same time eliciting *de novo* CTL responses to the variant virus [66]. The results of recent studies have also suggested that the specificity of responses may be critical for the durable control of HIV infection, with multiple studies showing that preferential targeting of Gag is associated with a better outcome [26, 67]. This observation may be at least partially explained by immune-induced mutations, which would be expected to have a greater impact on viral fitness when arising in key structural or functional proteins, as opposed to the envelope protein, which is able to accommodate extensive sequence variation.

Durable control of AIDS virus infection has also been achieved with live attenuated vaccines, which by far have had the most impressive effect of any vaccine tested. So far, a live attenuated SIV represents the most successful nonhuman primate vaccine approach, and has consistently protected rhesus macaques against challenge with a homologous, pathogenic SIV [68]. Whilst this is a critical model to understand in terms of the correlates of immune protection, thus far it remains unclear how such protection is achieved. Moreover, even this approach potentially falls short of what would be required, given the need for protection against heterologous strains of virus. The protective effect of this vaccine against a heterologous SIV challenge has been addressed in only a few smaller studies, with mixed results [69, 70].

1.5 Human Trials of AIDS Vaccines

To date, only two trials of AIDS vaccines have been conducted that have reached endpoints, and both have been failures.

1.5.1

Antibody-Based Vaccination

1.5.1.1 VaxGen Trial of AIDSVax

The first of these investigations was the VaxGen trial of AIDSVax, a recombinant HIV-1 gp120 vaccine consisting of two rgp120 envelope subunits derived from the subtype B isolates MN and GNE8. The hypothesis was that antibodies directed against the envelope would bind, neutralize, and clear HIV particles before infection became established. The vaccines generated antibody responses in almost 100% of recipients in Phase I and II trials [71], and protected chimpanzees from intravenous and mucosal challenge with homologous and heterologous HIV-1 variants [72, 73]. However, after completion of the Phase III trial in 2003, analyzing 3598 vaccine recipients and 1805 placebo recipients, no effectiveness in the reduction of HIV infection or levels of plasma viremia could be observed. Neither could any differences in the time to ART initiation or to virologic failure or pre-ART viral load and CD4 + lymphocyte count be found between the vaccine and placebo arms. Furthermore, antibody response levels were comparable among low-risk or high-risk vaccine recipients [74]. Although a larger trial is currently under way combining AIDSVax with a canarypox vector designed to induce T-cell responses, it is largely felt that the prospects for preventing infection or attenuating disease are modest at best with this vector, and unlikely to be achieved with any candidate neutralizing antibody vaccines in development today.

1.5.2

T Cell-Based Vaccination

1.5.2.1 The STEP Study

The second vaccine concept that has completed clinical efficacy studies involved immunization with three replication-incompetent recombinant adenovirus serotype 5 (Ad5) vectors expressing HIV-1 Gag, Pol, and Nef. The Ad5 vector-based vaccines have shown to be among the most immunogenic of available cell-mediated immunity vaccines in early-phase clinical trials [75, 76], surpassing immune responses generated by DNA plasmids [77] and many poxvirus vectors [78]. The antigens, HIV-1 Gag, Pol, and Nef, were selected because they are fairly conserved across different HIV-1 clades and commonly recognized during natural infection. In a promising Phase I trial, the study vaccine elicited immune responses in immunocompetent participants, independently of their Ad5 serostatus [75]. The aim of this collaborative study between Merck and the HIV Vaccine Trials Network (STEP study) was to elicit HIV-1-specific cellular immune responses, with the goal of preventing disease progression, but with little expectation that the vaccine would reduce the acquisition of infection.

The vaccine candidate was being studied in a Phase IIb clinical trial known as “STEP,” and was being extended to a cohort in Africa through the Phambili trial. STEP (HVTN 502, Merck V520/Protocol 023) was a multicenter, randomized, double-blind, placebo-controlled Phase IIb test-of-concept clinical trial. STEP included 34 clinical trial sites in North and South America, the Caribbean and Australia. The first of 3000 participants enrolled in the study in December 2004, and enrollment was

completed in March 2007. The second Phase II trial of this study vaccine, Phambili (HVTN 503, Merck V520 Protocol 026), began in 2007 in South Africa, the goal being to determine whether the study vaccine used would prevent infection or reduce viral loads in an area where HIV subtype C is common.

The STEP trial was successfully enrolled, and even expanded to include persons who were adenovirus seropositive, but was prematurely halted for futility based on an early review of incoming data. The first planned interim analysis showed that this vaccine failed to protect against infection, or to reduce viral loads after infection in participants with baseline Ad5 antibody titers of 200 or less, despite generating interferon- γ ELISPOT responses in most participants receiving vaccine. Surprisingly, the risk for infection was highest in the subgroups of men given vaccine who were both uncircumcised and had pre-existing Ad5 neutralizing antibodies when compared to the placebo cohort; the risk was intermediate in men with either one of these two factors [3, 79]. With the early termination of the STEP Study, the Phambili protocol team also stopped vaccinations. Several theories have been proposed to explain the apparently increased acquisition in the STEP trial:

- A replication-defective Ad5 vector is insufficient to stimulate cellular immune responses of sufficient breadth to control HIV-1 infection.
- Previous infection with Ad5 led to immunity against this virus, and a lack of induction of potent immune responses.
- Pre-existing Ad5-specific antibodies could reduce the number of infectious Ad5 particles, and therefore the amount of transgene-derived proteins produced by target cells.
- rAd5 vaccination of individuals with pre-existing Ad5-specific neutralizing antibodies may have resulted in activated memory Ad5-specific CD4 + T-lymphocytes that were increased targets for HIV-1 infection.
- In individuals previously exposed to an adenovirus, Ad5-specific memory CD8 + T-cells could potentially eliminate infected target cells and thereby reduce the potency and breadth of vaccine-induced HIV-1-specific CD8 + T-cell responses.
- The selection of HIV-1 antigens Gag-Pol-Nef may have been insufficient for inducing protective immune responses.
- Ad5 immune complexes facilitate dendritic cell (DC) maturation and also induce significantly higher stimulation of Ad5-specific cytolytic CD8 + T-cells. A recent study showed that Ad5 immune complexes caused significantly enhanced HIV infection in DC-T-cell cocultures than Ad5 vectors alone [80].

1.6

Recent Advances in Animal Models: Reasons for Optimism

Despite the failure of the first two large-scale vaccine trials for candidate AIDS vaccines, and particularly the concern regarding the apparent increased acquisition

in the STEP trial, there are reasons for optimism. These stem not only from the observation that some persons control HIV for more than 30 years without developing disease, but also from some recent results with animal models of T-cell-based vaccination.

1.6.1

Success against Heterologous Challenge

One of the greatest challenges for the development of an effective AIDS vaccine is the generation of broadly cross-reactive immune responses that will protect against a heterologous virus challenge. Recent data, albeit in a vaccination model that involves a live attenuated virus vaccine that is not likely to be a viable approach in humans, have suggested that this type of cross-protection may be achievable. The immunization of rhesus macaques with live-attenuated SIV has consistently induced protective immunity against a homologous pathogenic SIV challenge. However, an effective HIV vaccine should be able to protect against a wide variety of HIV isolates circulating worldwide. Only heterologous challenge studies can therefore try to predict the degree of protection that an HIV vaccine can achieve. A recent report addressed this issue in macaques when, following the administration of a live-attenuated SIV vaccine, the animals were re-challenged with a heterologous SIV isolate [68]. This strategy led to a 2-log reduction in viral replication in the vaccinated animals for up to 32 weeks post-challenge. Macaques expressing protective MHC class I alleles were even able to achieve complete suppression of the challenge virus in the acute phase. After depletion of peripheral CD8⁺ T-cells in four vaccinated animals during the chronic phase, an increase in virus replication was observed which supported the crucial role that CD8⁺ T-cells play in viral control. One drawback in these findings was the fact that the authors identified evidence of recombinant viruses emerging in some of the vaccinated animals. Taking this into consideration, the conclusion can be drawn that attenuated virus vaccines will not play a key role in future vaccine developments.

1.6.2

Heterologous rAd26 Prime/rAd5 Boost Vaccine Regimen

The disappointing results of the STEP trial were largely predicted by animal model experiments, which had shown that rAd5 vectors expressing SIV Gag failed to reduce peak or setpoint viral loads after SIV challenge of rhesus monkeys lacking the protective MHC class I allele Mamu-A*01 [81]. However, a recent study in monkeys [82] using two serologically distinct adenovirus vectors, demonstrated a substantially improved protective efficacy in this challenge model, and has raised hopes for a T-cell-based vaccine approach. The heterologous rAd26 prime/rAd5 boost vaccine regimen expressing SIV Gag elicited cellular immune responses with augmented magnitude, breadth, and polyfunctionality as compared to the homologous rAd5 regimen. Vaccinated monkeys, when re-challenged with SIV (MAC251), showed a 1.4-log reduction of peak and a 2.4-log reduction of setpoint viral load, as

well as decreased AIDS-related mortality, when compared to control animals. Of note, the breadth and magnitude of vaccine-elicited, Gag-specific cellular immune responses before challenge correlated with the control of set-point viral loads after challenge, which suggested a critical importance of Gag-specific T-lymphocyte responses in controlling viral replication. Furthermore, the vaccine used in this study expressed only a single SIV Gag antigen and did not include a homologous Env immunogen. It could therefore be argued that the observed protective effect was mediated by Gag-specific cellular immune responses rather than Gag-specific antibodies. However, the protective efficacy of this vaccine regimen against highly *heterologous* SIV challenges has to be examined in future studies.

1.6.3

Induction of Effector Memory T-Cell Responses at Viral Entry Sites

One major limitation of current vaccine candidates is the fact that induced memory T-cell responses transform increasingly into lymphoid tissue-based central memory cells. This might be due to the decreasing amount of antigen provided by the vaccine vectors, as most of these vectors are nonpersistent. Central-memory T-cells, however, are primarily lacking effector functions and require differentiation and expansion in the presence of antigen to respond effectively to virus. However, this might be too slow to prevent the systemic dissemination of HIV. A recent study therefore utilized a SIV protein encoding vector based on rhesus cytomegalovirus (RhCMV); this was chosen because these vectors induce lifelong effector memory T-cell responses. Rhesus macaques vaccinated with the RhCMV vector expressing SIV Gag, Rev/Nef/Tat, and Env maintained robust SIV-specific, CD4⁺ and CD8⁺ effector-memory T-cell responses, despite pre-existing RhCMV immunity. In addition, vaccinated Rhesus macaques were less susceptible to progressive SIV (mac239) infection upon intra-rectal challenge. Four animals were even able to control rectal mucosal infection and prevent the progressive systemic dissemination of the challenge virus [83].

1.7

The Current Vaccine Pipeline

The most immunogenic vaccine used to date has been the adenovirus; however, concerns regarding the possible enhancement of infection in persons with pre-existing adenovirus-specific immunity that were uncovered in the STEP trial, have made the future of this vector uncertain. In the following section we provide a brief review of the candidate delivery systems currently under investigation.

1.7.1

DNA

Although plasmid DNA vectors have elicited effective cellular immune and antibody responses in mice, HIV DNA vaccines could not replicate the same level of

immunogenicity in nonhuman primates, and have been even less immunogenic in humans. In order to improve the immunogenicity of these plasmid DNA vaccines, several approaches for improved *in vivo* expression and more robust protection against degradation have been investigated [84]. The coadministration of plasmid DNA immunogens with plasmids encoding cytokines has proven to increase vaccine-elicited cellular immune responses [85]. Furthermore, alternative strategies to deliver the DNA plasmids have been explored, including delivery of the plasmid by intramuscular injection followed by electroporation *in vivo* [86]. A recent study used DNA plasmids containing gp160, Rev, p17/24 Gag and RT from multiple HIV-1 subtypes and boosted with a heterologous vaccinia virus Ankara (MVA) containing Env, Gag, and Pol. Subsequently, 30% of the vaccinees developed CD8 + and CD4 + T-cell responses after the DNA prime. After the boost with MVA, T-cell responses could be measured in 92% of the vaccine recipients [87]. Further studies are required to investigate how to enhance DNA-induced immune responses.

1.7.2

Adenovirus

Adenovirus-vectored vaccines have the ability to induce strong cell-mediated immunity, and have therefore been considered primary candidates for further vaccine development. The results of early-phase trials were very encouraging, in that the rAd5 vector-based vaccines elicited cellular immune responses in monkey models and later in most human subjects, although these responses were partially suppressed in individuals with pre-existing Ad5-specific neutralizing antibodies [75]. However, even with the DNA prime–Ad5 boost, which was suggested to be more effective at controlling SIV replication than the Ad5 prime–Ad5 boost strategy, only macaques expressing a macaque MHC class I protein that had previously been associated with diminished viral replication (Mamu-A*01) showed some control of SIV (mac239) replication [81, 88]. In order to investigate whether cellular immune responses are able to control viral replication, eight Mamu-A*01-positive rhesus macaques were vaccinated with SIV Gag, Tat, Rev and Nef using a DNA prime–adenovirus boost strategy, excluding Env intentionally. A strong protective effect of the vaccine on peak viremia and viral set point was observed, which supported the idea that a vaccine that induces only cell-mediated immunity might be able to control viral replication [89]. These data, together with the recently demonstrated protective effect of an adenovirus prime boost regimen in macaques challenged with SIV, offers hope for this approach if concerns regarding possible enhancing effects can be overcome.

1.7.3

Peptides

The so-called OPAL study (Overlapping Peptide-pulsed Autologous Cells) did not seek to prevent new infection with SIV, but instead followed new pathways in eliciting potent cellular immune responses in already infected animals. Instead of delivering HIV antigens with vectors to induce CTL and Nab responses, the OPAL approach has

shown promising results in SIV (mac251)-infected macaques. After exposure to overlapping SIV peptides, autologous blood cells were reinfused into the animals. Following immunization, robust SIV-specific CD4⁺ and CD8⁺ T-cell responses were observed, and SIV levels were up to 10-fold lower for one year in immunized animals compared to controls. Furthermore, the AIDS-related mortality in immunized animals was significantly delayed. Interestingly, immunization with all SIV proteins did not show any better effect on viral outcome compared to animals which had been immunized against Gag alone, thus supporting the fact that Gag is an effective stimulator for T-cells [90]. A more advanced approach evaluated inactivated SIV pulsed fresh peripheral blood mononuclear cells in the same animal model. Strong SIV-specific CD4⁺ T-cell responses, but unfortunately lower SIV-specific CD8⁺ T-cell responses, could be measured. Interestingly, most of the functional responses were directed against Gag. In contrast to the previous study, no reduction in viral load was observed [91]. Human trials will undoubtedly follow.

1.7.4

Bacillus Calmette-Guérin

The attenuated, nonpathogenic *Mycobacterium bovis* (Bacillus Calmette-Guérin; BCG) is widely used as a vaccine for TB and leprosy [92], although its efficacy is rather limited. Nevertheless, mycobacteria have distinct characteristics that make them attractive as potential HIV-1 vaccine vectors; for example, their ability to stably express transgenes or to elicit longlasting cellular and mucosal immune responses [93, 94]. So far, recombinant BCG (rBCG) vaccine constructs have been used in multiple murine models to evaluate immunogenicity and protection against various infectious agents, including *Borrelia burgdorferi*, *Streptococcus pneumoniae*, *Bordetella pertussis*, rodent malaria, leishmania, and measles virus. Furthermore, rBCG was able to induce antibody as well as cellular responses against antigens derived from HIV and SIV in murine and monkey studies [95, 96]. A *Mycobacterium smegmatis* vector expressing full-length HIV-1 envelope protein was able to induce functional MHC-class I-restricted HIV-1 epitope-specific CD8⁺ T-cell responses in mice [97]. Furthermore, repeated immunization led to the expansion of central-memory virus-specific cells. Human studies with recombinant BCG-HIV vectors can be anticipated in the near future.

1.7.5

Listeria and Other Bacterial Vectors

1.7.5.1 *Listeria monocytogenes*

Listeria monocytogenes (Lm) is an intracellular bacterium with promising properties, as it infects and induces the maturation of DCs; it therefore has the capacity to stimulate innate as well as adaptive immune responses. Additionally, vaccine antigens encoded by *Listeria* are efficiently presented by both MHC class I and MHC class II molecules, as *Listeria* vectors deliver antigens directly to the DC cytosol, resulting in antigen-specific CD8⁺ and CD4⁺ T-cell activation [98, 99]. Another

advantage, although secondary, is the benefit that *Listeria*-derived vaccine vectors may be given orally as the natural route of *Listeria* infection involves oral exposure [100]. A *Listeria* vector containing two genes of feline immunodeficiency virus (FIV) showed that pre-existing immunity against Lm does not preclude the generation of immunity to foreign antigens expressed by the *Listeria* vector [101]. A recent study compared oral priming/oral boosting versus oral priming/intramuscular boosting of a live attenuated Lm expressing HIV Gag in rhesus macaques [102]. The latter was able to induce Gag-specific cellular immune responses as well as mucosal anti-Gag antibodies, whereas the former could only elicit cellular immunity. Another study showed that an attenuated, recombinant Lm-gag for priming, followed by a boost of a replication-defective rAd5-gag, induced a strong cellular immunity. By modulating the route of priming or boosting (oral, intrarectal, intravaginal, or systemic) differences in CTL activities in the different target tissues could be observed [103].

1.7.5.2 *Salmonella enterica*

Recombinant *Salmonella enterica* serovar Typhi can function as a live vector to deliver HIV antigens and induce both mucosal and systemic immune responses. Recombinant *Salmonella* Typhi vaccines are easy to produce and have been used as oral typhoid vaccines, which can induce mucosal, humoral, and cellular immune responses after immunizing via mucosal surfaces. A recent study used a recombinant *Salmonella* Typhi strain expressing HIV-1 Gag integrated into the bacterial chromosome and *gp120* gene carried by a plasmid, induced high titers of gp120 antibodies as well as Gag and gp120-specific CTL responses in mice [104].

1.7.5.3 *Shigella*

Invasive *Shigella* strains are able to gain access to the cytoplasm of infected cells, and are therefore attractive for DNA vaccine delivery. *Shigella* vectors with DNA encoding HIV gp120 were immunogenic after administration into mice [105]. Mice, which were immunized intranasally with live recombinant bacterial cells carrying a plasmid encoding HIV Gag, showed local and systemic immune responses [106]. *Shigella* vectors might become useful in prime-boost vaccination regimens with DNA vaccines, although additional more detailed studies, preferably in humans, are required.

1.7.6

Canarypox

In the past, poxvirus vectors have shown very variable efficiency in eliciting T-cell responses. In a study using recombinant DNA in combination with a modified vaccinia virus Ankara expressing Gag protein and some immunodominant CD8 + T-cell epitopes [107], only limited immunogenicity was observed. Although, using the vaccines in a higher dose resulted in an enhanced immunogenicity, the response rate based on *ex vivo* interferon- γ ELISPOTs remained limited and was due exclusively to CD4 + T-cells [108]. A very recent study, the EuroVacc 02 Phase I trial,

delivered a prime-boost regimen to evaluate the safety and immunogenicity of recombinant DNA and the poxvirus vector NYVAC, both of which were expressing Env, Gag, Pol, and Nef polypeptides from HIV clade C isolates. A functional analysis of the vaccine-induced T-cell responses indicated that the DNA/NYVAC vaccine combination elicited *ex vivo* T-cell responses in 90% of immunized volunteers, and that these responses were polyfunctional, broad, and durable. Env induced by far the strongest and most frequent T-cell responses (91% of vaccines), although in 48% of the vaccinees responses against Gag-Pol-Nef could also be observed. This led to the conclusion that regimens using DNA/NYVAC vaccine combinations are promising, and support the need for further clinical development [109].

1.7.7

Adeno-Associated Virus

Adeno-associated viruses (AAVs) are single-stranded DNA parvoviruses that infect both dividing and nondividing cells [110], but do not cause disease. The characteristic feature of the adeno-associated virus is a deficiency in replication, and thus its inability to multiply in unaffected cells. However, new AAV particles are successfully generated in the presence of selected proteins derived from the adenovirus genome [111], or other viruses such as HSV [112]. Although recombinant AAV (rAAV) vectors have been initially developed for gene replacement therapy, several characteristics have made these potential candidates for vaccine delivery [113]: (i) rAAV vectors are well tolerated; (ii) they fail to induce strong vector-directed inflammatory responses, although the induction of neutralizing antibodies has been described [114]; and (iii) rAAV vectors do not introduce any of their viral genes into the host cells. The latter point is due to the fact that the inverted terminal repeat (ITR) elements which flank the 4700 nucleotides of the AAV's single-strand DNA genome are minimally required in *cis* to generate rAAV vectors in which all other viral sequences are supplied in *trans* [115]. A Phase II clinical trial using a rAAV vector-based vaccine containing clade C HIV antigens was started in 2006 in South Africa (IAVI Report, <http://www.iavireport.org/Issues/Issue9-5/VaccineBriefs.asp>). However, a recent report suggested that T-cells generated by AAV vectors might have a limited proliferation potential [116].

1.8

Conclusions and Future Directions

The pathway to the development of a licensed HIV vaccine will no doubt be long. Even if a viable candidate vaccine were available today, the manufacture, testing and distribution of such a vaccine could easily take 10 years before it could be made available to healthcare providers for administration in endemic areas. The challenge of a fully preventive vaccine is not likely to be overcome with any of the products that are currently available, and thus a return to basic science to address fundamental concepts is needed. These were highlighted in a recent vaccine summit sponsored by

the US National Institute of Health, and prompted a re-allotment of funds to address some of the basic questions before any major advances are made in the HIV vaccine field. On the other hand, the recent success of a T-cell-based vaccine for an AIDS virus in a monkey model [68, 82] offers hope that the viral load set point might be altered by a vaccine that could function to delay or prevent disease progression. In the meantime, ongoing studies to determine the correlates of immune protection are of paramount importance, and the vigorous investigation of those persons who are able to control viremia in the absence of therapy may represent the most promise for providing the required insights. Meantime, major efforts should be expended not only to prevent new infections but also to implement procedures to slow the intersection of the TB and HIV epidemics.

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2

Immune Response to Tuberculosis as a Basis for Rational Vaccination Strategies

Stefan H.E. Kaufmann and Steffen Stenger

Mycobacterium tuberculosis (*Mtb*), the etiological agent of tuberculosis, is capable of replicating in resting macrophages. Protective immunity is mediated by T lymphocytes which activate antimycobacterial capacities in macrophages and kill the pathogen directly. Yet, even in the face of an active immune response, *Mtb* persists in a dormant stage. Thus, latent infection is the outcome of an ongoing immune response. When immunity weakens, reactivation of *Mtb* occurs and active disease develops. Recent advances in immunology form the basis of rational design of vaccines which are urgently needed because the current vaccine Bacillus Calmette-Guérin (BCG) protects only against miliary tuberculosis in newborn and fails to prevent pulmonary disease in adults as the most prevalent form. Current strategies can be divided into three levels. At Level 1.0, novel subunit vaccines and improved recombinant BCG vaccines have been generated; these prevent disease outbreak but fail to achieve sterile eradication of the pathogen, or to prevent infection. Subunit vaccines at this level are composed of secreted antigens and novel adjuvants which are capable of stimulating T cells. Recombinant BCG vaccines overexpress mycobacterial antigens and/or are endowed with improved immunogenicity. All of these vaccines are aimed at pre-exposure vaccination of a naïve target population. At Level 2.0, the vaccines are aimed at eradicating *Mtb*; these are combination vaccines that are given either pre- or post-exposure. The target population of latently infected individuals comprises two billion people; post-exposure vaccines for this target population are focused on latency antigens. At Level 3.0, vaccines are aimed at preventing infection, for example, by rapidly eliminating *Mtb* that enters organisms which enter promptly after arrival in the pulmonary alveoli. In order to accelerate clinical trials with novel vaccines, and combinations thereof, biomarkers which serve as surrogate endpoints of disease outbreak in the vaccinated study population are urgently needed. Biomarkers are most likely combined into a tailor-made biosignature comprising immunologic and biomic markers.

2.1

Introduction

Goal 6 of the Millennium Development Goals brought forward by the United Nations in the year 2000 focuses on the combat of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), malaria, and other diseases. It claims to halt and reverse the incidence of the global tuberculosis (TB) threat by 2015. This courageous statement has been further specified by the Stop TB partnership by stating that, in 2015, TB prevalence and mortality (including coinfection with HIV) should be half of the 1990 levels. Further along this line, the Global Plan to Stop TB proposes to eliminate TB by 2050 as a global threat – that is, to an incidence rate of less than one TB case per million. Today, at half-way through our journey towards the Millennium Development Goals, global morbidity and mortality have only slightly changed, from almost 310 per 100 000 people suffering from TB and 28 per 100 000 dying of the disease in 1990, to 219 per 100 000 people suffering from TB and 25 per 100 000 dying of the disease in 2006 [1]. The TB crisis has been fueled by the AIDS pandemic, and sub-Saharan Africa suffers most of this deadly combination [2]. In this region, TB is the number-one cause of death in HIV-infected individuals, and HIV is the driving force for the re-emergence of TB. Globally, 15 million individuals are coinfecting with the etiologic agents of AIDS and TB, and this results annually in more than 250 000 additional deaths. The ambitious goals set can, therefore, only be achieved if new drugs and new vaccines of high efficacy become available within the next few years.

TB is caused by the bacterial pathogen *Mycobacterium tuberculosis* (*Mtb*). Typically, it is a pulmonary disease that is transmitted via the aerogenic route. Although poverty is a driving force for the spread of TB, it is difficult to prevent solely by behavioral and cultural measures. On the other hand, we already have at hand effective drugs for the therapy, and a vaccine for the prevention, of TB. Drug treatment is complex and longlasting, comprising three to four drugs over a period of 6–9 months. Inherent in this treatment scheme is an unsatisfactory compliance, which has led the World Health Organization (WHO) to introduce the directly observed treatment short-term course (DOTS) regime [3]. Despite such efforts, 500 000 cases of multidrug-resistant (MDR) TB emerge annually, which cannot be treated by first-line drugs, while up to 40 000 cases of extensively drug-resistant (XDR) TB have been recorded in more than 50 countries. This disease is almost impossible to treat, and not only in poor countries; hence, new drugs are urgently needed.

2.2

Clinical Aspects of TB

Infection with *Mtb* occurs when small infectious droplets (ca. 10–30 μm) are inhaled by a susceptible individual. The droplets each contain up to 300 bacilli, which remain suspended in the air for several hours, so that the risk of infection is not limited to close or direct contact with a tuberculosis patient [4].

The risk of infection with *Mtb* is mostly dependent on exogenous factors, such as the epidemiology of TB in a given region, the social environment, and the quality of the healthcare system. In contrast, the likelihood of developing active disease is mostly determined by endogenous factors; nutritional status, general health, and immunocompetence are most critical in determining the outcome of infection. The vast majority of infected persons (ca. 90%) will not develop active TB. Rather, the bacilli will be contained in an immunological microenvironment termed “granuloma,” and a conversion of the tuberculin skin test will be the only feasible parameter to verify that an encounter with *Mtb* has occurred [5]. Nevertheless, few bacilli will survive and persist during the entire life span of the host, thereby posing a continuous threat to the infected individual. Any compromise of the cellular immune system – most frequently immunosuppressive therapy, HIV infection, or simply advanced age – may allow the resuscitation of dormant bacilli. These can then cause reactivation TB, which is clinically indiscernible from primary infection [6]. Most cases of TB (>70%) in industrialized countries are due to reactivation disease [5].

Primary TB is found predominantly in children aged less than 4 years, that appear to have an inherent deficit in the control of *Mtb*. Frequently, the local lymph nodes (hilar, mediastinal) and the pleura (effusions) are involved in the disease process [4]. The disease in children (primary infection) tends to be more severe than in adults (postprimary, or reactivation disease), and involves the complete lung or the meninges (miliary TB).

Reactivation TB is found in adults that have acquired the tubercle bacilli many years earlier. This form of TB is most frequently localized in the lung (>80%), even though other organs can be affected (lymph nodes, urogenital tract, bones). Reactivation TB is usually limited to defined anatomical compartments, and can be readily treated if the diagnosis is timely and appropriate treatment is initiated promptly.

While the current BCG vaccination prevents primary disease in children with acceptable success, it remains ineffective in preventing the much more frequent reactivation of latent TB in adults [7]. Therefore, one of the most challenging goals in TB vaccine research is to develop strategies that are efficient in the post-exposure stage of infection [8].

With the advent of molecular mycobacteriology it has become clear that different lineages of *Mtb* strains are prevalent in different parts of the world [9, 10]. The genetic make-up of mycobacteria is not only of importance to understand the epidemiology and distribution of *Mtb*, but also influences virulence and drug susceptibility [11, 12]. The most striking example is the recent emergence of the Beijing genotype family of *Mtb* that causes severe disease and accounts for a high proportion of MDR-TB strains [13]. This genotype family is currently spreading to all parts of the world, and its restriction poses a major challenge for global healthcare organizations [14, 15]. Despite intense efforts, details of which genetic, microbiological and functional specificities are responsible for the hypervirulence of this strain remain elusive.

Besides the emergence of hypervirulent strains, there are two threats that highlight the need for an effective vaccine to be developed:

- First, the AIDS pandemic is not under control, and appropriate therapy is only available to a minority of infected patients. AIDS and TB are most prevalent in very similar regions of the world and together form a deadly liaison, which may become a major cause of morbidity and mortality in sub-Saharan Africa [16]. The pathophysiology of HIV, with the hallmark of reduced quantity and quality of CD4 + T-cell responses, is an immediate risk factor for the reactivation of latent *Mtb* infection. The group of patients harboring both HIV and *Mtb* is one of the key target populations for post-exposure vaccines, and deserves particular attention due to the special immunological features in this group.
- The second development of concern in TB treatment is that the previously comfortable situation of having effective anti-TB drugs available has changed profoundly, since the implementation of multidrug treatment in the 1960s. Initially, the occurrence of drug-resistant *Mtb* strains (to mostly one drug, isoniazid; INH) was circumstantial and restricted to poor patient compliance. Subsequently, the susceptibility patterns became more worrisome and the number of rifampin- and INH-resistant strains – termed MDR-TB strains – has begun to increase [17]. Only limited treatment options remain available for MDR-TB-infected individuals, and those few drugs that are available are toxic and of inferior efficacy. Therefore, MDR-TB prolongs the duration of hospitalization, the number of fatalities, and the costs for the public healthcare system.

To add fuel to the fire, the problem of drug resistance is further escalating. XDR-TB strains are spreading throughout the world [18]. These strains are MDR-TB with additional resistance to the frequently used second-line drugs fluoroquinolones and injectable aminoglycosides. The majority of patients infected with these strains are AIDS patients, and the prognosis is extremely poor [19]. Yet more recently, increased incidences of XDR-TB have been recorded in HIV⁻ (and thus immunocompetent) individuals. It appears, therefore, that XDR-TB-causing organisms have learned to survive in the face of an active immune response. Only experimental treatment regimens are available to combat XDR-TB strains. The majority of XDR-TB strains belong to the Beijing family, which highlights the danger arising from specific *Mtb* lineages.

2.3

Immune Response to TB: Innate Immunity

The interaction of *Mtb* with the immune system can be conveniently divided into two different stages. First, the local cells in the lung – mainly alveolar macrophages and a few interstitial dendritic cells (DCs) – sense and respond to the invader. This innate response presents the first line of defense, and in parallel initiates the consecutive adaptive immunity. These two intertwined arms of the host response represent a refined program aimed at containing *Mtb* growth and preventing the development of active TB.

When *Mtb*-containing droplets reach the peripheral small airways of the bronchial system, the bacteria or bacterial components are coated with serum opsonins

and sensed by cell surface-bound receptors. These include complement receptor 3, mannose receptors, scavenger receptors and Toll-like receptors, which are expressed on pulmonary macrophages and/or DCs [20]. The mycobacteria are phagocytosed and then shuttle through a network of intracellular vesicles, which mature from endosomes to phagosomes to phagolysosomes. *Mtb* or mycobacterial fragments eventually reach the lysosome where an acidic pH, nitrogen radicals, oxygen radicals and antimicrobial peptides kill, destroy, digest, and eliminate the foreign particles [21]. Even though it is difficult to estimate the efficacy of these effector mechanisms, epidemiological evidence suggests that in up to 70% of infected individuals innate effector mechanisms are sufficient to arrest infection without the support of adaptive immunity.

The reason for the incomplete efficacy of macrophages in mycobacterial clearance is the special evasion program that mycobacteria activate once they are engulfed by a host cell. A key evasion mechanism is the active prevention of phagosome maturation by the release of the soluble kinase PknG and the recruitment of coronin 1 (formerly termed TACO) to the phagosome [20]. Without support from the cellular immune system these mechanisms favor the survival of *Mtb*.

In addition to directly killing, degrading, and removing *Mtb*, the endosomal pathway in macrophages serves as a link between the innate and adaptive immune systems. Small mycobacterial peptide fragments (12–16 amino acids) are delivered to major histocompatibility complex (MHC) class II compartments where they are loaded onto MHC class II molecules (Figure 2.1). The MHC class II/peptide

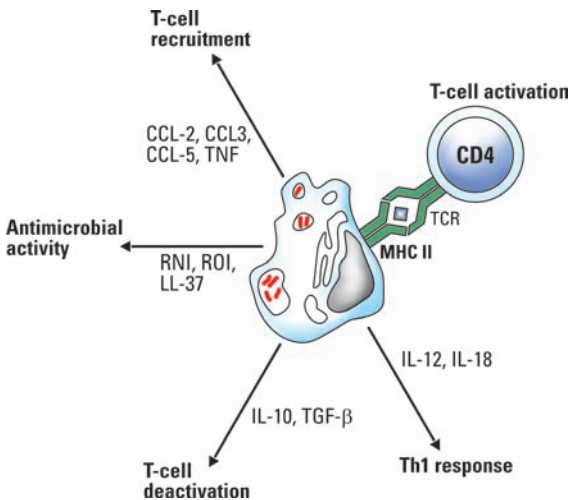


Figure 2.1 Function of macrophages and dendritic cells during infection with *Mtb*. Uptake of *Mtb* by macrophages or dendritic cells (DCs) induces synthesis of nitrogen (reactive nitrogen intermediate; RNI) and oxygen (reactive oxygen intermediate; ROI) radicals and antimicrobial peptides such as cathelicidin (LL-37) that directly kill the pathogen. Infected macrophages also link innate and adaptive immunity by releasing chemokines (CCL-2, CCL-3, CCL-5) and

cytokines (tumor necrosis factor; TNF) that initiate cell movement of DCs (to draining lymph nodes) and T cells (to the site of infection). The quality of the T-cell response is influenced by cytokines such as interleukin (IL)-12 and IL-18, which drive a T helper type 1 (Th1) response. IL-10 and transforming growth factor- β (TGF- β) are released at later stages of infection and suppress the release of inflammatory mediators that would eventually provoke tissue destruction.

complexes – which are still localized within intracellular vesicles – are then shuttled to the plasma membrane, where the cargo is offered to circulating T lymphocytes monitoring the host tissue for foreign peptides (Figure 2.1).

Alternatively, mycobacterial antigens may escape the vesicular network and gain access to the cytosol. Even though the exact mechanisms remain elusive, there is evidence that metabolites trapped inside the endosomes are not completely excluded from contact with the cytosol. Similarly, mycobacterial proteins and lipids can reach the cytosolic MHC class I molecules, which transport them to the cell surface for presentation to CD8 + T cells. The activation of CD8 + T cells may also be achieved by a process termed “cross-presentation,” where infected macrophages undergo apoptosis and are taken up by DCs which then present the peptides to CD8 + T cells via MHC class I molecules [22]. The simultaneous activation of both CD4 + T helper (Th) cells and CD8 + cytotoxic T cells is a major advantage of live vaccines, and is a guiding principle of an improved BCG vaccine (rBCG:Δ*ureC*:Hly), which is currently being evaluated in clinical trials [23] (see below).

2.4 Adaptive Immunity

2.4.1 T-Cell Subsets

The release of inflammatory cytokines and chemokines by infected macrophages creates a milieu that directs the migration of antigen-laden DCs into the draining lymph nodes (Figure 2.2). Within this immunological compartment, the DCs present antigens to T cells carrying the appropriate T-cell receptor (TCR). Responding T cells can be categorized into different T-cell subsets according to MHC restriction, TCR expression, and antigen specificity [2].

Conventional T cells recognize peptide antigens in the context of MHC class I (8–12 amino acids) or MHC class II (12–16 amino acids). The MHC class I-restricted cytotoxic T cells are CD8 +, and characterized by the ability to lyse infected macrophages and simultaneously kill the intracellular pathogen. The MHC class II-restricted Th cells are CD4 +, and release macrophage-activating Th1 cytokines such as interferon-gamma (IFN- γ) or tumor necrosis factor (TNF). These cytokines convert a resting “helpless” macrophage into a powerful effector cell by promoting phagosome–lysosome fusion and the production of antimicrobial effector molecules [24] (Figure 2.2).

Unconventional T cells comprise T cells that recognize lipid antigens and T cells carrying the $\gamma\delta$ -TCR. Mycobacterial lipid antigens (lipoarabinomannan, mycolic acids, sulfoglycolipid, monomycolates) are presented to the T cells by a unique class of antigen-presenting molecules, group 1 CD1 (CD1a,b,c), which have a special groove designed for fitting hydrophobic moieties [25]. The structure of group 1 CD1 molecules is reminiscent of MHC class I molecules. In contrast, the antigen-presentation pathway resembles that of MHC class II molecules, in that antigens

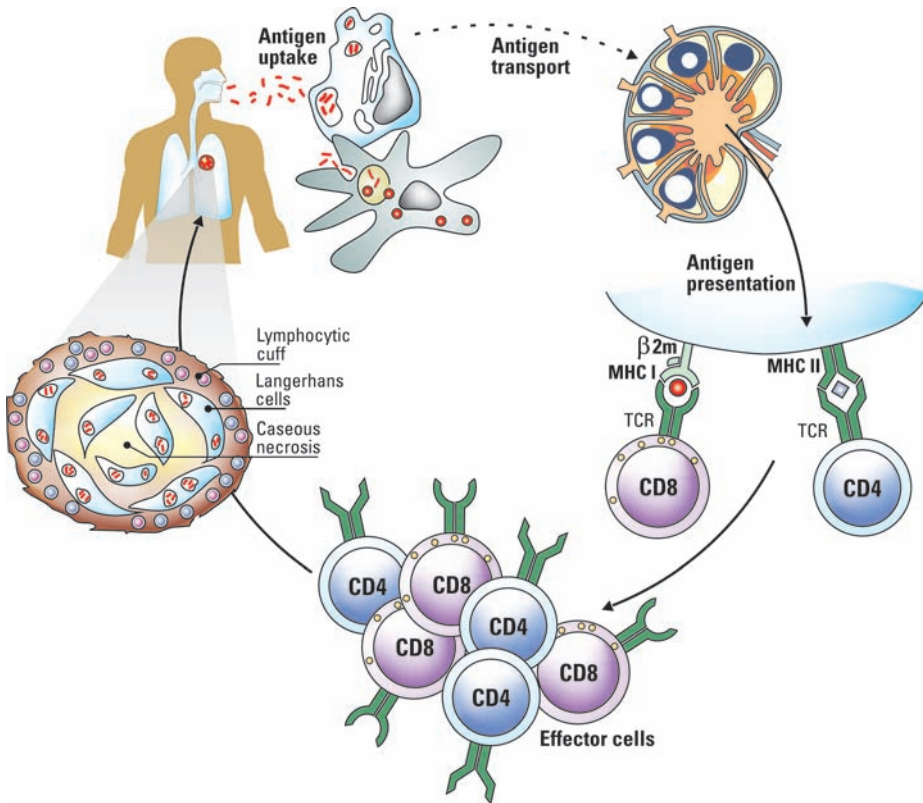


Figure 2.2 The link between adaptive and acquired immunity. Inhaled mycobacteria are taken up by macrophages and dendritic cells (DCs), a process that is mediated by cell surface receptors such as carbohydrate receptors, complement receptors, or lectins (e.g., DC-SIGN). Host cells digest mycobacteria, and TNF mediates the migration of antigen-laden DCs to the draining lymph nodes. Here, antigen is presented to T cells, which mature, proliferate,

and are then directed back to the site of infection where they support macrophages in eliminating the pathogen. This interaction takes place in a granuloma, consisting of central macrophages and Langerhans giant cells, which are surrounded by a lymphocytic cuff. If the immunological balance tips in favor of the pathogen, the center of the granuloma may liquefy, resulting in severe destruction of the lung tissue.

remain in vesicles during their passage through the macrophages. The responding T-cell populations are much more heterogeneous than initially thought, and are CD4⁺ and CD8⁺ and carry either $\alpha\beta$ - or $\gamma\delta$ -TCR [25]. The biology of $\gamma\delta$ T cells is less well understood, and to date the antigen-presenting molecule requires elucidation. There is no doubt, however, that mycobacteria contain small phospholipids that stimulate $\gamma\delta$ T cells. The most prominent functional features of both $\gamma\delta$ T cells and CD1-restricted T cells are found in cytotoxic and antimicrobial activity mediated by perforin, granzymes, and granulysin [26].

From animal models, genetic disorders and observations in HIV patients, it is clear that conventional CD4⁺ T cells are the key players in protection against TB [24].

Animal models suggest that CD8 + cytotoxic T cells complement Th cells, whereas the role of unconventional T-cell subsets is less clear. The lack of appropriate small animal models makes it difficult to investigate the contribution of $\gamma\delta$ T cells or CD1-restricted T cells *in vivo* [27].

2.4.2

T-Cell Activation

Activated T cells will proliferate, mature to an effector phenotype, and migrate back to the site of infection. Local lymphadenopathy detected by X-radiography or computed tomography (CT) scanning provide morphological correlates of the cellular accumulation and proliferation in the local lymph nodes. The quality of the T-cell response is dependent on the local cytokine environment that instructs lymphocytes to mature into either Th1 (protection against intracellular pathogens) or Th2 (anti-inflammatory) lymphocytes (Figure 2.2). Macrophage- or DC-derived mediators favor the development of macrophage-activating Th1 subsets [secreting for example interleukin (IL)-12, IL-15, IL-18] or immunosuppressive T cells [secreting for example IL-4, IL-10, transforming growth factor- β (TGF- β)] that antagonize macrophage activation. This fine-tuned balance is required to stimulate cellular immunity, without causing inappropriate tissue destruction [24, 28].

Productive T-cell responses require at least two signals: the first is mediated by the TCR, and the second is provided by costimulatory molecules present on antigen-presenting cells (APCs). The family of costimulatory molecules comprises the B7 family of ligands on T lymphocytes (CD28, CTLA4) and receptors on APCs [B7.1, B7.2 as well as ICOS/ICOSL and programmed death (PD) 1 : PD-L1/PD-L2]. Costimulation can trigger both positive (B7.1/B7.2: CD28) and negative (B7.1/B7.2: CTLA4; PD1/PD-L; B7.1 : PD-L1) signals [28]. Thus, the functional outcome of T-cell activation is determined by the combination of positive and negative costimulatory signals. The relevance of costimulation for shaping immune responses *in vivo* has been documented in autoimmune diseases and parasitic infections, but not in TB. However, *in vitro* PD/PD-L neutralization inhibited mycobacteria-specific Th1 responses [29]. Clearly, costimulatory molecules influence T-cell responses and their role must be considered, or better yet exploited, for the development of novel vaccines.

2.5

Cytokines as Mediators of Immune Function

2.5.1

IL-12 Family of Cytokines

The recognition and uptake of *Mtb* by macrophages induces the first burst of cytokines, including the release of IL-12, IL-15, and IL-18. IL-12 drives the development of Th1 cells that produce IFN- γ , which in turn activates antimicrobial macrophage functions including radical production, phagosome maturation, and

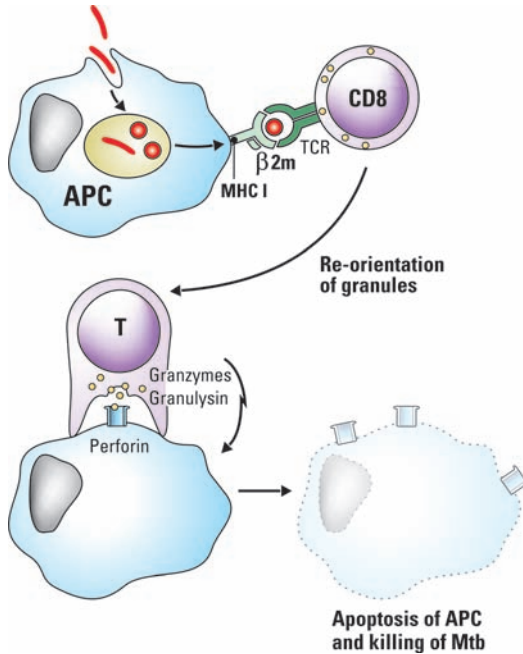


Figure 2.3 Activation and function of CD8 + cytotoxic T cells. Macrophages phagocytose *Mtb* and, in the case of appropriate activation (e.g., IFN- γ) the bacterial cargo is shuttled through a vesicular network involving early endosomes, late endosomes, phagosomes, and acidic phagolysosomes. In case macrophages receive appropriate activation signals, the mycobacterial antigens may also escape the endosomal network and gain access to MHC class I

molecules located in the cytosol. Antigen-specific CD8 + cytotoxic T cells recognize bacterial antigens in the context of MHC class I molecules, resulting in a reorientation of cytoplasmic granules to the contact zone of the T cell and infected macrophage (“immunological synapse”). Components within cytotoxic granules (e.g., perforin, granzymes, granulysin) then act in concert to induce apoptosis of the host cell and directly kill *Mtb*.

autophagy [30]. The relevance of this pathway in protection against TB is highlighted by genetic deficiencies, in which dysfunctional IL-12 or IFN- γ receptors predispose to severe mycobacterial infections [31] (Figure 2.2). IL-12, IL-15, and IL-18 each promote the activation of cytotoxic T lymphocyte (CTL) subsets, including CD8 + T lymphocytes, natural killer (NK) T cells, and NK cells. Granules within these lymphocytes contain perforin, granzymes, and granulysin that coordinately interact to lyse *Mtb*-infected host cells and kill the pathogen [32] (Figure 2.3).

IL-12 is member of a larger IL-12 family comprising IL-12, IL-23, and IL-27. For receptor signaling, IL-12 uses the IL-12R β 1/IL-12R β 2 complex, IL-23 uses an IL-12R β 1/IL-23R complex, while IL-27 uses a gp130/IL-27R α complex [33]. The common usage of receptor components results in overlapping functions of IL-12, IL-23, and IL-27, although recent studies in mice have begun to identify distinct functions. During chronic TB, IL-12 is required for the long-term maintenance of Th1 CD4 T cells in the lung, IL-27 promotes the production of IFN- γ by Th1 cells and limits inflammation, and IL-23 is required for the maintenance of a recently

identified T-cell subset, the Th17 cells [31]. The Th17 cells are characterized by an MHC class II-restricted, antigen-specific release of IL-17, and participate in granuloma formation without any direct impact on bacterial burden [34]. Even though IL-17 is not essential for the outcome of *Mtb* infection, this subset may be special in optimizing vaccine-induced chemokine and cytokine release [35]. Therefore, the targeting of Th17 cells may provide a novel strategy to guide the selection of vaccine antigens.

2.5.2

Tumor Necrosis Factor

Tumor necrosis factor is a macrophage and, to a lesser extent, a T-cell-derived cytokine that is a major player in host immunity to *Mtb*. TNF has been shown to be essential for protection against TB in mouse models of infection by triggering macrophage activation and coordinating cellular trafficking [36, 37]. Even though TNF fails to elicit antimycobacterial activity in human host cells *in vitro* [38, 39], a post-marketing observation with TNF-neutralizing drugs provided striking evidence for a critical protective role *in vivo*. Here, the treatment of patients suffering from severe forms of rheumatoid arthritis (RA), Crohn's disease, psoriatic arthritis and ankylosing spondylitis (AS) with anti-TNF antibodies led to a significantly increased risk of developing severe TB compared to controls [40, 41]. Circumstantial evidence on histological sections [40] and *in vitro* studies on leukocytes from treated patients indicated that the neutralization of TNF during latent TB interfered with T-cell function and cellular trafficking [41].

The pleiotropic functions of TNF in TB are further illustrated by the hyperinflammatory response (immune reconstitution inflammatory syndrome; IRIS) [42] that is observed shortly after the initiation of tuberculostatic drug therapy. Symptoms such as fever, nausea, and bone pain can be ameliorated by TNF-blocking agents such as thalidomide [43], which strongly suggests that TNF (over-) produced by the recovering cellular immune system may be the noxious molecule.

Taken together, these points highlight the delicate role of cytokines in determining the qualitative response to *Mtb*. A careful evaluation of the impact of vaccines on the local and systemic cytokine milieu is warranted to prevent unexpected effects on the immune system.

2.6

Vaccines against TB

2.6.1

From the Past to the Present

At the 10th Congress of Medicine, the claim was made that "... the possibility exists to inactivate pathogens in the host without major side effects – a possibility that was thus far considered extremely unlikely...". This statement raised enormous

excitement and expectations in both the scientific and medical community, as well as among the public [44]. The pathogen in the focus of this claim was *Mtb*, the major killer in Europe at those times, and the statement was made in 1890 by Robert Koch (1843–1910) who had discovered this pathogen 8 years earlier. Koch's claim to cure TB with a therapeutic vaccine was assessed in a clinical trial within a year of his public announcement. In a series of publications, Koch described the content and preparation of the vaccine: a partially purified glycerol extract of liquid cultures of *Mtb* [44]. When the first clinical trials were completed, all illusions of a remedy for TB were dashed, as less than 2% of the treated patients were cured. In other words, this therapeutic vaccine was ineffective. In fact, sporadic reports arose claiming the dissemination of *Mtb* leading to miliary TB in treated patients who suffered from pulmonary disease, and suggesting exacerbation rather than cure. Today, we know that this so-called “Koch phenomenon” is probably an IRIS caused by the vaccination of patients with active TB. Indeed, it continues to represent a major roadblock for a therapeutic – and perhaps even a post-exposure – vaccine.

Koch was the first to attempt the development of a subunit vaccine against TB, but was too ambitious in using the vaccine not as a preventive measure but as a therapeutic intervention. Louis Pasteur (1822–1895), who might be regarded as the forerunner of rational vaccine design, had previously developed a preventive vaccine against anthrax in 1881, that found broad application in veterinary medicine during the nineteenth century [45]. Although, in 1886, Pasteur developed a vaccine against rabies which could be given post-exposure, this was designed to prevent disease outbreak following exposure to the agent, but before the active disease occurred. In both cases, Pasteur had applied pathogen attenuation through specific *in vitro* manipulation as the developmental principle. In 1906, following in the steps of their mentor Pasteur, Albert Calmette (1863–1933) and Camille Guérin (1872–1961) initiated a 15-year series of experiments [46] in which they cultured the etiologic agent of cattle TB on potato slices soaked with ox bile and glycerol. From these studies was derived the original term, Bacille billié Calmette Guérin, later shortened to BCG. As their studies concluded that killed bacteria or bacterial products failed to induce an efficient protection against TB, Calmette and Guérin decided to attenuate a virulent mycobacterium (*Mycobacterium bovis*, the causative agent of cattle TB) through a painstakingly long number of serial passages. The first signs of success were obtained when passage 20 was used to vaccinate guinea pigs and rabbits via different routes of administration, including the oral route. Subsequently, after 13 years the 230th passage was obtained, by which time the mycobacterium had apparently lost all virulence, as measured by the development of lesions after administration to guinea pigs, rabbits, rats, and mice, and to larger animals such as horses, cattle, sheep, and nonhuman primates. It was shown that, in all cases, an immune response as well as protection against experimental human *Mtb* challenge infection had developed.

These positive results led to a preliminary vaccination study being conducted in July 1921, when a newborn child living with a tuberculous grandmother was given three oral doses of BCG. Despite the high risk that the child, if untreated, would develop TB, it remained healthy. As a consequence, 120 children were vaccinated

between July 1921 and June 1922, and 4 years later 80 were seen to be healthy, despite many living in highly contagious surroundings where a family member suffered from active TB. By February 1927, more than 20 000 babies had been vaccinated in total, yet only a few hundred died from TB. Whilst this mortality rate may appear high, the normal risk of death in babies born into a tuberculous household was one in four; in other words, about 5000 unvaccinated babies would have died without vaccination [47].

To date, BCG has been administered to about four billion individuals globally, and consequently is the most widely used vaccine ever [48]. As part of the expanded program of immunization (EPI) of the WHO and the United Nations Children's Fund (UNICEF), among other vaccination programs, 100 million newborns are vaccinated every year. Moreover, the vaccine is well-tolerated and protects against miliary TB in children. Due to its mass production and widespread use, the costs of BCG are very low, and range in the order of less than US\$ 1 per dose, including the cost of the needle and syringe. Numerous studies have assessed the vaccine's efficacy against adult pulmonary TB, which today is the most prevalent form of the disease. Although these analyses have revealed extreme variability, most importantly they have shown that BCG affords no or insufficient protection against adult TB in developing countries – that is, in regions where it is needed most. Hence, a general agreement exists that BCG is insufficient for TB control, and that novel vaccines are urgently needed.

Although, BCG is an attenuated viable vaccine, which can cause adverse reactions, it is generally safe. However, in immunocompromised children, BCG can disseminate and cause a disease termed “BCGosis.” With increasing incidences of HIV in young women, and the failure to provide antiretroviral therapy (ART) during pregnancy as well as during the cesarean section delivery of newborn in several developing countries, safety issues for BCG have gained increasing importance. Accordingly, the WHO has stopped recommending the BCG vaccination of HIV + newborn – irrespective of the potential threat of exposure to TB – if ART treatment cannot be provided [2]. Consequently, novel vaccines which are efficient in adults and safe in immunocompromised individuals – notably HIV + newborn – are urgently needed. In this regard, two strategies are currently being pursued: (i) vaccines which can replace BCG; and (ii) vaccines which are given in addition to BCG [8]. In the following section, an overview is first provided of the different types of vaccine under development, with emphasis placed on those that have already reached the clinical trial stage. The rationale behind the different vaccination strategies will then be discussed, together with details of the different target populations at which the vaccines are being aimed. Novel TB vaccination strategies will be viewed from different vantage points (see Box 2.1) and different levels of feasibility.

2.6.2

The Future

2.6.2.1 Goals of Vaccination

The general goal of all TB vaccines is efficacious prevention of disease [49].

Box 2.1**The three levels in TB vaccine development**

• Goals

Level 1.0: Delay disease outbreak

Level 2.0: Achieve sterile eradication

Level 3.0: Prevent infection.

• Strategies

Level 1.0: First generation, in clinical trials:

- a. Subunit boost on top of BCG prime
- b. Substitution of BCG by recombinant live vaccine.

Level 2.0: Second generation, in preclinical testing:

- a. Subunit boost on top of prime with recombinant live vaccine
- b. Improved adjuvant or carrier and optimal antigen combination
- c. Improved recombinant live vaccine expressing optimal antigen combination.

Level 3.0: Third generation, on the drawing board:

- a. Vaccine which causes sterile eradication
- b. Vaccine which induces mucosal immunity that prevents infection.

• Targets

Level 1.0: Pre-exposure vaccine in uninfected individuals

Level 2.0: Post-exposure vaccine in infected individuals

Level 3.0: Therapeutic vaccine in TB patients.

- *Level 1.0* is the least ambitious and attempts to delay disease outbreak. The *Level 1.0* strategy is based on our knowledge that 90% of all *Mtb*-infected individuals will not develop active TB due to efficacious containment of the pathogen in granulomas. Thus, it tries to mimic the acquired immune response against natural infection with *Mtb*. This is the most likely goal to be achieved, and all vaccines currently in clinical trials are aimed at this goal. As long as the immune response is of sufficient potency, *Mtb* can probably be contained. However, in immunocompromised individuals the immune system can no longer keep *Mtb* in check, and disease is likely to break out. With increasing incidences of HIV in *Mtb*-infected individuals this strategy needs to be improved [49].
- At *Level 2.0*, the vaccines attempt to further strengthen the acquired immune response so that it is superior to that stimulated by natural *Mtb* infection and achieves sterile eradication of *Mtb*. This strategy depends on T-cell responses of

superior quality and quantity as compared to those against natural *Mtb* infection. The advantage, and even necessity, of such a vaccine is obvious. An individual vaccinated with this vaccine would remain free from the risk of TB reactivation even when coinfecting with HIV, or when suffering from other immune-suppressive conditions [49].

- *Level 3.0* is even more ambitious and aims at preventing *Mtb* infection. It can be envisaged that high antibody titers in the lung are able to eliminate *Mtb* before it enters its safe niche, namely the alveolar macrophages and interstitial pulmonary DCs. This approach is currently without precedent but is obviously of greatest interest. The only caveat of this strategy is the need for continuous vaccine immunity through booster vaccinations, as *Mtb* will never engage with the immune system [49].

2.6.2.2 Vaccination Strategies

We will now turn to the three different strategies currently in development (see Box 2.1), all of which are aimed primarily at delaying disease outbreak and can, potentially, be optimized to achieve sterile eradication [49]. The design of a vaccine to prevent infection via the induction of mucosal antibodies has not yet been attempted.

Strategies at *Level 1.0* take advantage of a prime vaccination with conventional BCG to strengthen the immune response by booster with a subunit vaccine (Table 2.1). This strategy has been termed “heterologous prime–boost vaccination.” Several subunit vaccines have already entered Phase I or Phase II clinical trials [50–52]. These are composed either of a dominant protein antigen formulated in an adjuvant, which stimulates T cells efficiently, or is expressed by a recombinant viral carrier [53]. None of these vaccine candidates induces protection at a level better than BCG in preclinical models, but all increase the protective efficacy caused by a BCG prime.

An alternative strategy is to substitute BCG with a recombinant live vaccine (Table 2.1), and two candidates of this type have now entered clinical trials. The first candidate is a recombinant BCG (rBCG)-expressing Antigen 85 B [54]. The rationale behind this vaccine is that the overexpression of a dominant antigen will improve the immune response and, indeed, the results of preclinical studies support this assumption. The second candidate attempts to stimulate a stronger and qualitatively different immune response as compared to BCG. This rBCG : Δ ureC : Hly induces superior protection in preclinical models [23]. Most likely, this vaccine perforates the phagosomal membrane, thus allowing translocation into the cytosol of both antigens and lysosomal enzymes such as cathepsins. This results in a direct loading of antigenic peptides onto MHC I and/or apoptosis of infected macrophages, allowing for cross-priming. In this way, *Mtb* antigens can be presented by DCs to different T-cell populations.

Both of these strategies are aimed at delaying disease outbreak and, hopefully, providing sterile eradication. However, little evidence supports this hope. At *Level 2.0*, success may be achieved by combining a prime with the best recombinant live

Table 2.1 TB vaccine candidates currently undergoing clinical trial.

Candidate	Type	Status	Developer(s)
Subunit vaccines:			
Mtb 72 fusion protein in AS02A	Subunit (protein/adjuvant)	Phase II	GlaxoSmithKline (http://www.gsk.com)
Ag85B/Esat-6 fusion protein in IC-31	Subunit (protein/adjuvant)	Phase I	Staten Serum Institute (http://www.ssi.dk) and Intercell (http://www.intercell.com)
Ag85B/TB10.4 fusion protein in IC-31	Subunit (protein/adjuvant)	Phase I	Staten Serum Institute (http://www.ssi.dk), Intercell (http://www.intercell.com) and Aeras (http://www.aeras.org)
r-MVA-Ag85A	R-virus	Phase II	University of Oxford (http://www.ox.ac.uk)
R-Adeno-Ag85A/Ag85B/TB10.4	R-virus	Phase I	Crucell (http://www.crucell.com) and Aeras (http://www.aeras.org)
Improved BCG:			
r-BCG-Ag85B	Viable	Phase II	UCLA School of Medicine (http://www.cme.ucla.edu) and Aeras (http://www.aeras.org)
r-BCGΔure : Hly	Viable	Phase I	Max Planck Institute for Infection Biology (http://www.mpiib-berlin.mpg.de) and Vakzine Projekt Management (http://www.vakzine-manager.de)

vaccine candidate and a boost with the best subunit vaccine candidate [49]. In addition, the subunit vaccines can be further improved by optimizing the adjuvant or carrier and the antigen combination. Similarly, recombinant live vaccines could be further advanced by expressing dominant antigens contained in the subunit vaccine used for booster vaccination and further improving its immune stimulatory capacity. The latter option includes the introduction of genes encoding cytokines involved in protective immunity against TB, and the deletion of anti-apoptotic genes to further enhance cross-priming.

It is now hoped that a second generation of TB vaccination strategies will lead to a vaccine that achieves sterile eradication, and this is indeed a feasible goal. Nonetheless, time will tell whether it can indeed be achieved by vaccines currently in preclinical testing, and novel strategies must be designed that provide robust protection that will reliably causes the sterile eradication of *Mtb*. Although new approaches are now being considered, based on the most recent insights into immunology, all of these attempts are still on the “drawing board.” Alternatively, a vaccine which induces pulmonary mucosal immunity with high efficacy could prevent infection at the port of entry for *Mtb*. It is most likely that IgA antibodies, which first stimulate uptake by phagocytes in the lung and then block vital functions in *Mtb* as it enters the alveolar space, are the most likely candidates. Typically, the corresponding antigens are expressed on the bacterial surface. Evidence in other systems has shown that the blocking of vital bacterial functions, accompanied by

rapid uptake by neutrophils, monocytes and macrophages, can stimulate a prompt bacterial eradication and thus prevent infection [55, 56]. Unfortunately, this approach is the least developed, and reflects more “wishful thinking” than “concrete planning” at this stage.

2.6.2.3 Targets for Vaccination

The third viewpoint is concerned with the target populations for vaccination. Obviously, the uninfected newborn, which has not encountered *Mtb*, is the main target for a pre-exposure vaccine at *Level 1.0*. At *Level 2.0*, a post-exposure vaccine of already infected but healthy individuals is being envisaged; this population comprises two billion people globally.

It is likely that different sets of antigens will be required for the vaccination of naïve and latently *Mtb*-infected individuals. Secreted proteins are the main antigens for pre-exposure vaccines, and current research efforts are focused on analyzing the value of so-called “dormancy antigens” for post-exposure vaccines. As a combination of *Level 1.0* pre-exposure and *Level 2.0* post-exposure vaccination, it is also possible to consider a pre-exposure prime with a recombinant live vaccine expressing latency antigens (e.g., an improved rBCG), followed by a post-exposure boost with a subunit vaccine composed of the same latency antigens at later stages of age when individuals are frequently infected.

Finally, at *Level 3.0*, therapeutic vaccines for TB patients are being considered but are currently beyond the realm of feasibility. Such vaccines are thought to be administered as an adjunct to chemotherapy. With increasing incidences of MDR- or even XDR-TB, such therapeutic vaccines may gain importance if currently effective TB drugs become ineffective.

2.7

Biomarkers

Increasing efforts aim at the definition of biomarkers which allow the state of *Mtb* infection and of active TB disease, as well as of the efficacy of intervention strategies, to be monitored [2, 57]. Whilst such biomarkers first and foremost provide guidelines for novel diagnostics, they also provide insights into the mechanisms underlying the severity, progression, and cure of disease. Moreover, monitoring with biomarkers can shorten clinical trials for novel intervention strategies. There are numerous terms and definitions in the field of clinical trials and biomarkers; for vaccination against TB, the following are the most important. Currently, vaccine trials rely on a clinical end-point in Phase III trials; that is, the development of active TB. However, already in Phase I and II clinical trials, numerous immunologic and other biomarkers are measured which can provide the basis for correlates or surrogates of protection. In this context, biomarkers are considered as indicators of normal or pathological processes, and above all as indicators of the host response to vaccination [58]. Thus, all immune responses which are induced by a vaccine can be defined as “biomarkers.” Yet, biomarkers need not necessarily represent correlates of protection. To satisfy the

definition of a correlate of protection, the response must be directly correlated with the protective status against infection and prevention of active TB. The term “surrogate of protection” is further specified as a validated correlate of protection. Consequently, a surrogate endpoint is a biomarker that can substitute for the clinical endpoint – that is, active TB. Such a surrogate endpoint is defined by means of surrogates of protection, and should predict the emergence of active TB as early as possible [59]. Conventional vaccines, for example against diphtheria or tetanus, often place trust on the measurement of circulating antibody titers against the protective antigen. Such antibody titers serve as correlates of protection as they directly relate to protection against the disease – in this case, diphtheria or tetanus. However, protection against TB is mediated by T cells, and the rules for antibody-mediated protection do not hold true for T-cell-dependent protection. Thus, we are far from having reliable correlates of protection against TB [2].

First, in the arena of T-cell immunology, protective antigens cannot be readily defined, and it is most likely that several antigens participate in the protection. Second, a multitude of T-cell mechanisms contribute to protective immunity in TB; thus, IFN- γ -producing CD4 + T-cells with specificity for a dominant antigen such as antigen 85 of *Mtb* are important mediators of protection. Consequently, IFN- γ -producing CD4 + T-cells with a specificity for antigen 85 can be used as biomarkers to measure the response to a vaccine composed of this very antigen. Yet, it does not qualify as correlate of protection, because it neither proves protection against infection nor against active TB. It is known that vaccination with this very antigen stimulates IFN- γ -secreting CD4 + T-cells, which reduce the bacterial load in vaccinated animals. However, the situation becomes even more difficult for viable vaccine candidates which express a multitude of antigens and stimulate a combination of T-cell populations [60]. Whilst this broad response is considered an advantage of such vaccines, it is difficult to predict which antigen(s) should be chosen for defining the correlate of protection. Moreover, it has become clear that different T-cell populations are required for efficacious protection. Current knowledge favors memory T cells, which are phenotypically defined by the CD45RO marker. These coexpress multiple cytokines, including IFN- γ , IL-2 and TNF- α , the so-called “polyfunctional memory T cells.” The memory T cells segregate further into central and effector memory T cells, according to surface-expressed molecules that determine their migration pattern to lymphoid organs or affected tissue sites [61]. In conclusion, in the field of TB we are far from having a correlate of protection or a surrogate endpoint for active TB which could shorten clinical trials. On the other hand, with increasing vaccine candidates and combinations between different vaccine candidates being ready for clinical trials, we urgently need such markers. If it is assumed that a pre-exposure Phase III trial for TB vaccines lasts between five and 10 years, depending on the type of vaccination schedule and target population until the clinical endpoint is reached, the consecutive testing of different vaccine combinations could easily last for decades. This is far too long, and every effort must be undertaken to define the correlates of protection and surrogate endpoints of active TB. These endeavors need to be complemented by a definition of biomarkers which predict the risk of developing active TB in latently infected individuals. These

correlates of resistance/susceptibility would allow the identification of the approximate 200 million individuals at risk of active TB among the two billion latently infected people who should qualify for preventive measures, such as preventive drug therapy.

Specific immunologic markers are likely important for the monitoring of vaccine trials, for diagnosing latent infection versus active TB disease, and for predicting the risk of developing active TB. These specific responses need to be complemented by global biomics approaches, notably transcriptomics, proteomics, and metabolomics. Ultimately, a combination of different biomarkers may turn out to be most successful approach. As an added value, a combination of the different biomics from gene transcription via protein expression to catabolic and metabolic events, will provide novel insights into the crosstalk between host and *Mtb*, and thus help form the basis for the identification of targets for novel intervention strategies.

2.7.1

Immunologic

Because of the critical role of T lymphocytes in acquired immunity against TB, great efforts have been made to identify the most relevant antigens reflecting active TB disease and latent *Mtb* infection [57]. Antigen discovery is accompanied by the identification of relevant T-cell populations and functions. The currently preferred antigens are ESAT-6 and CFP-10, while the most widely used immune response is IFN- γ production by CD4 + T-cells to these antigens. Recent findings indicating a particular role of multifunctional T cells (secreting different cytokines) in protective immunity against intracellular pathogens warrant more complex assays [62]. Currently, a longitudinal study using different *Mtb* antigens and different cytokines is ongoing to elucidate the most reliable combination for immunologic biomarker discovery in TB (<http://www.biomarkers-for-tb.net/>) [63]. Although, antibodies apparently play a minor role in protection against natural infection with *Mtb*, a plethora of antibodies is produced during *Mtb* infection and, despite many failures, ongoing research exploits the antibody repertoire against *Mtb* for diagnostic purposes. Current attempts to test antibody responses against the whole proteome of *Mtb* in an unbiased way, may lead to a custom-made antibody assay for TB with higher sensitivity and specificity than its predecessors [64].

2.7.2

Transcriptomics

The transcriptomic assays allow the determination of RNA transcription from expressed genes, and hence can provide a genome-wide expression profile [57]. Generally, peripheral blood leukocytes are used as surrogate tissue. The relative distribution of cells in peripheral blood may vary according to the disease stage, which makes the deconfounding of exogenous influences a critical issue. Transcriptome analyses of peripheral blood leukocytes have revealed that global gene expression

profiling can be reduced to a handful of differentially expressed genes which allow the reliable distinction of latent *Mtb* infection from active TB. Ongoing studies will assess the value of transcriptomics for predicting TB risk in infected individuals, and ultimately for the monitoring of clinical trials [57, 65].

2.7.3

Proteomics

In TB, proteomic biomarker discovery is mostly focused on analyzing serum/plasma proteins [57, 66]. Because of the disproportionate distribution of serum proteins, particular efforts need to be made to detect low-abundant proteins [67]. Thus, fewer than 10 proteins in human serum represent more than 90% of its total protein mass. Nevertheless, proof-of-principle of the value of serum/plasma proteome profiling for TB biomarker discovery has been achieved by showing that a combination of four proteomic markers allowed for distinguishing active TB disease from controls.

2.7.4

Metabolomics

Metabolomics comprises small molecules derived from the host, the pathogen, or the environment [57, 68]. Thus, the identification of small *Mtb*-specific molecules (e.g., *Mtb* lipids) can provide the initial of a biosignature that allows a differential diagnosis of active TB from other chronic inflammatory diseases, such as Crohn's disease or sarcoidosis. Neither proteomics nor transcriptomics is suited for this purpose. Although blood serum provides the main source for metabolomics, the use of urine would be technically more feasible. A proof-of-principle study has shown that serum metabolomics allows for the reliable distinction between latent *Mtb* infection and active TB disease (unpublished results). *Mtb* is rich in distinct glycolipids; moreover, the lipids of *Mtb* have been identified in the serum of patients. Thus, lipidomics may represent a subdiscipline of metabolomics of particular value for TB biomarker exploration. *Mtb* also produces a variety of volatile compounds which can be measured in the breath [69]. Whilst this area is in its very early stages of development, it has the major advantage of easy accessibility of material.

2.8

Concluding Remarks

Biomarker studies are a relatively new field in TB research, and further studies are clearly required before a correlate or surrogate of disease susceptibility or of protection can be defined. Once this has been achieved, however, issues of technical feasibility will need to be tackled to allow easy performance under field conditions. Obviously there is a long way to go, but the rewards of having correlates for different stages of TB would be enormous. Notably, this approach could

significantly accelerate the onset of clinical trials for the novel drugs and vaccines that are urgently needed to bring the global threat of TB under control, as proposed by the Millennium Development Goals and the Stop TB partnership.

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3

BCG Vaccination in the HIV + Newborn

Willem A. Hanekom and Gregory D. Hussey

3.1

Bacillus Calmette-Guérin (BCG) and its Efficacy in Healthy Infants

Bacillus Calmette-Guérin (BCG) has been given to more than three billion persons since its first use in 1921. The routine is to administer a single dose of the vaccine at, or soon after, birth in countries where tuberculosis (TB) is endemic. Most industrialized countries administer BCG to selected high-risk groups only.

“BCG” constitutes a group of strains of live, attenuated *Mycobacterium bovis*. The parental strain was created in 1908 after serial subculture of the virulent bacterium over a period of 13 years. The different vaccines in clinical use today emerged after subculture of this parental strain in many laboratories; these strains have varying phenotypic and genotypic characteristics [1].

After its initial use as an oral vaccine, the intradermal administration of BCG is used most commonly today, as recommended by the World Health Organization (WHO) [2]. The vaccine affords approximately 80% protection against disseminated forms of TB in infancy – that is, miliary TB and TB meningitis [3–5]. It is estimated that, each year, BCG prevents approximately 30 000 cases of TB meningitis and about 11 000 cases of miliary TB worldwide [3, 6].

The effectiveness of BCG in preventing pulmonary TB in adults and in infants is highly variable, with efficacies ranging between 0% and 80% (average 50%) having been reported from multiple clinical trials performed during the twentieth century [6]. The reasons for such variable protection may include geographic location, BCG strain variation, patient age at vaccination, the dose of vaccine, interference by environmental mycobacterial and helminthic infections, patient nutritional status, and host genetic factors [1, 6–8]. A recent Phase IV trial of BCG vaccination has addressed whether the route of vaccination may impact on BCG efficacy [9]. For this, the Tokyo strain 172 was administered at birth via either the intradermal or percutaneous route, in a randomized study, to 11 680 infants. Subsequently, equivalence in TB disease incidence over the first two years of life was demonstrated between the two groups. It follows that, although BCG has been used for more than

80 years, many questions regarding the variability of its effective use remain unanswered. Irrespective of these findings, it is clear that BCG is generally poorly protective against the form of TB responsible for the spread of the bacillus, namely pulmonary disease.

BCG also has other medical benefits, however. For example, vaccination is associated with a lower mortality of TB disease, if this is not prevented by the vaccine [10]. The vaccine also protects against two other mycobacterial diseases, leprosy and Buruli ulcer, caused by *Mycobacterium leprae* and *Mycobacterium ulcerans*, respectively [11, 12]. BCG is also used to treat bladder carcinoma, and limited evidence is available that BCG may have some efficacy in preventing *Mycobacterium tuberculosis* infection [13]. The vaccination of infants with BCG may also improve their overall survival in lower socioeconomic environments, although this effect appears unrelated to mycobacterial infection and disease [14]. BCG has also been shown to enhance the antibody responses to other vaccines given early in the first year of life [15]. Finally, the vaccine may prevent allergy early in life, although conflicting results on this outcome have emerged [16, 17].

3.2

Adverse Events Caused by BCG in Healthy Infants

Following the intradermal administration of BCG, mild local cutaneous adverse events occur in virtually all recipients (Figure 3.1). After 3 weeks, a red macule



Figure 3.1 Normal evolution of the local skin reaction following intradermal vaccination with BCG.

Table 3.1 Revised classification of BCG disease.

Type of BCG disease	Characteristics
Local	A local process at the site of vaccination, which may include: <ul style="list-style-type: none"> • an abscess ($\geq 10 \text{ mm} \times 10 \text{ mm}$ – EPI definition), or • severe ulceration.
Regional	Ipsilateral involvement of regional lymph nodes beyond the vaccination site, including <ul style="list-style-type: none"> • axillary • supraclavicular • cervical • upper arm glands Involvement may include enlargement (EPI definition), suppuration or fistula formation.
Distant	Involvement of any site beyond a local or regional ipsilateral process. Isolation of <i>M. bovis</i> BCG from at least one site such as the: <ul style="list-style-type: none"> • lung (pulmonary secretions, including gastric aspirate) • cerebrospinal fluid • bone • urine • distant skin
Disseminated (original classification by Hesselning <i>et al.</i>) [18]	<i>M. bovis</i> BCG isolated from more than one distant site, and/or from at least one blood or bone marrow culture.
Disseminated (our proposed modification)	<i>M. bovis</i> BCG isolated from either a distant site, and/or from at least one blood or bone marrow culture.
BCG immune reconstitution inflammatory syndrome (BCG-IRIS)	BCG disease that occurs within 3 months of commencing combination antiretroviral therapy (cART).

The diagnosis of BCG disease, particularly when distant to the vaccination site, requires a high index of suspicion. Further, although ipsilateral axillary lymph gland involvement is likely due to BCG, supraclavicular or cervical lymph gland involvement requires the exclusion of other causes.

becomes visible, which develops into a papule by week 6. This papule then evolves into a shallow ulcer by week 10, which characteristically heals by week 14.

A practical classification system of more significant adverse events has recently been proposed by Hesselning *et al.* (Table 3.1) [18], with “local,” “regional,” “distant,” or “disseminated” disease being described (Table 3.1). Here, a modification of this classification is proposed, so that the term “disseminated” BCG disease may include any nonlocal or nonregional involvement (see Table 3.1).

BCG has proven to be extraordinarily safe in healthy infants, with local BCG disease in children aged less than one year being estimated to occur in <0.04% of the vaccine recipients [19, 20]. Disseminated BCG disease occurs in <0.002% of infant vaccine recipients [19, 20]. It is thought – and has often been demonstrated – that disseminated BCG disease occurs only in infants with underlying congenital immune deficiencies, such as those of the interleukin-12/interferon-gamma (IL-12/IFN- γ) pathway [21] that is critical for protection against mycobacteria.

Concern that the live attenuated organism in the vaccine may cause disease in the face of immune compromise also represents a reason for the current recommendation not to administer BCG to persons with impaired immunity, with any known or suspected congenital immunodeficiency, with leukemia, lymphoma or generalized malignant disease, who are receiving immunosuppressive therapy, or who are pregnant [2].

3.3

Specific Immunity Induced by BCG in Healthy Infants

The immune correlates of human vaccination-induced protection against TB are not known. Therefore, the term “vaccine take” would be most appropriate to describe immunity induced by BCG. Initially, BCG take was assessed by the tuberculin skin test (TST) reactivity, and diverse strains or different routes of vaccination did induce differential TST reactivity. However, it should be emphasized that TST reactivity has been shown not to correlate with protection against TB [22].

Recently, more detailed descriptions of BCG-induced immunity, particularly of the T-cell immunity thought to be important for protection against TB, have emerged. BCG vaccination of infants appears to induce a CD4 T-cell response in most recipients, if not all [7, 23, 24]. Multiple, diverse subsets of CD4 T cells are induced, based on a capacity to produce IFN- γ , IL-2 and/or tumor necrosis factor (TNF) (Figure 3.2) [23]. CD4 T cells capable of producing these type 1 cytokines may have a central role in protection against TB. CD4 T cells that produce only IFN- γ will dominate, although a “polyfunctional” subset, capable of producing all three cytokines together, is also induced. Polyfunctional T-cell induction is associated with an improved outcome in animal models of chronic intracellular infection [25], including TB [26].

The vaccine response appears to peak within the first three months of life, and to then wane towards one year of age (Figure 3.3) [27]. Throughout the first year of life, most specific CD4 T cells have an effector memory phenotype, based on CCR7 and CD45RA expression [23] (B. Kagina *et al.*, unpublished observations). CD8 T cells may also be important in protection against TB, and are also induced, albeit at a much lower frequency than CD4 T cells [23, 28]. These specific cells have been shown to have cytotoxic potential, or to produce IFN- γ [28]. BCG also appears to induce other T-cell subsets, such as IL-17-producing and regulatory CD4 T cells and $\gamma\delta$ T cells [29] (T.J. Scriba, B. Kagina and W.A. Hanekom, unpublished results). The present authors’ group is currently focusing their efforts on delineating which immune responses correlate with BCG-induced protection against clinical TB in infants.

Both, quantitative and qualitative differences in the BCG-induced immune response have been ascribed to geographic location, to the age of administration, and to strains used for vaccination [30–32]. Yet, there is an urgent need to confirm these results, as BCG is likely to remain the backbone of novel TB vaccination strategies.

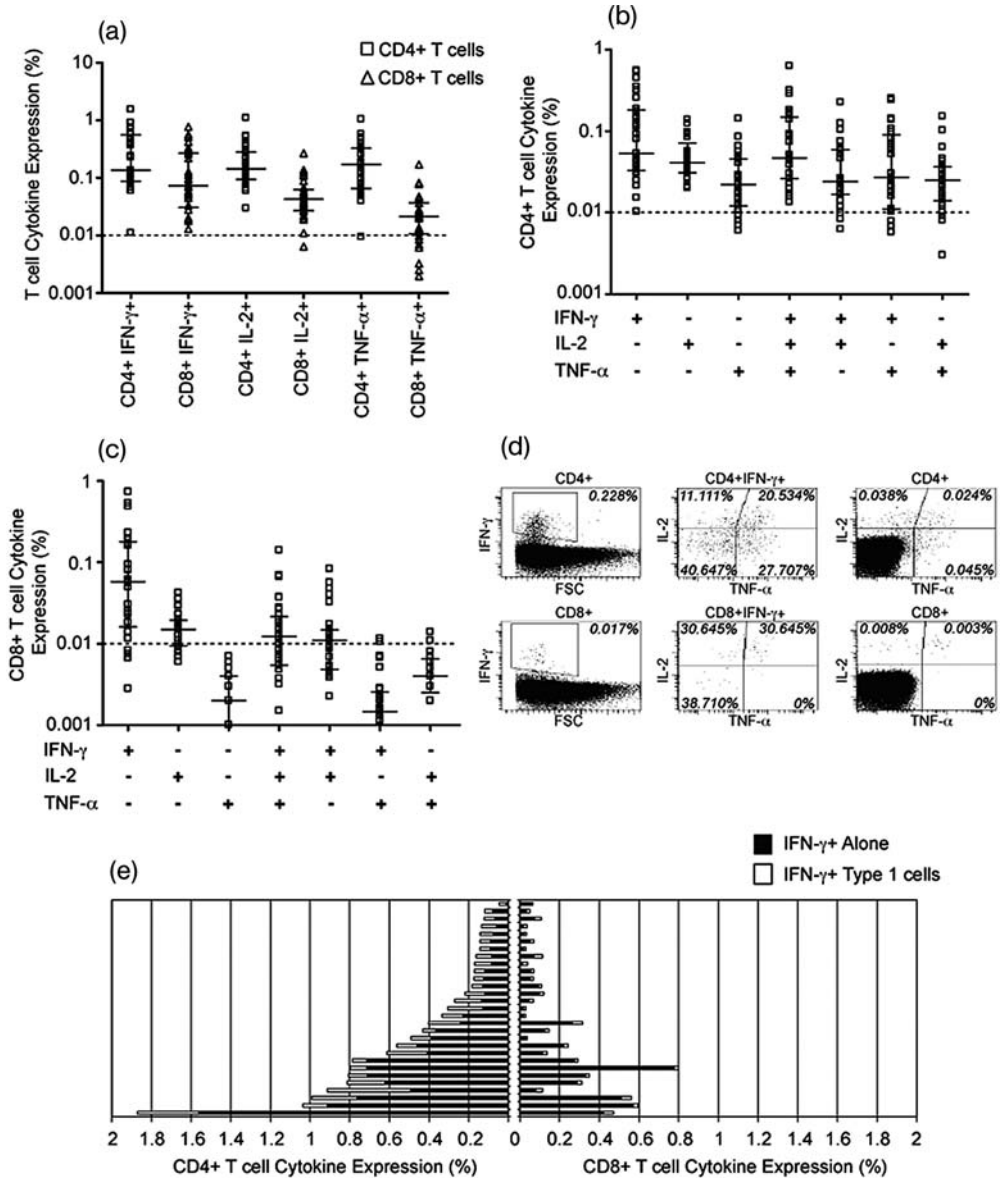


Figure 3.2 Cytokine profiles of BCG-specific T cells in HIV-unexposed 10-week-old infants, vaccinated at birth. (a) Frequency of CD4 + and of CD8 + T cells expressing individual Type 1 cytokines following incubation of blood with BCG for 12 h, in 29 infants, using a whole-blood intracellular cytokine assay [71]. Responses above 0.01% were considered positive. The horizontal line indicates the median, and the whiskers the interquartile range; (b) Frequency of BCG-specific CD4 + T cells expressing different combinations of Type 1 cytokines; (c) Frequency

of BCG-specific CD8 + T cells expressing different combinations of Type 1 cytokines; (d) Representative staining of intracellular Type 1 cytokines in BCG-specific CD4 + T cells and CD8 + T cells, from a single 10-week-old infant; (e) Comparison of frequency of CD4 + and of CD8 + T cells expressing IFN- γ (dark bars) with frequency of T cells expressing IL-2 and/or TNF- α without IFN- γ (light bars) in 29 BCG-vaccinated infants. Reprinted with permission from Ref. [23].

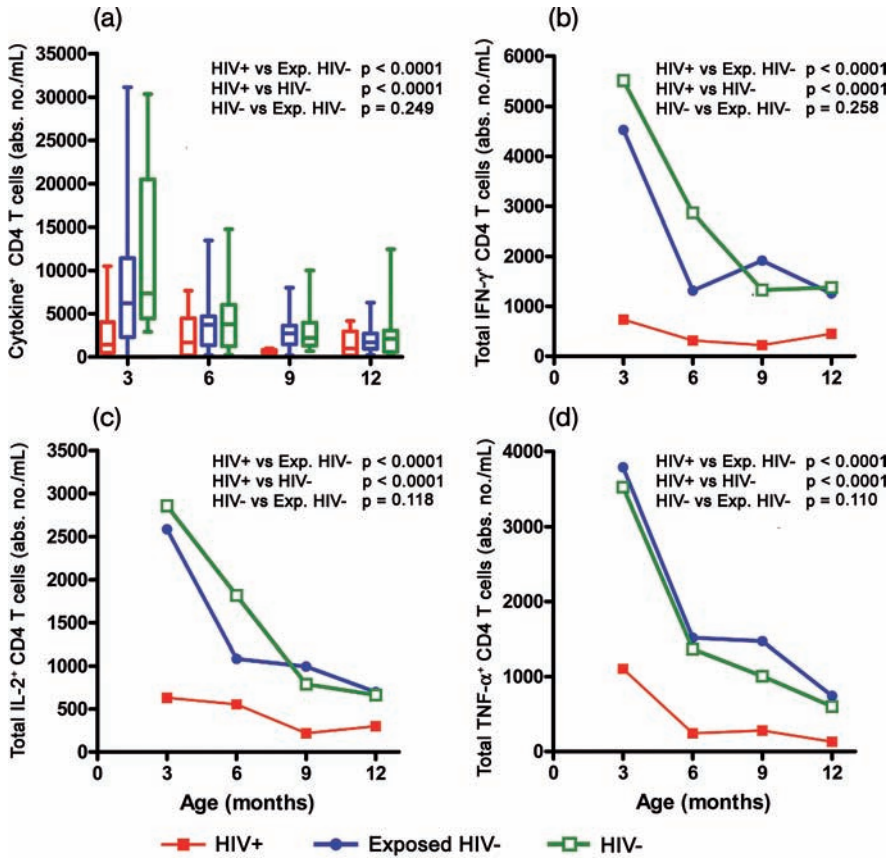


Figure 3.3 CD4 T cell cytokine responses induced by incubation of whole blood with BCG, as measured by the whole-blood intracellular cytokine detection assay [71], in HIV-infected (HIV+, $n = 20$), HIV-exposed but uninfected (Exp. HIV-; $n = 25$), and HIV-unexposed (HIV-; $n = 23$) infants [27]. (a–d) Absolute (abs.) cytokine-producing CD4 T-cell numbers (no.) in the three infant groups. Background values (unstimulated blood) were subtracted for each measurement. (a) Total cytokine response at each time point post-vaccination. For each

boxplot, the median is represented by the horizontal line, the interquartile range by the box, and the range by the whiskers; (b–d) Median total IFN- γ -producing CD4 T cells (b), median total IL-2-producing CD4 T cells (c), and median total TNF- α -producing CD4 T cells (d), in each infant, at each time point. Comparisons (p -values) were calculated from mixed effects maximum likelihood regression models on log-transformed responses that also included time as a continuous effect.

3.4

Efficacy of BCG to Prevent TB in HIV-Infected Infants

Tuberculosis is extremely common in HIV-infected infants living in areas where the disease is common. For example, between 2004 and 2006 the incidence in Cape Town, South Africa, was 1596 per 100 000, compared to 66 per 100 000 in HIV-uninfected

infants [33]. The incidence of disseminated TB in HIV-infected infants was 241 per 100 000, compared to only 14 per 100 000 in HIV-uninfected infants [33]. Consequently, there is a clear and urgent need to prevent TB among these infants.

The few studies that have addressed BCG-induced protection against TB in HIV-infected children and adults have either lacked adequate sample sizes, or were not designed appropriately to reach reliable conclusions [34–36]. The evidence suggests that BCG has no protective effect in HIV-infected persons. In the largest study of children reported to date, TB disease incidence was compared between 310 HIV-infected children with a history of BCG vaccination, and 64 such children who were not vaccinated [37]. Subsequently, 44 infants (14%) from the former group developed TB, whereas only seven (11%) from the latter group developed the disease; however, the inter-group difference was not significant.

BCG has not been shown to have any effect in preventing pulmonary or disseminated TB disease in HIV-infected adults; in contrast, a protective effect of BCG in preventing disseminated disease in HIV-uninfected controls could be demonstrated [36]. One small study has suggested a possible effect of newborn BCG in preventing *M. tuberculosis* bacteremia among persons with advanced HIV disease [34]. It is important to recognize that blood cultures have a poor sensitivity in the diagnosis of either local or disseminated TB disease.

3.5

Adverse Effects Caused by BCG in HIV-Infected Infants not Receiving Antiretroviral Therapy

The interpretation of most results from investigations into local cutaneous adverse effects following BCG vaccination to infants born to HIV-infected mothers is difficult because of the small sample sizes. Overall, when HIV-infected and uninfected infants were compared, local cutaneous adverse effects did not appear to be more severe in HIV-infected infants [38–41].

Multiple case reports and case series have described disease caused by BCG in infants who are HIV-infected. In one such case, an adult developed BCG disease after becoming HIV-infected, 30 years after vaccination [42]; this would suggest that *M. bovis* BCG may persist in a latent state for prolonged periods in healthy human hosts. Although no data regarding the latency of *M. bovis* BCG in humans exist, a single instance of a positive blood culture due to *M. bovis* BCG has been reported [43]. Following multiple case reports, two sentinel case series from the pre-antiretroviral therapy (ART) era have been published, both from Cape Town, South Africa.

The first series focused on further speciation of the *M. tuberculosis* complex, which contains *M. bovis* BCG, in 183 isolates from 49 HIV-infected children with a diagnosis of “TB” [44]. (The speciation of *M. tuberculosis* complex, which could include *M. tuberculosis* and *M. bovis* strains, had not been common practice early in the HIV epidemic in Cape Town.) Danish *M. bovis* BCG was isolated from five patients, all aged <12 months and all severely immunodeficient at presentation. Four patients had regional axillary adenitis ipsilateral to the vaccination site, and two had

pulmonary BCG disease. Two patients with regional BCG disease had simultaneous *M. tuberculosis* isolated from gastric aspirates (pulmonary disease).

The second series was a retrospective, hospital-based study of culture-confirmed BCG disease in HIV-infected and uninfected children aged <13 years over a three-year period [18]. BCG disease was diagnosed in 25 children; among these, 22 (88%) had local disease and eight (32%) had distant or disseminated disease; five children (20%) had both local and distant or disseminated disease. In addition, 17 children were HIV-infected and two had other immunodeficiencies. All eight children with distant or disseminated disease were severely immunodeficient, with a median CD4 lymphocyte differential of 7%; six children were HIV-infected. The mortality rate was 75% for children with distant or disseminated disease. Two unpublished hospital case studies from Argentina [37, 45, 46], and one study from Venezuela, have contributed to the awareness of the increased risk of BCG disease in HIV-positive infants. These studies collectively identified 79 cases of BCG complications in 1196 infants (6.6%), including 11 cases (0.9%) of disseminated BCGosis.

To summarize, BCG disease in HIV-infected infants most commonly presents as localized lymph node disease, although a disseminated disease often involving bone may also occur. Established bone disease at the time of presentation may suggest dissemination of the vaccine strain very early after vaccination, as mycobacterial bone disease takes time to develop. Cases of BCGosis with a presentation similar to pulmonary TB have also been described.

The Cape Town case series were followed by mathematical modeling to estimate the risk of disseminated BCG disease in HIV-infected infants in the region. Such risk was calculated to be extraordinarily high at between 329 and 417 per 100 000 vaccine recipients, assuming a 95% BCG coverage, an HIV prevalence of 12.4–15.4% among pregnant women, and a vertical HIV transmission rate of 5% [47]. This agrees with anecdotal experience of physicians working with HIV-infected infants, who invariably state that BCGosis is a very common problem in clinical practice and that, if anything, the calculated rates are an underestimation.

An intriguing hypothesis proposed by Hesselning *et al.* is that BCG may not only cause disease in HIV-infected infants, but also accelerate the HIV disease progression, due to immune-activating effects of the vaccine [48]. It has been well described that any vaccination may result in transient immune activation and increased viral replication in HIV-infected persons, although the clinical consequence may be negligible. Newborn infants have different immune responses, compared to adults (sometimes termed “immature,” although “appropriate” may be a better term, given the new environment outside the uterus) [49]. Therefore, the effects of an infection that is likely to be chronic in HIV infection, such as *M. bovis* BCG infection, may afford different outcomes in infants, compared to adults.

3.6

BCG Immune Reconstitution Inflammatory Syndrome (BCG-IRIS)

BCG can cause an immune reconstitution inflammatory syndrome (IRIS) in infants. An IRIS event may be defined as a paradoxical infectious or inflammatory

condition, temporally related to the initiation of ART [50]. The syndrome usually occurs within months of initiation of combination ART (cART) in infants or children, is most common in the presence of severe immunosuppression, and usually has an infectious etiology [51]. TB that manifests after commencing cART is the most common form of the “unmasking” type of IRIS in Africa; that is, in patients who had subclinical infection or disease prior to commencing antiretrovirals [52–54]. In contrast, BCG-IRIS is a “paradoxical” form of the syndrome [18, 44, 55].

A prospective Thai study of 153 children who commenced cART reported five children with IRIS associated with BCG [51, 56]. Interestingly, 14 of the 32 cases of IRIS described in this series were attributed to mycobacterial disease; the median time to the onset of IRIS was four weeks (range: 2–31 weeks), and most children had CD4 lymphocyte percentage $\leq 15\%$. Nuttall *et al.*, recently reported that 21 of 352 South African infants or children who commenced cART developed BCG complications [55]. The median age of commencing cART was five months, at a median baseline CD4 lymphocyte percentage of 12.3% and viral load of 6.1 log copies ml^{-1} . All of the children developed ipsilateral axillary lymphadenitis, and one child had suspected disseminated disease. Young age and a high baseline viral load were independent risk factors for the development of BCG complications. The bacterium was isolated in 70% of patients who underwent incision and drainage of abscesses at the vaccination site or regional lymph nodes. An unpublished conference report, also from the Cape Town region of South Africa, documented the characteristics of 33 cases of BCG-IRIS [57]. This translated to an incidence rate of 8.8% among patients followed at a tertiary care hospital. The details of presentation were largely similar to those reported by Nuttall *et al.*

3.7

Management of BCG Disease in HIV-Infected Infants

When BCGosis occurs due to severe immune compromise, the initiation of cART should be the cornerstone of intervention [58]. cART has been shown to result in an improved outcome of BCG disease, particularly when initiated as early as possible [57].

The use of antimycobacterial therapy may be also considered for BCG-IRIS, although recent evidence has suggested that this therapy does not improve the outcome of BCG disease when cART is also given [57]. Regardless, it would be wise to assess each case individually, and to consider antimycobacterial therapy especially when the disease is disseminated [18]. As is the case for therapy of TB, at least three antimycobacterial drugs should be used. It should be recognized that *M. bovis* BCG is inherently resistant to pyrazinamide, which is one of the primary agents used to treat TB. Isoniazid, rifampin, and ethionamide or ethambutal are most commonly used for the empiric treatment of *M. bovis* BCG disease. It is advisable to collect clinical specimens routinely for culture and sensitivity testing when mycobacterial disease is considered in HIV-infected infants. Further, speciation of the isolated *M. tuberculosis* complex into

M. tuberculosis and *M. bovis* should be performed routinely, so as to avoid assumption that the *M. tuberculosis* complex reflects *M. tuberculosis*. This is an important point, because BCGosis may have presentations that are very similar to those of TB disease, and the resistance of *M. bovis* BCG to pyrazinamide calls for alternate (or no) therapy [44].

The most common presentation of BCG complications is subaxillary lymph node disease [44, 55, 57]. Interestingly, these nodes are usually filled with neutrophils, rather than mononuclear cells, classical of TB abscesses in non-HIV-infected persons. The appearance of such lesions is therefore often that of an acute abscess, and the temptation exists to perform an incision and drainage. However, as with other mycobacterial abscesses, incision and drainage may be associated with chronic fistula formation and should best be avoided. Symptomatic therapy, such as the treatment of pain, may be all that is warranted. However, some experts would aspirate the contents of a very prominent abscess, but would carefully choose a region of entry for the needle away from the point of the abscess.

Steroids have been used anecdotally to treat TB-IRIS in adults, a condition associated with significant mortality and morbidity. A double-blind, placebo-controlled, randomized trial of the use of prednisone for TB-IRIS was recently completed in adults from Cape Town [59]. The median CD4 count in the 109 patients enrolled was 53 cells μl^{-1} prior to cART, and at enrolment 116 cells μl^{-1} . Prednisone reduced the need for hospitalization and procedures, and resulted in symptom improvement without any excess of corticosteroid side effects or severe infections. These results suggest that the role of corticosteroid therapy for treatment of BCG-IRIS in children should be evaluated.

Overall, the prognosis of BCG disease in HIV-infected infants is determined by the underlying immune deficiency. The median time of resolution of BCG-IRIS is approximately four months [57], and mortality due to BCG disease is very rare.

3.8

Specific Immunity Induced by BCG in HIV-Infected Infants

In order to guide a more comprehensive assessment of risks and benefits of BCG vaccination in HIV-infected infants, an assessment was recently made as to whether BCG could induce an immune response thought to be required to protect infants against TB [27]. As this study was completed before the routine availability of cART, none of the infants received cART. BCG induced a markedly lower frequency of specific CD4 T cells in HIV-infected infants, compared to uninfected infants (Figure 3.3). The “quality” of the T-cell response was also compromised, as very few polyfunctional T cells (see definition above) were induced (Figure 3.4). These changes persisted throughout the first year of life.

At present, no data are available on immune mechanisms underlying BCG-IRIS, although this is the focus of a large study proposed by the International Maternal Pediatric Adolescent AIDS Clinical Trials Group.

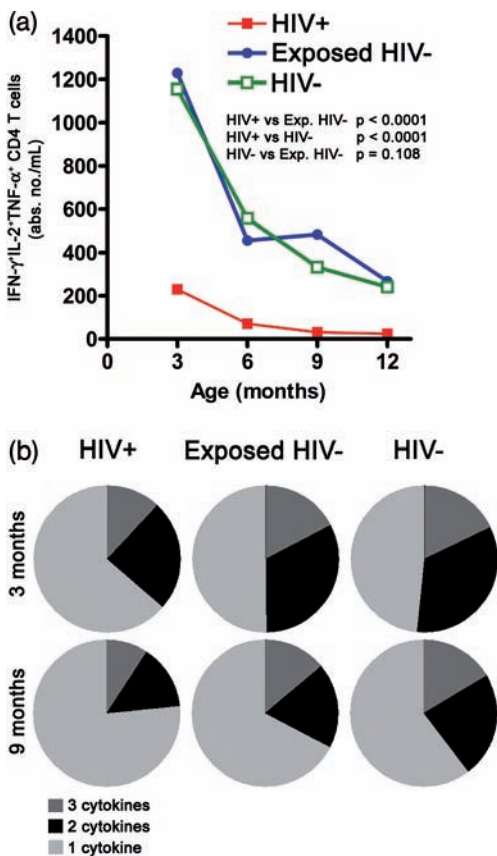


Figure 3.4 “Qualitative” differences of BCG-specific CD4 T-cell responses in the three infant groups described in Figure 3.3: HIV-infected (HIV+), HIV-exposed but uninfected (Exp. HIV-), and HIV-unexposed (HIV-) [27]. (a) Median absolute polyfunctional (IFN- γ^+ , IL-2 $^+$ and TNF- α^+) CD4 T-cell numbers at each time point. Comparisons (overall effect p-values) were calculated from mixed effects maximum likelihood regression models of log-transformed

responses that also included time as a continuous effect; (b) Pie charts represent the median proportions of polyfunctional (cells producing three cytokines, dark grey), bifunctional (cells producing two cytokines, black) and monofunctional (cells producing one cytokine, light grey) out of the total cytokine CD4 T-cell response for the three infant groups, at 3 and at 9 months post-vaccination.

3.9 Weighing up the Evidence: Should BCG be given to HIV-Infected or HIV-Exposed Infants?

The debate as to whether BCG should be given to HIV-infected or HIV-exposed infants hinges on two factors, namely: (i) protection against TB; and (ii) the adverse effects of the vaccine. To recap, there is no definitive evidence that BCG protects HIV-infected infants against TB, and BCG disease in the presence and in the absence

of cART is a common and morbid complication of vaccination. In addition, specific immunity induced by BCG is severely compromised in HIV-infected infants. The evidence therefore strongly suggests that BCG should not be given to HIV-infected infants, nor to HIV-exposed infants whose HIV status is not known.

This matter is not that simple, however, as indicated by statements of the Global Advisory Committee on Vaccine Safety of the World Health Organization and BCG Working Group of the Child Lung Health Section of the International Union Against TB and Lung Disease [60, 61]. The core issues relate to weighing up public health practices that promote health for the majority of childhood populations in underdeveloped settings, and the protection of a relatively small group of individual infants against ill health. The intervention of maternal to infant transmission of HIV with cART has resulted in a dramatic reduction in the numbers of infants who will be HIV-infected. If the most commonly used regimen to prevent vertical HIV transmission is used – that is, ziduvudine plus nevirapine – only about 5% of infants will become infected. As these infants often live in environments where TB is extraordinarily common, it is important to protect the approximate 95% of HIV-exposed infants who do not become infected with the virus against TB meningitis and miliary TB, through BCG vaccination. If the health service infrastructure cannot guarantee the return of most HIV-exposed infants for (and the routine availability of) a viral amplification test for HIV diagnosis at six weeks of age, then it would be in the best interests of the health of most infants to administer BCG at birth to HIV-infected infants. Unfortunately, this is the reality in virtually all underdeveloped countries and, as a result, BCG disease will for the time being continue to occur in HIV-infected infants. This is also the recommendation of the above-mentioned committees, who suggest that in resourceful settings, where BCG is given as a routine and where the return of an HIV-exposed infant and viral amplification testing for HIV infection can be guaranteed, BCG should not be given at birth. If the infant is HIV-negative at the time of the viral amplification test, then BCG may then be given; however, if the child is HIV-positive then BCG should not be given.

3.10

How Can We Protect HIV-Infected Infants Against TB, if BCG is Not Given?

Evidence is emerging that commencing cART in HIV-infected infants as soon as possible after an early diagnosis of the infection (at six weeks of age, or earlier) will significantly decrease mortality, compared to when cART is initiated based on clinical or CD4 T cell criteria. Violari *et al.* have recently shown that the reduction in mortality among South African infants, following early cART, was 76% [58]. In this study, which involved 252 infants with early therapy and 125 infants with deferred therapy, the incidence of TB disease was 8.3 per 100 person-years in the former group, and 20.2 per 100 person-years in the latter group. Unpublished data reported from Rabie *et al.* have suggested that the immune preservation may also affect the course of BCG-related illnesses, including BCG-IRIS, which may be less severe when treatment is initiated at an early stage [57]. The best means of

protecting HIV-infected infants against TB disease would therefore be to make the diagnosis of HIV infection as early as possible, and to institute cART as soon as possible.

An alternative approach would be to offer isoniazid prophylaxis to HIV-infected infants, as this has been shown to significantly reduce mortality and the incidence of TB in HIV-infected infants and children [62]. However, a more recent double-blind, randomized, placebo-controlled study of primary isoniazid prophylaxis for the prevention of TB disease and latent infection in 452 young infants with perinatal HIV-exposure, reported no benefit [63]. Many questions surrounding the proposed practice remain unanswered, such as the emergence of resistance against this cornerstone drug for the treatment of TB disease.

The question remains, could BCG be given to HIV-infected infants *after* commencing cART? If BCG-related disease were to be less severe after early cART, it might be hypothesized that immune reconstitution would be adequate to allow safe BCG vaccination, to protect these infants against severe forms of TB. Most experts agree that testing this approach remains fraught with unacceptable risks, and that approaches involving new vaccines may hold greater promise. These vaccines, which contain specific antigens delivered in specialized viral vectors, or with directed adjuvants, may prove safer and would constitute the most sustainable intervention [64]. Some novel vaccine approaches involve recombinant BCG, such as rBCG delta ureC hly + [65]. In animal models of immunodeficiency this vaccine has proven safer than the current BCG, suggesting promise for use in HIV-infected (or HIV-exposed) infants (S.H. Kaufmann, personal communication). It should be noted that BCG-IRIS appears as a significant complication after commencing cART in HIV-exposed infants. Therefore, the best test for safety would include an assessment of whether safer, whole, viable mycobacterial vaccines could cause this complication; however, no such animal models currently exist.

3.11

BCG Vaccination of HIV-Exposed, Uninfected Infants

HIV-exposed, uninfected infants may have systemic immune responses that differ from those of infants born to HIV-uninfected mothers. Typically, the exposed, uninfected infants demonstrate global T-cell activation and altered immune responses following exposure to multiple microorganisms [66, 67]. These factors may contribute to the increased mortality and morbidity reported in exposed infants, although other environmental factors, such as sociological compromise associated with chronic household disease, are also likely to contribute in poor socioeconomic environments. In addition, infants of HIV-infected mothers have a higher chance of being exposed to TB, compared to HIV-unexposed babies [68, 69]. To determine whether BCG can induce the immunity required by exposed, uninfected infants so as to protect them against TB, vaccination-induced immunity was compared to that induced in HIV-unexposed infants [27]. No difference was found in specific immunity, as measured by a short-term intracellular cytokine assay, between these two

infant groups, which suggested that uninfected HIV-exposed infants would benefit from vaccination (Figures 3.3 and 3.4). These findings were in agreement with those from another study, which showed no significant differences in BCG-specific IFN- γ release, as measured by ELISA in seven-day whole-blood assays at the age of six weeks [70]. However, when these authors used purified protein derivative (PPD) as the recall antigen for the same analysis, a lower IFN- γ response was observed in exposed HIV-uninfected infants.

A second important question relating to BCG in HIV-exposed, uninfected infants is whether the vaccine would still be effective if administration were to be delayed beyond the immediate newborn period, as recommended in high-resource areas. The present authors' group is currently examining this question in this population, by investigating induced immunity. However, recent results from a study of HIV-unexposed infants have suggested that vaccination-induced immunity may be more optimal if BCG is delivered at 10 weeks of age rather than at birth. It was shown that, at one year of age, the frequency of specific T cells induced by BCG (and particularly polyfunctional T cells), as measured by a short-term intracellular cytokine assay, was higher in infants who received their vaccine at 10 weeks of age (Figure 3.5). Thus, it was hypothesized that BCG would be at least as effective in preventing severe childhood TB if given to HIV-exposed, uninfected infants after the immediate neonatal period, as when given at birth.

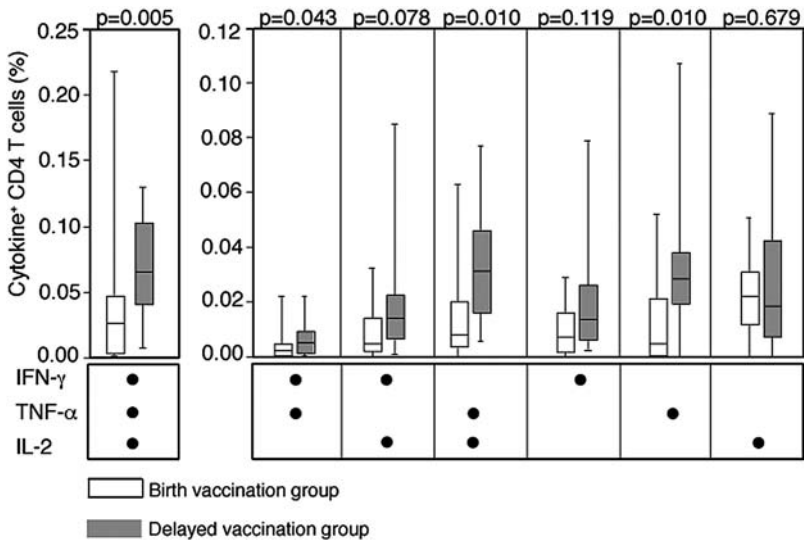


Figure 3.5 Frequencies of BCG-specific CD4 T cells induced by vaccination at birth ($n = 25$), or at 10 weeks of age (“Delayed vaccinated arm”, $n = 23$), as measured at 50 weeks of age. The whole-blood intracellular cytokine detection assay briefly described in Figure 3.1 was used.

Responses $>0.01\%$ were considered positive. The median is represented by the horizontal line, the interquartile range by the box, and the range by the whiskers. P-values indicate the statistical level of significance, using the Mann-Whitney U -test.

3.12

Conclusions

BCG is a safe vaccine in HIV-uninfected infants, and prevents severe childhood TB. In HIV-infected infants, the vaccine is associated with unacceptable safety risks, both in the presence and in the absence of cART; however, public health policies favor administration of the vaccine to all infants from low-resource settings who are born to HIV-infected mothers, in order to protect the majority of infants – who will not be infected by HIV – against severe TB. The risk of BCG disease following this routine neonatal BCG vaccination would be reduced significantly in settings where HIV and TB are endemic, if programs to prevent the transmission of HIV from mothers to infants, as well as TB control strategies, could be strengthened. Once an infant has been diagnosed with HIV infection, the best approach to protect them against TB might be to initiate cART as soon as possible. As yet, many questions surrounding the use of BCG in HIV-exposed and uninfected infants remain unanswered, however.

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Part Two
Drugs

4

HIV/AIDS Drugs

Roy M. Gulick

4.1

Introduction

Currently, a total of 25 FDA-approved drugs are available for the treatment of HIV infection (Table 4.1; Figure 4.1). Approved antiretroviral drugs comprise six mechanistic classes (in chronologic order of development): nucleoside analogue reverse transcriptase inhibitors (NRTI); non-nucleoside reverse transcriptase inhibitors (NNRTI); protease inhibitors (PI); fusion inhibitors; chemokine receptor (CCR5) antagonists; and integrase inhibitors (see Figure 4.2). Following the successful development of combination antimicrobial treatment regimens for both tuberculosis (TB) and Gram-negative bacterial infections, strategies for antiretroviral therapy evolved from monotherapy using single-nucleoside analogues during the late 1980s to early 1990s, to two-drug combination therapy using dual nucleoside analogues in the early to mid 1990s, and to three-drug combination therapy using dual nucleoside analogues together with an HIV PI or an NNRTI, beginning in the mid to late 1990s. The development of three-drug antiretroviral therapy led to a marked decrease in HIV-related morbidity and mortality, with an approximate 60% decrease in HIV-related deaths from 1995 to 1997, and a continued decline thereafter.

Over the past 10 years, effective combination antiretroviral regimens became more convenient, better tolerated, less toxic, and have demonstrated durable virologic, immunologic, and clinical responses. The introduction of drugs with activity against drug-resistant viruses and, in particular, the approval of three new classes of antiretroviral drugs since 2003 – namely, fusion inhibitors, CCR5 antagonists, and integrase inhibitors – allows the design of effective treatment regimens even for patients with drug-resistant viral variants, and also challenges the current standard paradigms of HIV antiretroviral treatment. The development of effective antiretroviral therapy has reduced HIV-related mortality to rates approaching that seen in the general population in developed countries [1, 2]. Worldwide, the intersecting epidemics of HIV disease and TB pose challenges for the optimal

Table 4.1 FDA-approved antiretroviral drugs.

Drug class/Generic name	Abbreviation	Trade name	Year of FDA approval	Standard oral dose	Concomitant use with TB medications
HIV reverse transcriptase inhibitors					
Nucleoside analogues					
zidovudine	ZDV, AZT	Retrovir	1987	300 mg bid	OK
didanosine	ddI	Videx	1991	400 mg qd	Overlapping toxicity (peripheral neuropathy)
zalcitabine	ddC	Hivid	1992	(withdrawn)	Overlapping toxicity (peripheral neuropathy)
stavudine	d4T	Zerit	1994	40 mg bid	Overlapping toxicity (peripheral neuropathy)
lamivudine	3TC	Epivir	1995	300 mg qd	OK
abacavir	ABC	Ziagen	1998	600 mg qd	OK
tenofovir	TDF	Viread	2001	300 mg qd	OK
emtricitabine	FTC	Emtriva	2003	200 mg qd	OK
Non-nucleoside analogues					
nevirapine	NVP	Viramune	1996	200 mg bid	Overlapping toxicity (drug hepatitis); significant drug-drug interaction (rifampin); increased virologic failure rate
delavirdine	DLV	Rescriptor	1997	400 mg tid	Significant drug–drug interaction (rifampin, rifabutin) – contraindicated
efavirenz	EFV	Sustiva	1998	600 mg qd	OK; overlapping toxicity (drug hepatitis); dose adjustments may be required
etravirine	ETR	Intence	2008	200 mg bid	Overlapping toxicity (drug hepatitis); significant drug–drug interaction (rifampin)

HIV protease inhibitors					Overlapping toxicity (drug hepatitis), significant drug–drug interaction (rifampin); rifabutin dose adjustment required
saquinavir	SQV	Invirase	1995	1000 mg with RTV 100 mg bid	See above
ritonavir	RTV	Norvir	1996	rarely used alone	See above
indinavir	IDV	Crixivan	1996	400 mg with RTV 100 mg bid	See above
nelfinavir	NFV	Viracept	1997	1250 mg bid	See above
amprenavir	APV	Agenerase	1999	(withdrawn)	
lopinavir/ritonavir	LPV/r	Kaletra	2000	400/100 mg bid or 800/ 200 mg qd	See above
atazanavir	ATV	Reyataz	2003	[300 mg with RTV 100 mg qd] or 400 mg qd	See above
fosamprenavir	FPV	Lexiva	2003	[700 mg with RTV 200 mg bid], [1400 mg with RTV 100 mg qd], or 1400 mg bid	See above
tipranavir	TPV	Aptivus	2005	500 mg with RTV 200 mg bid	See above
darunavir	DRV	Prezista	2006	600 mg with RTV 100 mg bid or 800 mg with RTV 100 mg qd	See above
HIV entry inhibitors					
Fusion inhibitors					
enfuvirtide	ENF, T-20	Fuzeon	2003	90 mg bid by subcutaneous injection	OK
CCR5 antagonists					
maraviroc	MVC	Selzentry	2007	300 mg bid or [150 mg bid with RTV-containing regimens]	Drug–drug interaction (rifampin) re- quiring dose adjustment; no data with rifabutin
HIV integrase inhibitors					
raltegravir	RAL	Isentress	2007	400 mg bid	Significant drug–drug interaction (rifampin)

bid, twice daily; qd, once daily; tid, three times daily.

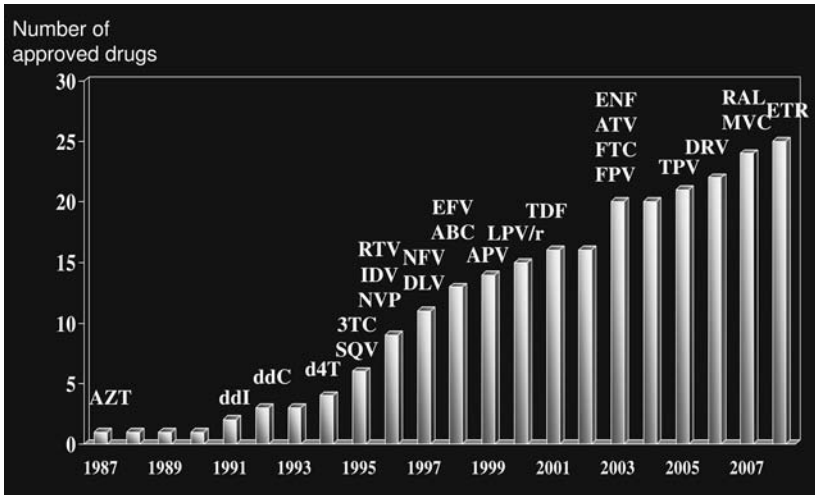


Figure 4.1 Timeline of the FDA approval of antiretroviral drugs, 1987–2008. For drug abbreviations, see Table 4.1.

concomitant treatment of both infections. However, both infections may be comanaged effectively, and the expanded arsenal of antiretroviral drugs better addresses drug–drug interactions and overlapping toxicities. Moreover, clinical strategy trials are under way.

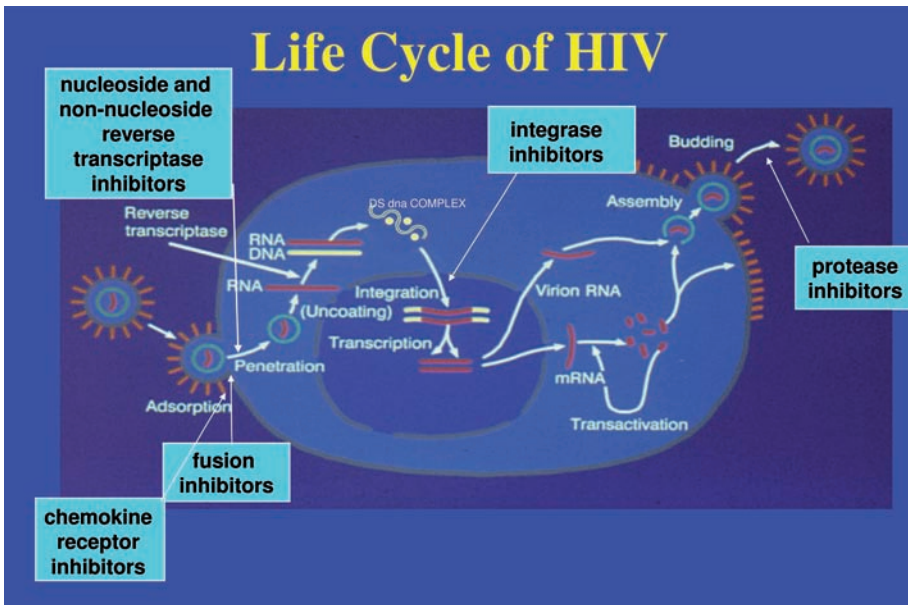


Figure 4.2 The life cycle of HIV and sites of action of the six classes of inhibitor.

4.2

Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs)

The eight approved NRTIs are analogues of the native DNA bases, adenine, cytosine, guanine and thymine, with substitutions at the 3' position of the ribose ring (Figure 4.3). The first antiretroviral agent to be approved, in 1987, was zidovudine (azidothymidine; AZT). One of the first studies of antiretroviral therapy was the BW002 study, in which 288 patients with AIDS or symptomatic HIV disease (then known as AIDS-related complex or ARC) were enrolled and then randomized to receive zidovudine 250 mg every 4 h around the clock, versus placebo [3]. After a median of 127 days, only a single patient in the zidovudine arm died, compared to 19 patients in the placebo group ($p < 0.001$). In addition, 24 patients taking zidovudine had opportunistic infections compared to 45 receiving the placebo ($p < 0.001$). Side effects and toxicities associated with zidovudine were common, and included nausea, neutropenia, and anemia [4]. Subsequent studies showed that zidovudine doses as low as 300 mg per day were as effective but less toxic [5, 6], and 600 mg (300 mg twice daily) remains the current standard dose. Although, when used as monotherapy zidovudine demonstrated only short-term benefits, the drug remained a common component of both two- and three-drug combination regimens. In fact, only recently has zidovudine fallen out of favor among NRTIs due to its gastrointestinal and hematologic toxicities and associated lipoatrophy (fat loss of the face or extremities) [7].

The next three approved NRTIs – *didanosine* (ddI), *zalcitabine* (ddC), and *stavudine* (d4T) – became known as the “d-drugs” and, while similarly effective virologically to zidovudine, they were associated with peripheral neuropathy and pancreatitis. Dual-nucleoside regimens of zidovudine and either didanosine or zalcitabine demonstrated clinical benefits over monotherapy regimens [8–10], and quickly became the standard of care in the early to mid 1990s. In contrast, the combination of zidovudine and stavudine (two thymidine analogues) was antagonistic and caused declines in CD4 cell counts [11]. Ongoing concerns about both antiretroviral activity and toxicity led to the withdrawal of zalcitabine in 2006, while high rates of lipoatrophy associated with stavudine have caused a major decline in its use. Didanosine continues to be used today in a once-daily enteric-coated formulation that must be taken while fasting. The overlapping drug toxicity of peripheral neuropathy complicates the concomitant use of d-drugs and TB drugs.

The development of the fifth NRTI, *lamivudine* (3TC), and its use in combination with zidovudine, was a notable step forward in antiretroviral therapy. Lamivudine is a potent agent with activity against both HIV and hepatitis B virus and little, if any, associated toxicity. Unfortunately, complete HIV drug-resistance is conferred by a single amino acid substitution in reverse transcriptase at position 184 (M184V, methionine → valine). The combination of zidovudine + lamivudine demonstrated potent, durable virologic activity [12, 13], and these two drugs ultimately were approved as a coformulated pill in 1997, the first of several popular coformulated agents that reduced the pill count and improved the convenience of antiretroviral therapy regimens (Table 4.2).

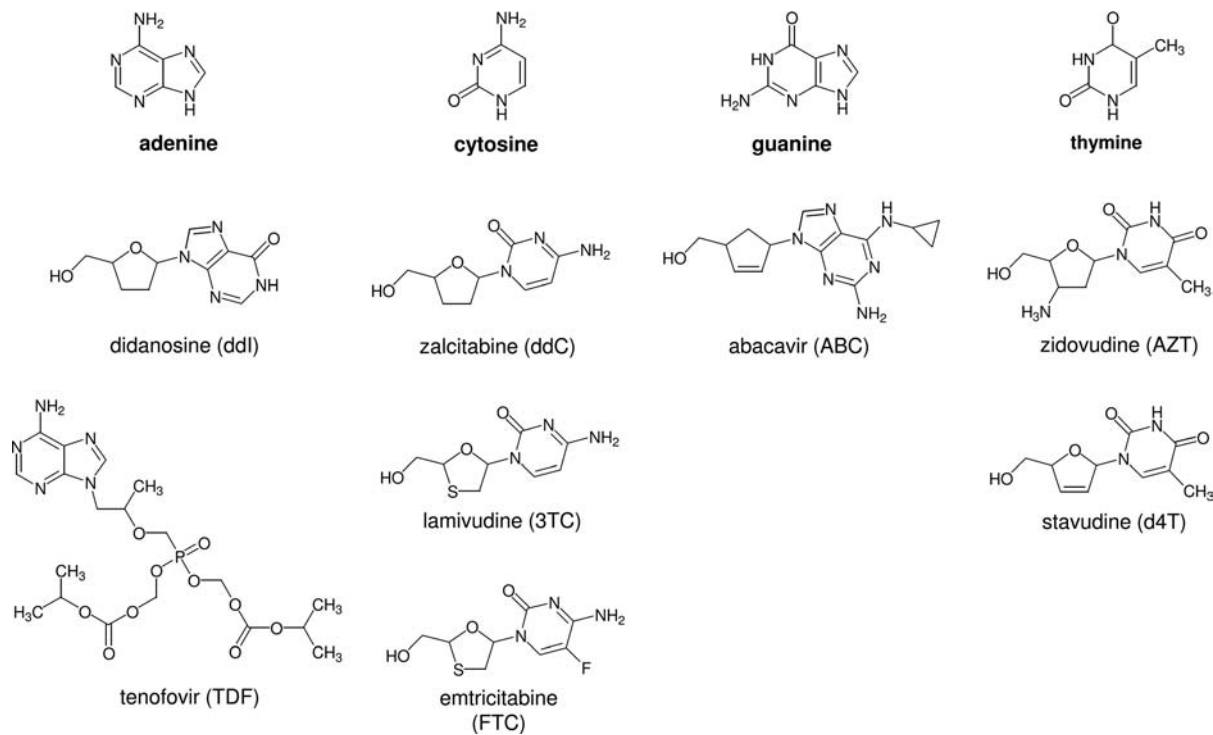


Figure 4.3 Structure of the nucleosides and corresponding nucleoside analogue HIV reverse transcriptase inhibitors (NRTIs).

Table 4.2 Approved coformulated antiretroviral drugs.

Generic names	Abbreviation	Trade name	Year of FDA approval	Standard oral dose
zidovudine + lamivudine	ZDV/3TC	Combivir	1997	300/150 mg bid
abacavir + zidovudine + lamivudine	ABC/ZDV/3TC	Trizivir	2000	300/150/300 mg bid
lopinavir + ritonavir	LPV/RTV	Kaletra	2000	400/100 mg bid or 800/200 mg qd
abacavir + lamivudine	ABC/3TC	Epzicom	2004	600/300 mg qd
tenofovir + emtricitabine	TDF/FTC	Truvada	2004	300/200 mg qd
tenofovir + emtricitabine + efavirenz	TDF/FTC/EFV	Atripla	2006	300/200/600 mg qd
stavudine + lamivudine + nevirapine	d4T/3TC/NVP	Triomune	^a	40/150/200 mg bid

^aGeneric formulation, not FDA-approved for use in the U.S. bid, twice daily; qd once daily.

Abacavir, a guanosine analogue, is a potent antiretroviral drug that demonstrated similar virologic but superior CD4 responses, compared to zidovudine, when each was used as part of a three-drug regimen [14]. Abacavir is associated with a hypersensitivity reaction in 5–8% of patients; this is a life-threatening toxicity that may be greatly decreased (if not eliminated) by the use of a genetic screening test for HLA-B* 5701 and avoiding the drug in patients with this genetic marker [15]. Initially thought to be free of other side effects, abacavir use recently was associated with myocardial infarction in two large retrospective studies [16, 17], although this finding requires further confirmation. Additionally, ACTG study 5202 included a head-to-head comparison of abacavir/lamivudine and tenofovir/emtricitabine, each combined with efavirenz in treatment-naïve subjects with pre-treatment HIV RNA levels >100 000 copies/mL, found that the time to virologic failure was significantly shorter with the abacavir-containing regimen [18]. Currently, abacavir is available as a coformulation with lamivudine alone and as part of a three-drug formulation with zidovudine and lamivudine (Table 4.2).

Tenofovir was approved in 2001 on the basis of its potent antiretroviral activity [19], and more recently was approved for the treatment of hepatitis B virus infection. The toxicity of tenofovir is limited to an uncommon renal toxicity—proximal renal tubular dysfunction (Fanconi's-like syndrome) that is characterized by glycosuria, hypophosphatemia, proteinuria, and elevated serum creatinine. The pharmacokinetics support once-daily dosing of tenofovir. Tenofovir was coformulated with the eighth approved NRTI, *emtricitabine* (a drug that is similar to lamivudine in terms of virologic activity, lack of toxicity and drug resistance, but with a longer intracellular half-life), and the coformulation was approved as a once-daily dual-nucleoside component in 2004. In 2006, a three-drug coformulation of tenofovir + emtricitabine + efavirenz became the first one-pill, once-daily antiretroviral regimen for HIV infection, and remains among preferred first-line regimens today [20, 21].

In summary, NRTIs remain a component of most antiretroviral drug regimens today, although their diverse toxicities, which are thought to be due mitochondrial

toxicity, limit the use of some members of the class. Of notable concern is the overlapping toxicity of peripheral neuropathy that is common with some NRTIs (e.g., didanosine, stavudine) and some TB drugs (e.g., isoniazid). In recent years, less-toxic, convenient once-daily coformulated NRTIs have become the standard of care. The lack of drug–drug interactions between NRTIs and TB drugs makes NRTIs an excellent choice for inclusion in antiretroviral regimens for the concomitant therapy of HIV infection and TB. Triple NRTI regimens are less potent than NNRTI-based regimens [22], and are used uncommonly in developed countries. Quadruple NRTI regimens have shown promise, but limited data are available to support their widespread use [23]. Hence, most commonly, two NRTIs are used with an NNRTI in the setting of concomitant HIV and TB infections.

4.3

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Whilst the NNRTIs also inhibit the HIV reverse transcriptase enzyme, they bind to the enzyme at a site remote from the active site where the NRTIs bind, and have diverse, unrelated structures (Figure 4.4). The NNRTIs are potent antiretroviral drugs and, despite their unrelated structures, share common toxicities of rash and hepatic transaminase elevations. They are metabolized by the cytochrome P450 enzyme system, so that drug–drug interactions with other hepatically metabolized drugs (including anti-TB drugs) are common occurrences. Drug resistance develops easily to most NNRTIs, and concerns about increasing transmitted NNRTI-resistance in the community exist [24].

The first NNRTI to be approved, in 1996, was *nevirapine*. Although potent, a single amino acid substitution in reverse transcriptase at position 181 (Y181C,

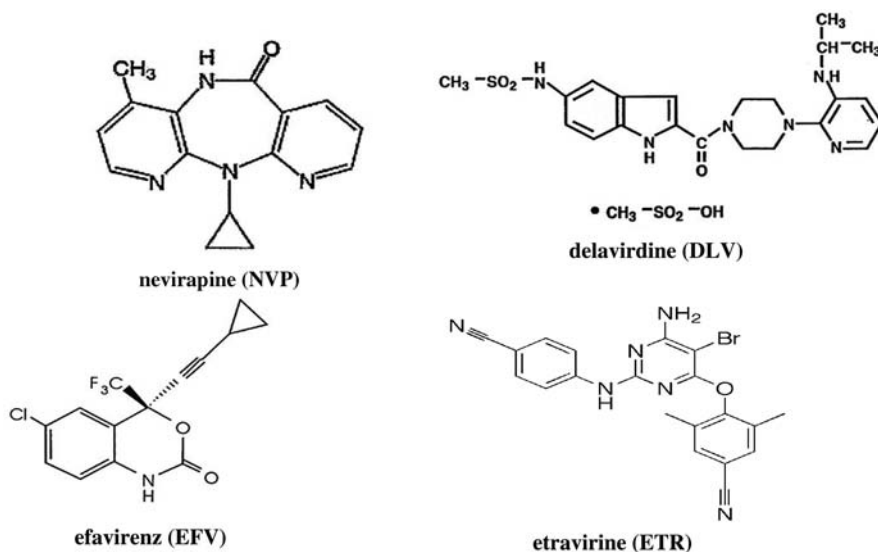


Figure 4.4 Structure of the non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs).

tyrosine → cytosine) within the NNRTI binding site is enough to confer, rapidly, complete resistance [25]. An early study of three-drug therapy with zidovudine + didanosine + nevirapine was conducted in patients with prior NRTI experience, but the virologic effect was modest [26]. A subsequent investigation of the same three-drug therapy in treatment-naïve patients was one of the first studies to show that a majority of patients could achieve maximal and durable virologic suppression [27]. Subsequent studies demonstrated elevations of hepatic transaminases, with rash occurring more commonly (and surprisingly) in patients with higher CD4 cell counts. A 10-fold increase was identified in women with CD4 cell counts $>250 \mu\text{l}^{-1}$, and a sixfold increase in men with CD4 cell counts $>400 \mu\text{l}^{-1}$, compared to those with lower CD4 cell counts [28]. Nevertheless, the generic coformulated combination of stavudine + lamivudine + nevirapine is likely the most commonly used regimen worldwide, although ongoing concerns remain regarding the toxicity of both nevirapine and stavudine. The use of a nevirapine-based regimen with concomitant treatment for TB is complicated both by an unfavorable drug–drug interaction with rifampin, that reduces nevirapine concentrations by 20–58%, as well as overlapping hepatotoxicity [20]. One prospective South African study identified inferior virologic responses in HIV-infected patients taking concomitant nevirapine-containing antiretroviral and TB treatment compared to patients taking nevirapine-containing antiretroviral treatment without TB [29].

The second approved NNRTI, *delavirdine*, requires three-times daily dosing, has suboptimal potency, and is used rarely. Because of unfavorable drug–drug interactions with rifamycins that lead to marked decreases in delavirdine concentrations, concomitant use is contraindicated [20].

The third NNRTI to be approved, in 1998, was *efavirenz*. In a seminal Phase III study (the 006 study), 450 treatment-naïve patients were randomized to receive zidovudine lamivudine + indinavir (control arm), zidovudine + lamivudine + efavirenz, or a NRTI-sparing arm of efavirenz + indinavir [30]. At 48 weeks, the two NRTIs + efavirenz arm proved superior virologically, well-tolerated and convenient, and led this combination rapidly to become the standard of care for antiretroviral therapy during the late 1990s. As above, the one-pill, once-daily coformulation of tenofovir + lamivudine + efavirenz is recommended among preferred initial antiretroviral

Table 4.3 Recommended initial antiretroviral regimens: Two NRTIs + [NNRTI or PI].

	Two NRTIs	NNRTI	PI
Preferred	TDF/FTC	EFV	ATV + RTV DRV + RTV FPV + RTV bid LPV/RTV
Alternative	ABC/3TC ddI + [FTC or 3TC] ZDV/3TC	NVP	ATV FPV FPV + RTV qd SQV/RTV

For drug abbreviations, see Tables 4.1 and 4.2.bid, twice daily; qd once daily.

regimens [20, 21] (Table 4.3), and this combination has become the most commonly prescribed initial regimen in many countries today. Efavirenz is associated with central nervous system toxicities (e.g., vivid dreams, somnolence) in about 50% of patients, although these symptoms tend to diminish over several weeks [31]; rash may occur in about 10% of patients, and hepatotoxicity in about 8%. As with nevirapine, a single amino acid substitution in reverse transcriptase confers resistance to efavirenz. An antiretroviral regimen with two NRTIs and efavirenz is commonly used with concomitant TB treatment, although there is potential for overlapping hepatotoxicity and because of a drug–drug interaction with rifampin, the optimal dose of efavirenz (600 versus 800 mg daily) is not known [20]. A prospective study found similar virologic outcomes in patients treated with efavirenz-based regimens either with or without concomitant TB treatment [29].

The fourth NNRTI, *etravirine*, was approved in 2008 and is the most recently introduced antiretroviral drug. Etravirine is the first NNRTI with demonstrated activity against NNRTI-resistant viral strains. The Phase III DUET studies included patients with prior NRTI, NNRTI, and PI treatment experience, and constructed an optimized background regimen that included the protease inhibitor darunavir along with a choice of NRTIs and/or enfuvirtide. The patients were then randomized to receive etravirine, or not [32, 33]. At 48 weeks, 40% of the group with the optimized background alone and 61% of the group with an optimized background and etravirine had HIV RNA suppressed to <50 copies ml^{-1} . This led to the approval of etravirine for treatment-experienced patients. However, etravirine is contraindicated with concomitant rifampin because of a significant drug–drug interaction [20].

In summary, NNRTIs are potent, convenient, and generally well tolerated. Hence, NNRTI-based regimens are the most commonly used antiretroviral regimens worldwide. However, ongoing concerns regarding transmitted NNRTI-drug resistance exist, while drug–drug interactions with rifamycins and overlapping hepatotoxicity complicate the concomitant use of NNRTI-based antiretroviral therapy with TB drugs.

4.4 HIV Protease Inhibitors

The HIV PIs were designed as peptidomimetic compounds to bind in the active site of the protease enzyme, and were the most potent antiretroviral agents described when they entered clinical development during the early 1990s. As a class, the PIs have complicated structures (Figure 4.5) and are associated with some class toxicities, such as transaminase elevations. Some PIs are also associated with lipohypertrophy and hyperlipidemia. The PIs are metabolized by the cytochrome P450 hepatic enzyme system, and drug–drug interactions are a common occurrence. For example, rifampin decreases the concentrations of all PIs by $>75\%$ and their concomitant use is contraindicated, although rifabutin can be substituted with careful dose adjustment [20]. The protease inhibitor *ritonavir* is the most potent inhibitor of the CYP 3A4 enzyme isoform ever described; it is used routinely at low doses to “boost” the levels of the other HIV PIs, allowing once- or twice-daily dosing with improved pharmacokinetic properties.

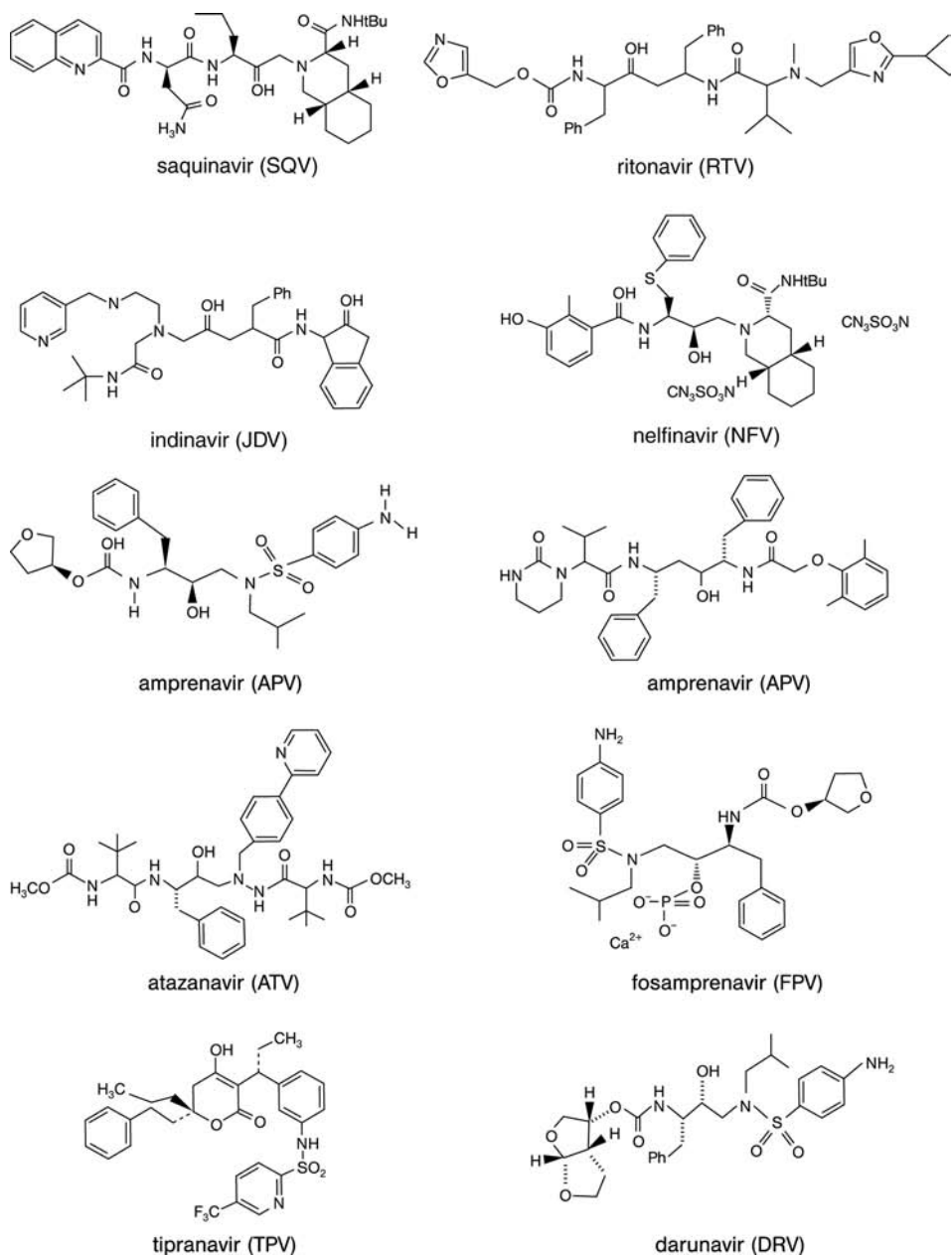


Figure 4.5 Structures of the HIV protease inhibitors (PIs).

The first three PIs to be approved were *saquinavir*, *ritonavir* and *indinavir*, during late 1995-early 1996. Approval was on the basis of landmark studies that paved the way for the development of effective antiretroviral therapy regimens that subsequently led to dramatic reductions in HIV-related morbidity and mortality [34]. The

first study to show that an HIV PI-containing regimen led to notable clinical effects included 1090 patients with CD4 cell counts $<100 \text{ mm}^{-3}$, all of whom received none, one, or two NRTIs and then were randomized to add the PI, ritonavir, or matching placebo [35]. After a median follow-up of 29 weeks, 22% of the patients receiving ritonavir compared to 38% of those receiving placebo had an AIDS-defining illness or died ($p < 0.001$). Thus, ritonavir use was associated with a near-50% reduction in clinical events over the short term.

The 035 Study was the first to utilize an effective three-drug combination antiretroviral therapy for the treatment of HIV infection [36]. In this pilot study, 97 zidovudine-experienced patients were enrolled and randomized to receive zidovudine + lamivudine, indinavir alone, or the three-drug combination of zidovudine lamivudine + indinavir. At 24 weeks, 0% (zidovudine + lamivudine) versus 43% (indinavir monotherapy) versus 90% (three drugs) of the patients had HIV RNA $<50 \text{ copies ml}^{-1}$. The ACTG 320 Study tested a similar strategy in 1146 zidovudine-experienced patients who received zidovudine + lamivudine with randomized indinavir or placebo [37]. After a median follow-up of 38 weeks, AIDS or death occurred in 11% (two drugs) versus 6% (three drugs) of patients ($p < 0.001$). Thus, the indinavir-based regimen was associated with a 50% reduction in clinical events. These two studies defined zidovudine + lamivudine + indinavir as the standard of care HIV treatment in the mid-1990s.

Unfortunately, the initial three PIs had limitations based on their bioavailability and potency (saquinavir), tolerability (ritonavir), and convenience (indinavir). Consequently, additional studies were conducted with newer PIs which included *nelfinavir* (approved in 1997), *amprenavir* (1999), and *lopinavir/ritonavir* (2000). Nelfinavir demonstrated virologic activity [38], was better tolerated (toxicity was limited to diarrhea in ca. 15–20% of patients), and more convenient in that it could be taken with food; these benefits led to its widespread use in three-drug combinations. Amprenavir also was potent and reasonably well tolerated, but had a high pill count, which limited its acceptance.

The first coformulated PI was lopinavir/ritonavir. Early Phase II studies showed not only its potency when combined with NRTIs in treatment-naïve patients [39], but also its significant activity in patients who had experienced prior virologic failure when receiving other PIs [40]. The coformulation improved convenience over other PIs, and a head-to-head study showed that a lopinavir/ritonavir-based regimen was superior to a nelfinavir-based regimen [41]. On this basis, lopinavir/ritonavir was recommended as a preferred PI in antiretroviral treatment guidelines [20, 21] (Table 4.3), although the side effects of diarrhea and hyperlipidemia were well recognized.

In a more recent head-to-head study, ACTG 5142, a total of 757 patients were randomized to receive two NRTIs together with either lopinavir or efavirenz, or an NRTI-sparing arm of efavirenz + lopinavir/ritonavir. Although virologic superiority of the efavirenz-based regimen was demonstrated, there were significantly better CD4 responses with the lopinavir-based regimens, and less drug resistance following virologic failure with the two NRTI + lopinavir/ritonavir regimen [42]. Surprisingly in this study, efavirenz was associated with more lipodystrophy than lopinavir/ritonavir, particularly when used with stavudine or zidovudine [43]. Currently, both lopinavir/

ritonavir- and efavirenz-based regimens are recommended among preferred initial antiretroviral regimens in U.S. treatment guidelines [20, 21] (Table 4.3), and both are used commonly internationally.

Having recognized the convenience, tolerability, and toxicity challenges of the prior PIs, atazanavir was developed and approved in 2003 as a once-daily PI without significant lipid increases, and performed favorably when compared with an efavirenz-based regimen [44]. One unique laboratory abnormality – an increased indirect bilirubin (Gilbert’s-like syndrome) – is due to atazanavir inhibiting the uridine 5'-diphospho-glucuronosyl-transferase (UGT) 1A1 enzyme; the drug has few other side effects. The addition of ritonavir boosting improves the pharmacokinetic profile of atazanavir, although some of the lipid benefits appear to be lost. Atazanavir + ritonavir compared favorably with lopinavir/ritonavir in both the CASTLE study of 883 treatment-naïve patients [45], and also another study of 358 PI-experienced patients [46]. The results of these studies supported the addition of atazanavir + ritonavir to the list of preferred protease inhibitors in treatment guidelines [20, 21] (Table 4.3).

Fosamprenavir is a pro-drug of amprenavir with reduced pill count that was approved in 2003 and subsequently prompted the withdrawal of amprenavir (with the exception of the liquid formulation) from the market. In the KLEAN study, 878 treatment-naïve patients who took NRTIs and were randomized to fosamprenavir + ritonavir- or lopinavir/ritonavir-based regimens did comparably well [47], supporting the use of amprenavir + ritonavir as a preferred PI in the treatment guidelines [20, 21] (Table 4.3).

Although patients who experienced virologic failure on potent PI-containing regimens in clinical trials often showed few PI-associated mutations, other patients developed numerous mutations; consequently, additional compounds were sought that would demonstrate activity against PI-resistant viral strains. The two most recently approved HIV PIs (*tipranavir* in 2005 and *darunavir* in 2006) share the property of virologic activity against PI-resistant strains. Each has been approved for the treatment of PI-experienced patients and, importantly, these PIs are not routinely cross-resistant to one another.

Tipranavir was approved based on the results of the Phase III RESIST studies in which 1483 treatment-experienced patients were enrolled and their background regimen optimized on the basis of treatment history and drug resistance testing. Patients were then randomized to receive either tipranavir or placebo [48]. The tipranavir regimen performed significantly better, with 23% of patients having HIV RNA <50 copies ml^{-1} at week 48 compared to 10% of those on placebo ($p < 0.0001$). Tipranavir is associated with hepatic transaminase elevations and some important drug–drug interactions, including its contraindication with the use of etravirine.

Darunavir was tested in a similar way in the Phase II/III POWER studies which included 596 treatment-experienced patients who received an optimized background regimen, with or without darunavir [49, 50]. Ultimately, darunavir at the standard dose led to 39–53% of patients with HIV RNA <50 copies ml^{-1} at 48 weeks, compared to 7–18% in the placebo arm ($p < 0.001$). More recently, darunavir was tested in patients in earlier stages of HIV disease. Darunavir + ritonavir was compared to lopinavir-ritonavir in 595 PI-experienced, lopinavir/naïve patients in

the TITAN study [51], and in 689 treatment-naïve patients in the ARTEMIS study [52]. In both studies, the darunavir regimen performed similar virologically to the lopinavir regimen, with darunavir having fewer liver function test and lipid abnormalities, fewer discontinuations for diarrhea in the treatment-experienced study, and fewer gastrointestinal side effects and treatment-related discontinuations in the treatment-naïve study. These data support the use of darunavir-based antiretroviral therapy among the preferred initial treatment choices [20, 21] (Table 4.3).

In summary, HIV PIs constituted the backbone for the first potent three-drug combination antiretroviral regimens for the effective treatment of HIV disease. Limitations of convenience, tolerability, toxicity, and drug resistance have been overcome with the development of newer members of this drug class. However, drug–drug interactions and overlapping toxicities such as hepatotoxicity complicate their use with concomitant TB medications. The high cost of PIs also creates a challenge for their routine use in resource-poor settings.

4.5

Newer Classes: Entry Inhibitors and Integrase Inhibitors

Drugs with new mechanisms of action would be expected to show activity against HIV strains that are resistant to the conventional classes of drugs, such as the NRTIs, NNRTIs, and PIs.

4.5.1

Entry Inhibitors

The first member of a new class of antiretroviral drugs to be approved for almost a decade, in 2003, was the fusion inhibitor, *enfuvirtide* (*envelope fusion viral peptide*, T-20). HIV entry is a three-step process: (1) HIV recognizes its target cell through the binding of HIV outer membrane glycoprotein (gp) 120 to the CD4 receptor; this binding induces a conformational change in gp 120 allowing (2) binding of gp 120 to the coreceptor or chemokine receptor (either CCR5 or CXCR4). This, in turn, induces a second HIV outer membrane glycoprotein (gp 41) to insert in the cell membrane, anchoring the viral particle to the CD4 cell (3a). This is followed by a folding of gp 41 on itself in a coil-on-coil interaction (3b) that, in turn, allows fusion of the viral membrane and the CD4 cell membrane (3d) [53] (Figure 4.6). Enfuvirtide is a small peptide that binds to gp41 and prevents membrane fusion. Although the landmark Phase III TORO studies demonstrated potent virologic activity of enfuvirtide in treatment-experienced patients [54, 55], enfuvirtide (a peptide) requires twice-daily subcutaneous dosing, is costly, and therefore is used only rarely today.

The second inhibitor of HIV entry to be approved, in 2007, was *maraviroc*. This is a CCR5 antagonist that binds not to a viral target but rather to the CC-chemokine receptor 5 (CCR5) on the surface of the host CD4 cell (Figure 4.6). In binding CCR5, maraviroc prevents entry of R5, but not of X4 viruses [56]. In the MOTIVATE studies, 1049 treatment-experienced patients with documented R5 viruses (only) using a

HIV Entry Mechanism

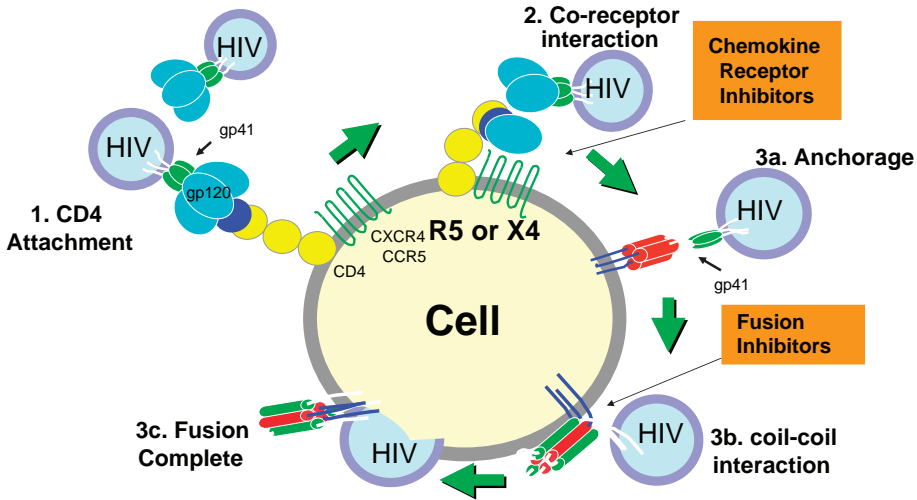


Figure 4.6 Mechanism of action of the HIV entry inhibitors.

tropism assay optimized their background regimens on the basis of treatment history and drug-resistance testing, and then were randomized to receive either maraviroc (at one of two doses) or matching placebo [57]. In total, 46% of the maraviroc twice-daily recipients compared to 17% of placebo recipients had HIV RNA <50 copies ml^{-1} at week 48 ($p < 0.001$). Since maraviroc was the first antiretroviral drug to target a host immune cell receptor, concerns about immune effects or unusual toxicities have been raised. In patients congenitally lacking the CCR5 receptor, there are demonstrable immune effects, e.g. increased morbidity and mortality following West Nile Virus infection [58]. However, no excess hepatic toxicity, unusual infections or malignancies were associated with maraviroc use in the MOTIVATE studies, in contrast to other investigational CCR5 antagonists [59, 60]. Of note, the Food and Drug Administration (FDA) required that all study subjects who received a CCR5 antagonist in a clinical trial be followed for clinical events for five years. The use of CCR5 antagonists in developing countries represents a major challenge due to a need for the viral tropism assay. The concomitant treatment of TB and HIV with a maraviroc-containing regimen is also complicated by a significant drug-drug interaction, whereby rifampin cause a $>60\%$ decrease in maraviroc concentrations that would necessitate the maraviroc dose to be adjusted. At present, no data are available regarding any interactions of maraviroc with rifabutin [20].

4.5.2

Integrase Inhibitors

The most recently approved member of a new class of drugs was the strand transfer integrase inhibitor, *raltegravir*. The HIV integrase enzyme catalyzes three steps of

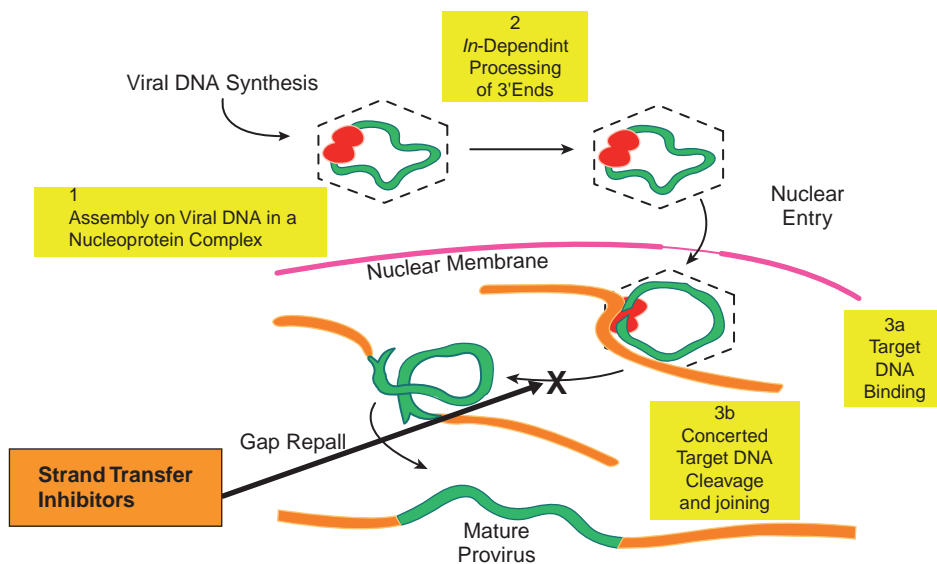


Figure 4.7 Mechanism of action of the HIV integrase inhibitors.

integration: (1) formation of the double-stranded viral DNA complex; (2) the 3'-processing of the DNA; and (3) transport and insertion of the viral DNA into the host cell DNA in a process called "strand transfer" [61] (Figure 4.7). Raltegravir was approved in 2007 on the basis of demonstrating significant virologic activity in the benchmark studies of 699 treatment-experienced patients in the BENCHMRK studies, whereby 62% of raltegravir versus 33% of placebo recipients had HIV RNA <50 copies ml^{-1} at 48 weeks ($p < 0.001$) [62]. However, the majority of patients who experienced virologic failure also developed drug resistance to raltegravir [63]. Raltegravir also was compared to efavirenz, both in combination with NRTIs, in a Phase II study of treatment-naïve patients and demonstrated comparable virologic suppression rates with 85–95% of patients having HIV RNA <50 copies ml^{-1} through 48 weeks [64, 65]. Raltegravir demonstrated a side-effect profile similar to that of placebo in all of these studies. Whilst raltegravir is metabolized by glucuronidation, concomitant administration with rifampin causes a 40–60% decrease in raltegravir concentrations, thus complicating the concomitant treatment of TB [20].

In summary, drugs with new mechanisms of action, such as HIV entry inhibitors and integrase inhibitors, have demonstrated significant virologic activity in patients with resistance to NRTIs, NNRTIs, and PIs. Consequently, new drugs have revolutionized the goals and management of treatment-experienced patients, setting a new standard of virologic response: HIV RNA levels suppressed to <50 copies ml^{-1} [20, 21]. Unfortunately, these newer drugs are limited by their higher cost, the need for parenteral administration of enfuvirtide the restriction of activity to R5 virus (only) and the need for the tropism assay, in the case of maraviroc, and a low barrier to resistance for raltegravir. Significant drug–drug interactions of maraviroc or raltegravir with rifampin also further complicate the use of these drugs.

4.6 Newer Strategies

While current combination antiretroviral therapy regimens are highly effective, their limitations of convenience, tolerability, toxicity, drug–drug interactions and activity against drug-resistant viral strains continue to prompt the search for new strategies and newer antiretroviral agents. The current preferred initial therapy for HIV infection is a regimen consisting of two NRTIs in combination with an NNRTI or a ritonavir-boosted PI [20, 21] (Table 4.3), on the basis of results from large randomized, comparative clinical trials [7, 14, 30, 41, 45, 47]. Studies comparing preferred NNRTI-based regimens with PI regimens have demonstrated distinct benefits to both strategies [42, 44], and support either approach.

Newer strategies for treatment-naïve patients include some novel approaches. All-NRTI regimens have the potential to avoid drug–drug interactions, particularly with the hepatically metabolized NNRTIs and PIs and TB drugs. However, three-NRTI regimens are less potent than NNRTI-based regimens [22], while four-NRTI regimens have not yet been studied extensively [23] and could have toxicity problems of their own. An NRTI-sparing approach to avoid NRTI-related toxicities also has been explored, typically consisting of an NNRTI together with a ritonavir-boosted PI. However, these regimens have been associated with toxicity [30] and/or increased rates of drug resistance at virologic failure [42], continue to have significant drug–drug interaction issues, and have not been adopted widely.

Newer compounds may challenge the existing drugs and treatment paradigms (Table 4.4). When combined with dual NRTIs, an investigational NNRTI – *rilpivirine* – showed comparable virologic responses to efavirenz but was associated with fewer rashes and central nervous system toxicity in a Phase II study for up to 96 weeks [66]. Rilpivirine is currently under investigation in large Phase III studies. Rilpivirine is also available in an investigational nanoparticle formulation with a prolonged drug

Table 4.4 Investigational antiretroviral agents in clinical development (partial list only).

Stage of development	NRTIs	NNRTIs	PIs	Entry inhibitors	Integrase inhibitors	Maturation inhibitors
Phase III		rilpivirine		vicriviroc	elvitegravir	
Phase II	apricitabine	BILR-355		ibalizumab		bevirimat
	dexelvucitabine	UK-453,061		PRO 140		
Phase I/II	amdoxovir	IDX-899		HGS004	GSK compounds	MPC-9055
	elvucitabine	RDEA-806		PRO 542		
Phase I			PPL-100	PF-232798	INH-1001	
			SPI-256	SCH-532706		
				TBR 652		
				TRI-1144		

half-life that allows dosing as infrequently as once a month [67]. Additional newer formulations of antiretroviral agents may allow infrequent dosing of antiretroviral therapy.

Newer drugs approved for treatment-experienced patients also may offer benefits to treatment-naïve patients as part of combination regimens. For example, darunavir was non-inferior virologically to lopinavir/ritonavir and had fewer side effects [52]. Maraviroc was non-inferior to efavirenz as part of a three-drug regimen for the HIV RNA <400 copies ml⁻¹ endpoint, but not for <50 copies ml⁻¹ [68], although tropism assay sensitivity most likely played a role. A raltegravir-based regimen had comparable virologic responses to an efavirenz-based regimen over 96 weeks, and a statistically significantly more rapid time to virologic suppression [64, 69]; similarly designed Phase III raltegravir studies provided similar preliminary results [65].

With the development of these newer agents in treatment-naïve patients, new potentially paradigm-shifting strategies could be tested that could spare several classes of drugs. For example, the combination of a PI with an integrase inhibitor or a CCR5 antagonist could spare both NRTIs and NNRTIs. Similarly, a standard combination regimen of NRTIs and an NNRTI could be used first and then, after regimen failure, a new regimen consisting of a PI with an integrase inhibitor or a CCR5 antagonist could be used. This would define a sequence of two fully potent antiretroviral therapy regimens with distinct mechanisms of action and nonoverlapping drug resistance profiles, although drug–drug interactions (including those with TB medications) would complicate these new approaches. Consequently, pilot studies investigating such possibilities are currently in progress.

For patients with significant treatment experience, the current guidelines recommend reviewing the treatment history, performing drug-resistance testing, and designing a regimen with two (or preferably three) fully active agents in the next regimen [20, 21]. A number of investigational compounds in existing classes (NRTIs, NNRTIs, PIs), in newer approved classes (CCR5 antagonists, integrase inhibitors), or in investigational classes (CD4 attachment inhibitors, CXCR4 antagonists, maturation inhibitors) have demonstrated activity against drug-resistant viruses, and may be particularly useful for treatment-experienced patients if they are proved safe and effective (Table 4.4). Today, the “pipeline” for new antiretroviral agents appears full.

4.7

Concomitant Treatment of HIV Infection and Tuberculosis

The current guidelines emphasize that the treatment of HIV-infected patients with active TB should follow the same principles as for HIV-infected patients without TB [20]. Although the optimum time to start antiretroviral therapy in patients with active TB is not known, a number of clinical trials are under way [70]. Neither are the optimal antiretroviral regimens to treat HIV-infected patients with TB known, although NRTI combinations that do not cause peripheral neuropathy (e.g., abacavir/lamivudine, tenofovir/emtricitabine, or zidovudine/lamivudine), in

combination with a NNRTI with manageable drug interactions and a lower potential for overlapping hepatotoxicity (e.g., efavirenz), have demonstrated virologic outcomes that are not different from those in treated HIV-infected patients without TB [29]. The treatment of HIV and TB can be successfully managed, and the integration of care and treatment for both infections is clearly critical. In addition, clinical trials are in progress that will continue to define the optimal strategies for concomitant treatment [70].

4.8

Conclusions

The development of effective antiretroviral therapy changed the natural history of HIV disease, with dramatic decreases in morbidity and mortality worldwide. Today, the life expectancy of HIV-infected people receiving treatment is approaching that of the general population. With effective treatment, HIV has been transformed into a chronic, manageable disease; consequently, more convenient, more tolerable and less-toxic medications are critical for long-term adherence and clinical responses. More recently, the development of new drugs in existing classes with activity against drug-resistant virus (e.g., NNRTIs, PIs), and of drugs with new mechanisms of action (HIV entry inhibitors, integrase inhibitors), has offered greatly improved treatment options for individuals with treatment experience and/or drug-resistant viral strains. Newer compounds and strategies continue to be tested and will ensure further progress in the field. The concomitant treatment of HIV infection and TB is complicated by overlapping drug toxicities, drug–drug interactions, and the inconvenience of multidrug regimens. However, effective comanagement strategies are possible, and clinical trials are either planned or under way to ensure further progress in this area.

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5

***Mycobacterium tuberculosis*: Drug Resistance and Genetic Mechanisms – Facts, Artifacts, and Fallacies**

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5.1

Introduction

The aim of this chapter is to question current perceptions of mycobacterial drug resistance, to briefly review the underlying molecular mechanisms, to critically reflect the principles involved in drug susceptibility testing of *Mycobacterium tuberculosis* in the diagnostic laboratory, and to discuss possible implications for therapy. Epidemiological aspects – for example, the evolution and spread of drug resistance or limitations of current control programs – will not be addressed specifically, as these have been discussed in part recently (e.g., Refs [1–4]).

The treatment of tuberculosis (TB) disease faces three problems: (i) the interruption of further transmission; (ii) curing of the acute disease; and (iii) preventing relapse. Antituberculosis drugs were first introduced for TB therapy in the 1940s [5, 6]. However, despite a positive initial clinical response, single drug therapy was observed to result in the rapid emergence of drug-resistant strains [7]. Yet, by combining the two antituberculosis drugs available at that time – that is, streptomycin (SM) and *para*-aminosalicylic acid (PAS) – the emergence of resistance was reduced to approximately 10% [8, 9]. When isoniazid (INH) was introduced in the early 1950s, and combined with SM and PAS, this combination effectively prevented the emergence of resistance but required an 18-month of treatment to ensure cure of the disease. Subsequently, pyrazinamide (PZA), rifampicin (RMP), and ethambutol (EMB) were introduced for TB treatment (for an overview of antituberculosis agents and drug targets, see Table 5.1). Extensive studies were carried out by the British Medical Research Council to define the optimal drug combination and minimal duration of therapy [10] (for reviews, see Refs [11, 12]). The outcome consisted of a therapeutic scheme which comprised an initial two-month treatment with INH, RMP, and PZA, followed by a four-month treatment with INH and RMP. Combination therapy is necessary for a successful treatment of the acute disease and for impending resistance to emerge, while a minimum treatment length of six months is required to prevent relapse of the disease [13]. This protocol, further developed and now termed the standard therapy short course (SSC), is still in use today, and is

Table 5.1 Antituberculosis drugs: mechanisms of action and cellular targets.

Drug	Cellular function inhibited	Target
First-line drugs		
Isoniazid	Mycolic acid synthesis	e.g., Enoyl reductase
Rifampicin	RNA synthesis	RNA polymerase
Ethambutol	Arabinogalactan synthesis	e.g., Arabinosyl transferase
Pyrazinamid	Unclear	Unclear
Second-line drugs		
Streptomycin	Protein synthesis	30S ribosomal subunit
Chinolones	DNA supercoiling	DNA gyrase
2-deoxystreptamine minoglycosides (e.g., anamycin, Amikacin)	Protein synthesis	30S ribosomal subunit
Capreomycin	Protein synthesis	30S/50S ribosomal subunit
Ethionamid	Mycolic acid synthesis	Enoyl reductase

recommended with slight modifications (including the possible addition of EMB or SM) by the International Union Against Tuberculosis and Lung Disease (IUATLD), the World Health Organization (WHO), and the American Thoracic Society (ATS).

The ongoing TB pandemic is a serious threat, in particular for developing countries which carry most of its burden. Worldwide, the current situation is characterized by vastly increasing numbers of drug-susceptible TB disease and by emerging drug resistance [14–16]. Much of the current situation has been fuelled by the ongoing HIV epidemic [17, 18]. Although drug-resistant TB has been reported in the past before the emergence of HIV, the susceptibility of HIV patients to infections with *Mycobacterium tuberculosis* has certainly contributed greatly to the current situation.

5.2 Genetic Aspects of Drug Resistance

Plasmid-mediated mechanisms of resistance are absent in *M. tuberculosis*, but acquired drug resistance is exclusively due to chromosomal alterations such as mutations or deletions. These chromosomal alterations affect either the drug target itself or the bacterial enzymes activating the pro-drug. Drug resistance in *M. tuberculosis* occurs when resistant mutants that occur naturally in the mycobacterial population are selected out by inadequate or interrupted treatment with antituberculosis agents. Mutants resistant to a given drug occur approximately in every 10^{-6} to 10^{-8} cell. In support of these considerations, it was observed that monotherapy led to the emergence of drug resistance more frequently in cases of smear-positive multi-bacillary infections (e.g., cavitary lung disease, which contains abundant tubercle bacilli, up to 10^{10} per lesion) than in cases of smear-negative paucibacillary infections

(e.g., noncavitary lung disease or tuberculous lymphadenitis, which contains relatively few bacilli, about 10^4 per lesion) [19]. In theory, the effective occurrence of a mutant which is resistant to two drugs would require a population of 10^{12} – 10^{16} mycobacterial cells. This mathematical concept provides the basis for the successful use of combination drug therapy for preventing emergence of resistance.

During the past 15 years, significant knowledge has been acquired concerning the mechanisms of mycobacterial drug resistance at the molecular level (for reviews, see Refs [20, 21]; see also Table 5.2). These studies have established, unequivocally, that

Table 5.2 Mechanisms of drug resistance in *Mycobacterium tuberculosis*.

Drug	Gene(s) involved in resistance	Role in resistance	Phenotypic resistance
Isoniazid	<i>katG</i> ^a <i>inhA</i> ^b	Pro-drug conversion Drug target	Variable ^c (always $>1 \text{ mg l}^{-1}$) Mostly low-level ^c ($<1 \text{ mg l}^{-1}$)
Rifampicin	<i>rpoB</i> ^d	Drug target	High-level ^e
Pyrazinamid	<i>pncA</i> ^f	Pro-drug conversion	— ^g
Ethambutol	<i>embB</i> ^h	Drug target	Low- to intermediate-level ⁱ
Streptomycin	<i>rpsL</i> ^j	Drug target	High-level ^k
	<i>rrs</i> ^k	Drug target	Intermediate-level ^k
	<i>gldB</i> ^l	Drug target	Low-level ^{k,l}
Amikacin, Kanamycin	<i>rrs</i> ^m	Drug target	Variable ⁿ
Fluoroquinolones	<i>gyrA</i> ^o <i>gyrB</i> ^o	Drug target	— ^p
	Ethionamid	<i>inhA</i> ^q	Drug target
		<i>ethA</i> ^r	Pro-drug conversion

^aSee Ref. [75].

^bSee Ref. [66].

^cSee Refs [7, 67, 76, 78] and unpublished data.

^dSee Ref. [79].

^eSee Refs [63, 80] and unpublished data.

^fSee Refs [81–85].

^gInherent difficulties in phenotypic assays of *in vitro* drug susceptibility testing have largely precluded the determination of precise MIC levels; most mutations result in a loss of pyrazinamidase (PncA) activity and thus mirror the natural nonsusceptibility of *M. bovis*, which presumably corresponds to high-level drug resistance.

^hSee Refs [86–90].

ⁱSee Ref. [91] and unpublished data.

^jSee Ref. [22].

^kSee Refs [22, 53, 92–94].

^lSee Ref. [95].

^mSee Ref. [96].

ⁿSee Refs [96–99]; the MIC is dependent on the underlying genetic alteration (e.g., A1408G high-level resistance, C1409U low- to intermediate-level resistance).

^oSee Refs [100, 101].

^pPrecise MIC data are largely not available.

^qSee Ref. [66].

^rSee Refs [102, 103].

^sSee Ref. [64] and unpublished data.

the chromosomal loci responsible for the resistance to various drugs in general are not linked. Thus, poly- or multidrug resistance in *M. tuberculosis* is not due to a single genetic locus, but rather due to an accumulation of different mutations [22]. Despite clear regimens and strategies to treat TB disease and to successfully prevent the emergence of resistance, drug-resistant strains of *M. tuberculosis* are present worldwide, and in many areas constitute a serious threat to the efficacy of TB control programs. The disappointing lesson here is that drug resistance in *M. tuberculosis* is entirely man-made, whether for individual (lack of compliance), social (poverty, malnutrition, under-education), societal (inadequate policies, poor policy makings, unresponsive governments) or even criminal (falsely labeled drugs) reasons. It has become clear that the amplification of resistance with the known disastrous multi-drug-resistant (MDR) and extensively drug-resistant (XDR) situation as a consequence, might have very much been fuelled by blindly adhering to a policy that was (in hindsight) destined to produce resistant cases, owing to a failure to test properly for resistance prior to treatment [23].

In general, there is a clear correlation between the genetic mechanism and the phenotypic expression of resistance (see Table 5.2 and Figure 5.1). For example, mutations in *rpsL* (streptomycin), *rpoB* (rifampicin), or 16S rRNA (2-deoxystreptamine aminoglycosides) are associated with high-level drug resistance, and mutations in *gldB* (streptomycin) and *inhA* (isoniazid) with a low-level resistance phenotype. In addition, for defined genetic alterations, significant variability at the level of phenotypic drug susceptibility may exist (see Figure 5.1). This is particularly noteworthy for the most frequent isoniazid resistance mutation found in clinical strains, that is, the KatG S315T replacement [24–26]. This genetic alteration may be associated with a surprisingly heterogeneous phenotype of resistance, with minimum inhibitory concentration (MIC) values ranging between 2 and $>10 \text{ mg l}^{-1}$ ([65] and unpublished data). Given the isoniazid concentrations present in the infected (see Table 5.3), it is unclear whether a corresponding mutation is unanimously associated with clinical resistance.

Resistance-conferring chromosomal alterations in a drug target gene are highly restricted, so as to maintain gene function. In contrast, resistance-conferring chromosomal alterations in genes involved in pro-drug conversion, for example *pncA* and *ethA*, often display a wide diversity, indicating that there is little functional constraint as a loss of gene-function phenotype is apparently well tolerated. The predominance of a single resistance mutation in the isoniazid conversion enzyme *katG* (S315T) reflects the enzyme's critical function in detoxifying reactive oxygen species (ROS). Under those rare conditions in which *katG* expression is completely lost, this loss occurs in conjugation with a mutation in the *aphC* promoter that upregulates the expression of AhpC, an enzyme also involved in antioxidant defense [27].

Intuitively, a strain's background – that is, nucleic acid sequence polymorphisms and unknown genetic alterations – would be expected to affect the phenotypic expression of a chromosomal resistance determinant. Presumably, the best-demonstrated example here is the role of the 23S rRNA polymorphism 2057-2011 in ketolide resistance [28]. Given this paradigm, it is perhaps surprising that the

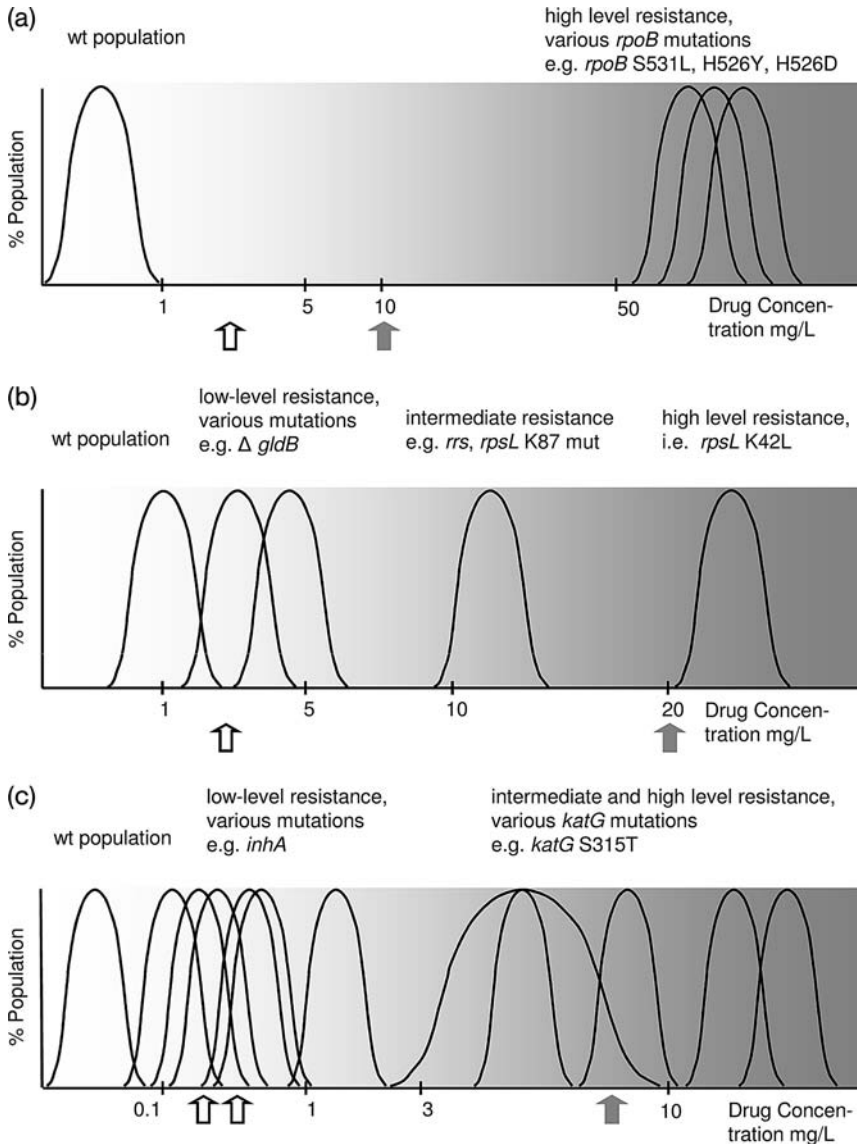


Figure 5.1 Schematized changes in drug susceptibility upon mutational alterations – exemplary Gaussian distributions of a population’s drug susceptibility. \Uparrow = “critical concentration”, \uparrow = drug serum level. (a) Rifampicin: predominantly high-level drug resistance associated with mutations in *rpoB*; (b) Streptomycin: various levels of phenotypic resistance – low-, intermediate-, and high-level drug resistance; distinct phenotypic resistance

levels are associated with distinct chromosomal mutations; (c) Isoniazid: various levels of phenotypic resistance – low-, intermediate-, and high-level drug resistance; different chromosomal mutations are associated with distinct phenotypic resistance levels; in addition, a given resistance mutation may be associated with variable phenotypic expression of drug resistance.

Table 5.3 Mycobacterial drug susceptibility testing: the critical concentration.

Antimicrobial agent	MIC of susceptible <i>M. tuberculosis</i> (mg l ⁻¹)	Concentration in serum (mg l ⁻¹)	Concentration used for testing (mg l ⁻¹)	
			Low	High
Isoniazid	0.05–0.2	5–10	0.1	0.4
Rifampicin	0.5	10	2	—
Pyrazinamid	20	40–50	100	—
Ethambutol	1–5	2–5	2.5	7.5
Streptomycin	1–2	25–50	2	6
Ethionamid	0.5–2.5	2–20	5	—
Amikacin	1	20–40	1	—
Chinolones	0.5–3.0	2–10	2	—

resistance level associated with a defined resistance mutation is a rather stable characteristic. Significant levels of phenotypic heterogeneity for a given resistance mutation have been observed only rarely, for example the *katG* S315T alteration and isoniazid resistance. Also, most of the known genetic resistance mechanisms are associated with a characteristic cost of resistance phenotype [1]. Along the line of arguments given above for the association of genetic mechanism of resistance and phenotypic resistance expression, exceptions from this rule are to be expected; that is, variability in the fitness cost associated with a defined resistance determinant due to different genetic backgrounds.

Resistance to a single drug (e.g., isoniazid) may involve various genetic alterations locating to different genes, for example *inhA*, *katG* (e.g., Ref. [29]), as well as multiple genetic alterations within a single gene, that is *katG*. Presumably, this accumulation of various resistance mutations in a single strain, all associated with resistance to a single drug, will either affect (increase) phenotypic resistance [30] or ameliorate the fitness cost associated with a defined resistance mutation [31, 32]. Rarely, strains (or, more precisely, a bacterial population recovered from a single patient) may show mixed resistance alleles, reflecting the stochastic nature of resistance mutations. Under the conditions of large inocula of microorganisms present (as in cavitary lung disease), various resistant mutants, each carrying a distinct chromosomal alteration, are simultaneously present in the bacterial population. Competition within the infected patient will select for those resistant mutants which are the most fit [33–35].

5.3

Principles of Drug Susceptibility Testing in the Laboratory

During the 1950s, the establishment of laboratory methods for the drug susceptibility testing of *M. tuberculosis* represented a tremendous challenge. At that time, when diagnostic procedures for drug susceptibility testing of bacteria were a largely

unexplored area, the sensitivity and resistance in *M. tuberculosis* were defined as follows: “‘Sensitive’ strains are those that have never been exposed to antituberculosis drugs (‘wild’ strains). ‘Resistant’ strains are those that differ from sensitive strains in their capacity to grow in the presence of higher concentrations of the drug” [36]. Fortuitously, it was found that drug-susceptible strains of *M. tuberculosis* that have not been exposed to antituberculosis drugs (wild-type strains) do not exhibit much variation in the MIC to those drugs. Depending on which laboratory method was used for drug susceptibility testing, significant differences were found in the drug concentrations which discriminate most efficiently between susceptible wild-type strains and probably resistant strains. For example, in the case of SM and with proportion testing, a maximum discrimination was achieved with a resistance proportion of 1% on 4 mg l^{-1} dihydrostreptomycin, while with the absolute-concentration method the maximum discrimination was at 16 mg l^{-1} [36].

Current procedures for drug susceptibility testing of mycobacteria are characterized by two peculiarities: (i) the critical concentration; and (ii) the critical proportion (for reviews, see Refs [37, 38]). The drug concentration which categorizes a clinical *M. tuberculosis* isolate as either susceptible or resistant is defined as the concentration that inhibits the growth of wild-type strains, without appreciably affecting the growth of strains with alterations in drug susceptibility [36, 39]; this concentration is termed the “critical concentration.” Proportion testing in mycobacteriology is based on the observation that “. . . all strains of tuberculosis contain some bacilli that are resistant to antibacillary drugs – in resistant strains the *proportion* of such bacilli is considerably higher than in sensitive strains” [39]. The drug susceptibility of a bacterial wild-type population follows a Gaussian distribution. Thus, depending on the drug concentration used, a small fraction of the population will show phenotypic resistance (see Figure 5.2). This observation forms the basis for combining proportion testing and critical concentrations (see Table 5.4).

Standardization of the critical drug concentration has not been without controversy. For example, the recommended concentrations for SM and EMB underwent adjustments over time [40, 41]. In part, the recommended concentrations are near the MIC for susceptible strains [42, 43] (see also Table 5.3). The WHO and IUATLD have initiated quality assurance programs for the susceptibility testing of *M. tuberculosis* in their supranational laboratory network [44, 45]. These studies confirmed previous findings that drug susceptibility testing procedures for INH and RMP – the two first-line TB drugs that define MDR TB – are highly reproducible. In contrast, it was found that the interlaboratory reproducibility of susceptibility testing for SM and EMB, two other first-line drugs, was significantly lower. Most likely, this is due to the very small difference between the concentration used for *in vitro* drug susceptibility testing and the natural drug susceptibility of wild-type isolates of *M. tuberculosis* (see Table 5.3). Thus, minute changes in drug susceptibility will have a major impact on the interpretation of the *in vitro* test result, within only a narrow range between the MICs of susceptible isolates and of resistant isolates.

The testing of PZA, another first-line drug, is particularly problematic. PZA, an analogue of nicotinamide, is a rather unique and unconventional antibiotic in that it

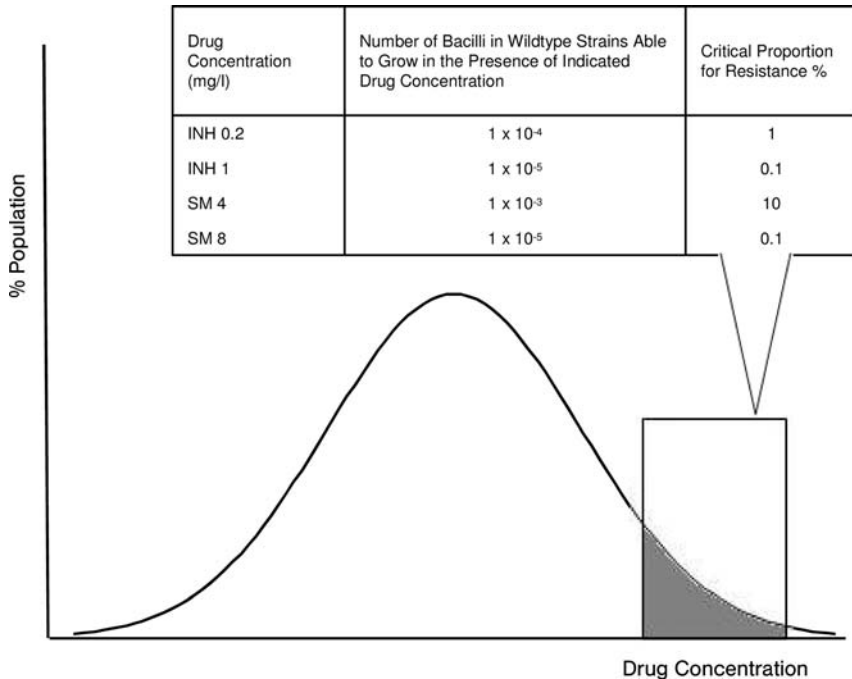


Figure 5.2 Critical concentration and proportion testing: the drug susceptibility of a wild-type bacterial population follows a Gaussian distribution.

is not active *in vitro* under normal culture conditions [46]. A paradox limiting analysis of PZA resistance is that the critical concentration used for *in vitro* testing largely exceeds the drug concentrations present *in vivo* (see Table 5.3). While the conditions established for PZA testing *in vitro* are highly artificial, they allow for a prompt recognition of resistance – in particular in combination with a determination of pyrazinamidase activity [47–50]. However, the quantitative analysis of resistance levels, for example low-level versus high-level resistance, is not possible with the procedures currently in place.

Table 5.4 Critical concentration and critical proportion.

Antimicrobial agent	Critical concentration used for testing (mg l^{-1})	Critical proportion ^a
Isoniazid	0.1	1%
Rifampicin	2.0	1%
Ethambutol	2.5	1%
Pyrazinamid	100	10%

^aThe critical proportion of drug-resistant mutants is defined as proportion of bacilli resistant to the antituberculosis drug *in vitro*, above which a clinical response is unlikely.

Among mycobacteriologists a misperception of critical proportion and clinical resistance frequently prevails. While the critical proportion of cells able to grow in the presence of the critical concentration is mostly defined as equal to or greater than 1% of the population (1 in 100) [37, 38], the frequency of mutational resistance is much lower, at approximately 10^{-7} (i.e., 0.00 001%, or 1 in ten million). It is, however, the mutational resistance which is responsible for treatment failure and for the emergence of resistance under conditions of inappropriate drug regimens, for example monotherapy (see below). The critical proportion of resistance is a technical term, and should not be confused with mutational resistance. In combination with the critical concentration, the critical proportion is a mere laboratory term used in *in vitro* drug susceptibility testing.

5.4

Clinical Implications of Drug Resistance

MDR-TB, by definition, implies a resistance to at least isoniazid and rifampicin, the two cornerstone drugs of standard short-course therapy. Therefore, a treatment based on isoniazid and rifampicin cannot be expected to cure or substantially improve TB in patients infected with MDR-TB; nor should ineffective treatment reduce the transmission of MDR-TB. Surprisingly, in some studies standard short-course therapy has been found to be an effective cure for 30–50% of patients with MDR-TB [51], and implementation of a DOTS (directly observed treatment, short course) strategy has been reported to reduce the transmission of MDR-TB [52]. The question remains, then, of how to explain these counterintuitive observations.

While meta-analyses of the impact of drug resistance on treatment outcome and transmission are complicated by the use of different methods and drug concentrations for phenotypic drug susceptibility testing in various countries, it is noteworthy to recall the definition of drug resistance in mycobacteriology. This definition dates back to 1962, and is as follows:

“Resistance is defined as a decrease in sensitivity of sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of human type that have never come into contact with the drug. . . This definition is based on the laboratory response; strains that are resistant in this sense do not *necessarily* fail to respond” [36]. Thus, rather than based on clinical outcome, this definition is an epidemiological one. Already in 1969 had the prognostic significance of *in vitro* determined drug resistance been found to be limited. “There is evidence that the presence of resistance to a single drug has little or no effect on the outcome of treatment with the three drugs isoniazid, streptomycin, and *para*-aminosalicylic acid. Furthermore, even in the presence of primary resistance to two first-line drugs, a bacteriological response is not infrequently obtained with the three drug regimen” [39].

The “gold standard” in bacteriology for drug susceptibility testing is MIC testing. This procedure is defined by two elements:

- The *drug concentration*: here, a series dilution is used to titrate the minimal amount of the drug needed to inhibit bacterial growth *in vitro*.
- The *inoculum*: the inoculum and the test conditions are chosen such that a single resistant cell in the population tested (1 in 10⁵ bacteria) is sufficient to give a resistant test result.

In contrast, in the diagnostic mycobacteriology laboratory, proportion testing and mostly a single drug concentration – the “critical concentration” – is used to study drug susceptibility and to categorize a clinical isolate of *M. tuberculosis* as being susceptible or resistant [37]. This “critical concentration” in part bears little relationship to the drug concentrations which are present *in vivo* in the patient (see Table 5.3). For example, the serum concentrations for INH and SM are 10- to 20-fold higher compared to the “critical concentration.” This contrasts with common procedures established in antibiotic therapy which take pharmacokinetic properties into account, and where the relationship between phenotypic resistance *in vitro* and drug concentration *in vivo* is addressed by the definition of breakpoints. Thus, the resistance phenotype determined *in vitro* is related to the drug levels which are present *in vivo*.

For SM and INH, a significant fraction of clinical TB isolates categorized as resistant in the diagnostic laboratory exhibits only a low-level-resistant phenotype [53, 54]. While the use of INH for the treatment of INH-resistant TB has been discussed controversially [55–57], several studies have demonstrated a benefit of adding INH to treatment regimens of INH-resistant tuberculosis, especially at high doses [58–60]. A successful treatment outcome despite a resistant phenotype – as determined by routine drug susceptibility testing – most likely reflects limitations of the procedures used to determine drug susceptibility, and indicates that low-level drug resistance may not correspond to clinical resistance [54, 57, 61]. The NCCLS subcommittee has incorporated parts of these considerations in its guidelines:

“In the case of isoniazid, if an isolate is resistant to the critical concentration of 0.1 µg/ml but susceptible to the higher concentration of 0.4 µg/ml, the following comment should be given – the test results indicate low-level resistance to isoniazid: some evidence suggests that patients infected with corresponding strains may benefit from continuing therapy with isoniazid” [62].

Data on quantitative drug susceptibility testings of clinical isolates and correlation with mutational analyses are sparse [53, 63, 64]. Over the past five years, we have systematically subjected clinical strains of *M. tuberculosis*, categorized as drug resistant on the basis of critical concentration testing, to quantitative measurements of drug susceptibility and molecular profiling [65]. The outcome can be briefly summarized as follows:

1. **Isoniazid**: mutations in *katG* are associated with intermediate- to high-level drug resistance, mutations in *inhA* are found in isolates with low-level drug resistance;

a significant fraction of clinical TB strains categorized as resistant to INH exhibits a low-level resistant phenotype with MIC values less than 1.0 mg l^{-1} .

2. **Rifampicin:** resistance predominantly is associated with *rpoB* mutations and a high-level drug-resistant phenotype (MIC values $>50 \text{ mg l}^{-1}$).
3. **Ethambutol:** in approximately 60% of clinical strains categorized as resistant, mutations in *embB* codon 306 are present; phenotypic drug resistance is of a low to intermediate type (MIC $5\text{--}25 \text{ mg l}^{-1}$).
4. **Streptomycin:** at the phenotypic level low- (MIC $\geq 1 \text{ mg l}^{-1} < 10 \text{ mg l}^{-1}$), intermediate- (MIC $\geq 10 \text{ mg l}^{-1} < 50 \text{ mg l}^{-1}$) and high-level (MIC $\geq 50 \text{ mg l}^{-1}$) drug resistance is found; intermediate- and high-level drug resistance is associated with mutations in *rpsL*.
5. **Ethionamide:** drug resistance is of a low- to high-level resistant phenotype (MIC 5 to $>25 \text{ mg l}^{-1}$), and is frequently associated with alterations in *inhA*, in particular C15T.

It is conceivable that low-level drug resistance does not correspond to clinical resistance; conversely, in the presence of a high-level resistant phenotype (e.g., *rpoB*, *rpsL*) the drug presumably is of little, if any, clinical benefit. Resistance in *M. tuberculosis*, as categorized by current procedures of drug susceptibility testing, apparently is a heterogeneous bag. While low-level resistance may not correspond to clinical resistance, the failure to properly recognize high-level drug resistance may result in a further build-up of resistance. The clinical implications of intermediate levels of resistance (in particular *katG*, *embB*) are less clear. The virtual absence of high-level EMB resistance has brought us to recommend the inclusion of EMB in drug regimens, despite resistance at the critical concentration.

The consequences of erratic drug susceptibility testing are particularly severe in terms of treatment options for apparent MDR or XDR TB. Isoniazid resistance reported by the diagnostic laboratory may lead to the use of second- or even third-line antibiotics – compounds which are compromised by severe toxicity and which almost certainly have inferior activity than INH against low-level INH-resistant strains. The thioamide drugs, ethionamid (ETH) and prothionamid (PTH), are reasonable treatment options for MDR TB. Isoniazid and the thioamide drugs share *InhA* as the primary target of action [66]. In contrast to INH, the thioamides do not require activation by *KatG*, and therefore strains with high-level INH resistance due to mutational *katG* alterations typically retain thioamide susceptibility. Newly introduced molecular diagnostic tests (e.g., the GenoType MTBDR assay) offer the rapid determination of genotypic resistance, as they allow for the direct detection of the most frequent and relevant *rpoB*, *katG* and *inhA* resistance mutations in smear-positive specimens [67]. These tests may assist in therapeutic decisions in the treatment of drug-resistant TB, for example whether to use INH or the alternative thioamide drugs.

While an enormous amount of knowledge has been gained during the past 20 years on drug resistance in *M. tuberculosis*, covering the vast majority of molecular mechanisms involved, some of these remain to be elucidated. Consequently – and

also given the complexity involved – a negative genetic screening test does not *per se* imply drug susceptibility, while a positive genetic test result may guide therapy. Having said this, it is a relief to note that a genetic screening assay which is negative for both *rpoB* and *katG* mutations (e.g., GenoType MTBDR assay) practically rules out MDR and XDR TB, the resistance patterns which are most problematic.

Streptomycin-resistant *M. tuberculosis* are particularly prevalent in many regions of the developing world. For example, in a recent study a resistance to SM accounted for >75% of monoresistance and was involved in >90% of poly- and multidrug resistance [68]. Given this prevalence, it is difficult to grasp that some retreatment regimens recommend a five-drug approach that includes INH, RIF, SM, EMB, and PZA. It is not surprising that such recommendations result in high levels of resistance amplification.

5.5 Outlook and Perspectives

The emergence and rise of drug-resistant TB urgently requires the development of new antituberculosis drugs. While there are promising candidate compounds in the pipeline [69–73], it will probably take years for a new drug to reach infected patients in the clinics. Hence, for the time being the best strategy is to make optimal use of the drugs that are available. This involves the widespread use of molecular tests for the rapid genotyping of resistance, in order to guide rational decisions on therapeutic regimens, as well as changes in laboratory drug susceptibility testing procedures. Following 40 years of proportion-based testing at critical concentrations, it is time to adapt mycobacterial drug susceptibility testing to standard bacteriology procedures, as are used for other microbes. Whilst the procedures in place are appropriate for screening, they must be complemented by measures of quantitative drug susceptibility testing [65, 74], in particular for those drugs where heterogeneity in phenotypic resistance is frequently present (low-, intermediate-, high-level resistance), such as isoniazid, streptomycin, and kanamycin. The widespread implementation of standardized protocols for quantitative drug susceptibility testing for both first- and second-line drugs will be the most important prerequisite to correlate data from quantitative resistance testing with clinical outcome, and to address the biological relevance of low and intermediate drug resistance. Hopefully, this will allow the assembly of available compounds into effective regimens so as to fully exploit their potential in the treatment of what is currently perceived as drug-resistant TB. Given the limited number of drugs available for the management of MDR TB, it is essential to take advantage of those that could possibly be used in a multidrug regimen to treat a significant proportion of corresponding cases.

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6

HIV–TB Drug Interactions¹⁾

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6.1

Important Concepts and Definitions [1, 2]

- **Combination antiretroviral therapy** Antiretroviral therapy where typically three or four drugs, in most cases from different drug classes, are given in combination.
- **Cytochrome P450 (CYP)** Membrane-associated hemoproteins, located either in the inner membrane of the mitochondrion or in the endoplasmic reticulum of the cells. CYPs metabolize endogenous and exogenous compounds, such as hormones (estrogen and testosterone) and xenobiotics.
- **P-glycoprotein** An energy-dependent efflux pump that exports substrates out of the cell, and is expressed in the epithelial cells of the gastrointestinal tract, the liver, the kidneys, the blood–brain barrier, and in CD4 + lymphocytes.
- **Drug interaction** Said to occur when the disposition of one drug is altered by another.
- **Pharmacokinetic drug interaction** Involves alteration in absorption, transport, distribution, metabolism or excretion of a drug, the results of which can be a decreased or an increased exposure, leading to reduced efficacy or increased toxicity, respectively.
- **Pharmacodynamic drug interaction** Pharmacological response to the drug is directly altered, leading to potentiation of effect (including toxicity) in either an additive or synergistic manner, or antagonism.

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6.2

Background

As of December 2007, two billion people globally are infected with *Mycobacterium tuberculosis*-mediated tuberculosis (TB) [4], and 33.2 million with human immunodeficiency virus (HIV) [5]. Where these pandemics intersect, they are the most common cause of death among young adults in many countries. In 2007, 2.1 million acquired immune deficiency syndrome (AIDS)-related deaths occurred worldwide [5], while in 2006 1.7 million deaths were attributed to TB [6]. The developing world accounts for the majority of the 11 million people coinfecting with HIV and *M. tuberculosis* [4]. In 2006, there were an estimated 709 000 new HIV-infected TB cases, 85% of which occurred in Africa [6]. TB and AIDS are inexorably entwined; the annual incidence of TB disease doubles within the first year of HIV infection [7], and may reach 30% per annum in the profoundly immune-suppressed [8]. Likewise, TB disease synergistically accelerates the progression of HIV infection to AIDS, by promoting viral replication in immunologically activated CD4 cells [9–11]. Soon after the commencement of TB therapy, combination antiretroviral therapy (cART) is often required to ameliorate the profound immune suppression of AIDS. In addition, simultaneous treatment(s) for associated AIDS-defining illnesses and complications is/are often necessary. While dual AIDS-TB therapy appears associated with improved survival compared to delayed antiretroviral therapy [12], several substantial challenges exist, namely drug–drug and drug–disease interactions, shared drug toxicities, immune reconstitution inflammatory syndrome (IRIS), and high pill burdens [3]. In particular, the possibility for drug–drug interactions that have clinically important consequences increases in HIV-infected patients receiving cART if three or more comorbid illnesses occur simultaneously, or three or more antiretroviral agents are taken concurrently [13]. In this chapter we describe the current therapy for AIDS-TB, and discuss the clinically relevant drug–drug and drug–disease interactions that may occur in HIV-infected patients receiving dual AIDS-TB therapy. A prediction is also made of the potential drug interactions that are likely to occur in the developed world.

6.3

Current Therapy for TB and AIDS

Tuberculosis therapy is a multidrug regimen prescribed for a minimum of six months. Multidrug treatment needs to be prolonged as single-agent TB therapy rapidly gives rise to drug-resistant organisms [14]. The advent of rifampin and pyrazinamide allowed the development of highly effective “short-course” TB regimens – usually a two-month intensive phase with rifampin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E) – followed by a four-month continuation phase with RH (2RHZE/4RH) [15]. Multi-drug resistant (MDR) TB, which is defined as TB resistant to RH, is treated with less-effective agents for up to 18 months after sputum conversion. Treatment is often initially empiric whilst awaiting drug

susceptibility results, and later individualized when such results are known. Regimens may last for up to 24 months and include (according to susceptibility profile): pyrazinamide, ethambutol, terizidone, ethionamide, an oral fluoroquinolone (ofloxacin/ciprofloxacin/gatifloxacin/moxifloxacin), and an injectable agent (kanamycin/amikacin/capreomycin). Extensively drug-resistant (XDR) TB, defined as MDR TB with resistance to both an oral fluoroquinolone and an injectable agent [16], also requires individualized treatment regimens [17, 18]. In addition, drugs to which *M. tuberculosis* remains susceptible, such as capreomycin, *para*-aminosalicylic acid (PAS), co-amoxycylav and linezolid, may be added.

Combination antiretroviral therapy (cART) is a lifelong, multidrug regimen. In patients infected with HIV, millions of virions are produced daily, and the reverse transcriptase target rapidly mutates. Hence, initial therapies with single or dual nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine (AZT) and didanosine (ddI), were only partially effective and rapidly led to viral drug resistance [19]. Effective therapy only became possible when non-nucleoside reverse transcriptase inhibitor (NNRTI) and viral protease inhibitor (PI) drugs were developed. Combinations of these three drug classes may lead to a prolonged suppression of HIV replication, and ultimately to a degree of immune recovery. Adherence to cART is crucial for successful viral suppression, which is very closely related to immune restoration and survival [20].

6.4 Potential Drug–Drug and Drug–Disease Interactions

Numerous concurrent diseases occur in profoundly immune-suppressed AIDS-TB patients, including *Pneumocystis jiroveci* pneumonia, toxoplasmosis, Kaposi's sarcoma, deep-vein thrombosis, seizure disorders, and sepsis [21]. The potential for drug–drug and drug–disease interactions increases when treatment for these illnesses is prescribed concomitantly with AIDS and TB therapy (Figure 6.1). While cART partially restores immune function and prolongs life, HIV-infected patients are at increased risk of metabolic and vascular disorders; these disorders may occur as a direct result of HIV infection [22], as side effects of AIDS therapy [23–25], or because prolonged survival allows HIV-infected patients to develop diseases that would otherwise have occurred later in life. Simultaneous treatment for AIDS, diabetes mellitus and diseases due to atherosclerosis, will challenge many clinicians, especially as a subgroup of patients will still have an increased risk of TB disease [26] and require treatment with rifamycins.

Potential drug–drug and drug–disease interactions should always be considered in patients infected with HIV-*M. tuberculosis*, especially as many of the drugs used to treat AIDS-TB and comorbid diseases are either inducers, inhibitors, or substrates of cytochrome p450 (CYP). Drugs that induce a particular CYP isoenzyme increase the rate at which the CYP isoenzyme metabolizes its substrate to metabolites. Conversely, inhibitors decrease the rate of metabolite production, resulting in increased substrate. Rifampin is a potent inducer of CYP3A4, whereas ritonavir is an inhibitor

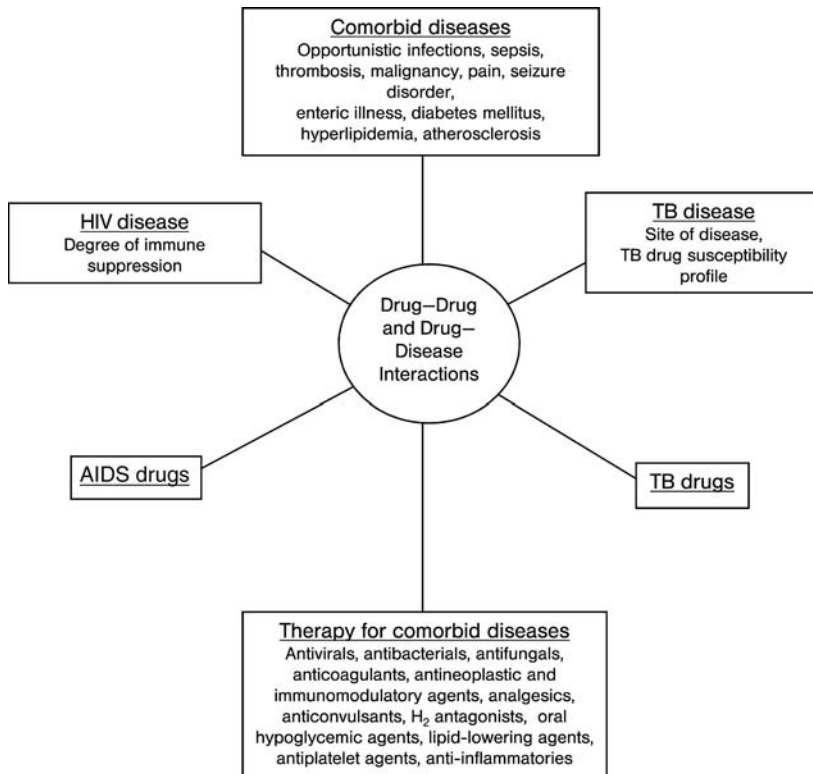


Figure 6.1 Potential contributors to AIDS-TB drug–drug and drug–disease interactions: TB, AIDS and comorbid diseases and therapy.

of CYP3A4. When warfarin and oral contraceptives are coadministered with CYP inducers, suboptimal anticoagulation and contraceptive failure, respectively, may occur. Similarly, when statins, calcium antagonists and benzodiazepines are coadministered with CYP inhibitors, rhabdomyolysis, symptomatic hypotension and excessive sedation, respectively, may result.

6.5 Treatment of Tuberculosis

Combination TB therapy with PAS and streptomycin was first reported in 1950 [27], it having been recognized at an early stage that monotherapy rapidly gave rise to bacterial resistance. With the later advent of rifampin, isoniazid, and pyrazinamide, large studies demonstrated that the best chance of curing TB would be provided by the combined use of rifampin and isoniazid for six months, with pyrazinamide and ethambutol added for the first two months. Rifampin results in a faster sputum conversion [28] and a shorter treatment duration [29]. The use of

rifampin in a multidrug regimen reduces the emergence of drug-resistant strains. In order to optimize pharmacotherapy, factors affecting the absorption, distribution, metabolism and elimination of anti-TB drugs should be considered, as should their pharmacokinetics and significant interactions (see Table 6.1 and below).

6.5.1

Rifampin

As rifampin interacts with a wide range of drugs, it is useful to understand its pharmacokinetics in order to predict possible drug interactions. An important mechanism of drug interactions with rifampin is the induction of drug-metabolizing enzymes such as CYP 3A4 [30] in the small intestine and liver. Rifampin also induces the CYP 2C isoenzymes, and therefore has the potential for interaction with CYP 2C substrates, including sulfonylureas such as gliclazide [31]. However, other possible mechanisms have been sought, as not all drug interactions can be explained by an induction of the cytochrome P450 system (see Table 6.1). In particular, the ATP-binding cassette (ABC) efflux transporter P-glycoprotein, located in the apical membrane of enterocytes, has been found to have a role in the elimination and bioavailability of certain drugs [32, 33]. Rifampin has been shown to be an inducer of P-glycoprotein, and this represents another mechanism through which drug–drug interactions can occur; indeed, this is thought to be the mechanism for rifampin–digoxin drug interaction [34]. Such induction is thought to be tissue-specific, as renal P-glycoproteins do not appear to be induced by rifampin [34], possibly because only enterocytes are locally exposed to high concentrations of orally administered drug, and not the kidneys or liver. In addition, it was found that human MRP2 (part of the multidrug-resistant protein family, a member of the ABC transporters, and expressed also in small intestine enterocytes) is induced by rifampin, thus alluding to another possible mechanism for drug interaction of rifampin with other drugs [35].

Rifampin may also activate the human glucocorticoid receptor by acting as a ligand and binding to the receptor, and is therefore a potential immunosuppressive [36]; however, this point has attracted controversy [37]. Rifampin may reduce the concentration of drugs metabolized by uridine diphosphate glucuronosyl transferase (UDPGT) and sulfotransferase (e.g., moxifloxacin) [38].

Trimethoprim-sulfamethoxazole is commonly used in HIV-infected patients as prophylaxis against *Pneumocystis jiroveci* pneumonia (PJP) and toxoplasmosis. In one clinical study it was suggested that rifampin could reduce the efficacy of cotrimoxazole as prescribed for toxoplasmosis prophylaxis [39]. A pharmacokinetic study conducted subsequent to this study showed reduced concentrations of both trimethoprim and sulfamethoxazole in the presence of rifampin, with the suggested mechanisms being rifampin's induction of either the CYP450 system, the induction of UDPGT, or by the induction of hepatic acetylation of sulfamethoxazole [40]. The effect on clinical outcome of this reduction in plasma concentration, however, is not known. Data are also available suggesting that rifampin levels increase after the

Table 6.1 Pharmacokinetics of TB drugs.

Drug name	CYP			P-glycoprotein			Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer	Inhibitor		
First-line TB drugs								
Rifampin	No	3A4, 1A2, 2C, 2D6			Yes		Metabolized by deacetylation induces UDPGT and sulfotransferase	85% protein bound
Rifapentine		3A4, 2C8/9						97% protein bound
Rifabutin	3A	3A, 2D						Least protein bound -70%
Isoniazid	No	2E1	2C9, 2C19				Metabolized by NAT2 and 2E1 Inhibits MAO	
Pyrazinamide	No	No					Metabolite inhibits uric acid secretion by renal tubules	
Ethambutol	No	No					15% metabolized to aldehyde and dicarboxylic metabolites	
Streptomycin	No	No	No				No identified metabolites	

Second-line drugs					
Kanamycin	No	No	No		No identified metabolites
Amikacin	No	No	No		No identified metabolites
Ofloxacin				Yes	
Ciprofloxacin [85]			1A2	Yes	20–40%
Moxifloxacin [86]				Yes	40–50%
Ethionamide	3A				
Capreomycin					
Clarithromycin [87–89]	3A		3A1		Hydroxylation and oxidative N-demethylation >50% acetylated
PAS [90]					
Augmentin					
Linezolid [79, 91]					Metabolized by nonenzymatic oxidation, reversible inhibitor of MAO A/B
					31%, albumin
Cycloserine					
Terizidone					

Abbreviations: CYP: cytochrome p450 isoenzyme; PAS: *para*-aminosalicylic acid; TB: tuberculosis; UDPGT: uridine diphosphate glucuronosyltransferase.

administration of co-trimoxazole [41] but, again, the clinical significance of this is unknown.

Dapsone is another drug used in the prophylaxis of PJP. It has been suggested that rifampin decreases the serum concentration of dapsone through the induction of CYP3A4 and, indeed, one study has shown dapsone clearance to be increased by between 69% and 122% [42]. It was not clear whether this reduction in concentration would result in a decreased efficacy in clinical practice [43], though some experts believed this to be the case [44]. Rifampin is itself metabolized by deacetylation, and is therefore unaffected by the CYP450 system; it is known to be capable of inducing its own metabolism, however [45].

6.5.2

Rifapentine

Rifapentine is a long-acting cyclopentyl-derivative of rifampin, and is an inducer of CYP3A4 and CYP2C8/9 of the same order of magnitude as rifampin [46]. Rifapentine does not possess autoinductive properties [47] after repeated administration, however. In the plasma, rifapentine has been shown to be highly protein-bound (97–99%), primarily to albumin, in healthy volunteers [48]. Such protein binding may be important for the drug's pharmacodynamics when compared to rifabutin, which is the least protein-bound rifamycin and shows little reduction in efficacy with dose reduction [49].

The few drug interaction studies performed with rifapentine have shown the most significant interaction to be with indinavir; coadministration of the two drugs led to a reduction of 55% in the maximum plasma concentration, and of 70% in the area under the concentration–time curve (AUC) [50].

6.5.3

Rifabutin

Rifabutin not only induces but is also metabolized by CYP 3A4; this results in complex interactions with inhibitors of CYP450, such as protease inhibitors, antifungal agents, and macrolide antibiotics. Rifabutin is also a potent autoinducer. Some data are available suggesting that microsomal cholinesterase is also involved in the metabolism of rifabutin [51].

6.5.4

Isoniazid

Isoniazid inhibits CYP isoenzyme systems and monoamine oxidase (MAO), and so is associated with some drug interactions [52]. Isoniazid is mainly metabolized by hepatic *N*-acetyltransferase 2 (NAT2) and CYP450 2E1. Unlike the rifamycins, it is mostly excreted via the urine. It is believed that isoniazid kills the largest population of *M. tuberculosis* in the rapidly growing phase. Several studies have shown that the acetylator status of individuals may play a role in determining clinical outcome, with

slow acetylators showing a reduced enzyme activity. Despite this, it has been shown that when isoniazid is given at least twice-weekly, the clinical outcome is independent of acetylator status, although slow acetylators are more prone to hepatotoxicity [53]. These results have led some to suggest that NAT2 genotyping might be used to ascertain acetylator status in the monitoring of TB treatment [54]. Acetylator status appears relevant in isoniazid's interaction with paracetamol (acetaminophen), with some data showing rapid acetylators to have an increase in the levels of paracetamol metabolites. It is thought that the induction of the CYP450 system leads to an increase in hepatocellular injury due to an increased formation of toxic metabolites of paracetamol [55]. A study conducted in mice showed that aspirin antagonized isoniazid treatment, with possible implications for the coadministration of salicylate-based anti-inflammatories and isoniazid.

6.5.5

Pyrazinamide and Ethambutol

Pyrazinamide and ethambutol each have a limited drug interaction profile [56].

The main metabolite of *pyrazinamide*, pyrazinoic acid, inhibits the renal tubular secretion of uric acid and hence may induce hyperuricemia. There is a scarcity of data regarding possible drug interactions of pyrazinamide; hepatotoxicity following its administration has also been demonstrated, although the mechanism involved is not clear [57]. An allopurinol–pyrazinamide interaction has been reported that causes a build-up of pyrazinoic acid and reduces the renal secretion of uric acid [58].

Ethambutol, as an anti-TB drug is predominantly bacteriostatic, and is administered during the intensive phase of TB in an attempt to prevent further drug resistance (see Table 6.1 for further information on metabolism). Various data have suggested an interaction with aluminum-magnesium antacids, leading to a reduction in plasma ethambutol concentrations [59].

6.5.6

Ethionamide

Ethionamide is thought to be metabolized by the cytochrome P450 enzymes, and may potentially have interactions with inducers or inhibitors of this system. However, there is a paucity of data on the pharmacokinetics of this drug.

6.5.7

Fluoroquinolones

Ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin, as fluoroquinolones, are used to treat TB via their inhibitory effect on DNA gyrase. As a class, the fluoroquinolones are not significantly affected by coadministration with food [60]. One known adverse effect of fluoroquinolones treatment is that of dysglycemia; this is especially the case when gatifloxacin is administered to patients receiving concomitant treatment for diabetes, to elderly patients, and to those who are renally

impaired [61]. The most common drug interactions with fluoroquinolones in TB therapy include malabsorption interactions associated with multivalent cations, and cytochrome P450 interactions with ciprofloxacin [62, 63]. The combination of pyrazinamide and ofloxacin appears to cause increased rates of asymptomatic hepatitis and gastrointestinal intolerance [64, 65], while combined ofloxacin and cycloserine may lead to an increased incidence of central nervous system (CNS) - mediated effects, possibly due to altered γ -aminobutyric acid (GABA) binding [66]. Modest, but potentially important, drug–drug interactions affecting the concentrations of gatifloxacin and rifampin have been reported [67]. Importantly, the Rv2686c-Rv2687c-Rv2688c genes of *M. tuberculosis* encode an ABC transporter responsible for fluoroquinolone efflux [68]; this efflux was shown to lead to a reduced accumulation of fluoroquinolone by its active removal, thereby potentially contributing to fluoroquinolone resistance in *M. tuberculosis*.

6.5.8

Streptomycin/Amikacin/Kanamycin/Capreomycin

The aminoglycoside antibiotics consist of sugar and amino moieties. Among these, streptomycin is used to treat drug-sensitive TB, while amikacin and kanamycin are used for MDR TB. As the cytochrome P450 system neither induces nor inhibits aminoglycoside activity, interactions with potent CYP inducers (rifampin) and inhibitors (protease inhibitors) do not occur. Aminoglycosides must be administered parenterally as they are poorly absorbed via the intestine. They are also not metabolized and are excreted unchanged, predominantly in the urine. The side effects of aminoglycoside are dose-dependent, and include nephrotoxicity (potentially reversible), ototoxicity (usually irreversible), and neuromuscular blockade. Thus, the concomitant administration of aminoglycosides with diuretics, radiographic contrast, angiotensin-converting enzyme (ACE) inhibitors, nonsteroidal anti-inflammatory drugs, amphotericin, and cisplatin is usually avoided [69–71]. *Capreomycin* is a peptide antibiotic that is used extensively to treat drug-resistant TB. However, as its adverse effects include nephrotoxicity and ototoxicity, its coadministration with other nephrotoxic or ototoxic agents is not advised.

6.5.9

Terizidone/Cycloserine

Terizidone is a combination of two molecules of cycloserine. A comparison of cycloserine and terizidone showed the blood levels of terizidone to be higher at all time points than those of cycloserine, although the difference was not proportional to two molecules of cycloserine being contained in one molecule of terizidone [72]. The high concentration of terizidone in urine suggests that it may be of benefit in genitourinary TB [72]. Evidence from South Africa has indicated that terizidone causes fewer adverse effects (incidence ca. 1%) than cycloserine (ca. 11%) [73]. Terizidone is a valuable companion drug to prevent resistance to other second-line

drugs, as it does not share any cross-resistance with other active TB drugs. Pyridoxine may decrease CNS-related effects, and a dose of 150 mg should be prescribed to all patients receiving terizidone or cycloserine. Terizidone should be avoided in patients with a history of epilepsy, alcoholism, and mental illness (especially depression) [73].

6.5.10

Linezolid

Linezolid does not induce cytochrome P450, and is not metabolized by this process. This is important given the possibility of its use in patients concurrently taking antiretrovirals [74, 75]. Linezolid is associated with mitochondrial toxicity, and as a result causes side effects such as peripheral neuropathy and lactic acidosis [76]. It is also known to cause reversible myelosuppression, thrombocytopenia and anemia in some patients [77]. The oral absorption (by AUC) of linezolid is unaffected by the presence of food in the intestine [78]. Drug interactions based on MAO inhibition are limited to increases in blood pressure with coadministered adrenergic agents, and are unlikely to be of any significant magnitude [79].

6.5.11

Co-Amoxyclav

The early bactericidal activity of amoxicillin/clavulanate is comparable to that reported for antituberculous agents other than isoniazid [80]. However, it has been reported unlikely that the combination of amoxicillin/clavulanic acid would have an important role in the treatment of TB, with the exception of those patients with MDR TB who otherwise are “therapeutically destitute” [81].

6.5.12

PAS

Para-aminosalicylic acid is metabolized to acetyl-PAS [82], and both compounds are excreted renally; consequently, PAS should be avoided in renal failure. The gastrointestinal toxicity of PAS also limits its use, especially as other anti-TB drugs are less likely to cause gastrointestinal side effects. Currently, PAS is formulated as granules and taken with food [83].

6.5.13

Clarithromycin

Clarithromycin is a macrolide antibiotic. Potent inhibitors of CYP3A may alter the metabolism of clarithromycin and its metabolites, while clarithromycin itself can increase the steady-state concentrations of drugs that depend primarily upon CYP3A metabolism [84].

6.6

Treatment of HIV Infection

6.6.1

Fusion Inhibitors

Enfuvirtide is a recently registered antiretroviral that inhibits HIV fusion to CD4 cells. It is not a substrate for CYP isoenzymes and neither inhibits nor induces CYP3A; thus, no significant interactions with rifamycins exist [100–102].

6.6.2

Nucleotide/Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

The NRTIs are predominantly excreted via the renal system (tubular secretion), and interactions based upon CYP are infrequent [17]. However, drugs influencing renal clearance or intracellular phosphorylation may interact with the NRTI. Significant pharmacokinetic interactions have been demonstrated when zidovudine is prescribed with probenecid, naproxen, and fluconazole [103].

Tenofovir is not a substrate, inducer or inhibitor of human cytochrome P450 enzymes (see Table 6.2). Tenofovir and rifampin may be used without dosage adjustment for the treatment of TB in HIV-infected patients [104]. However, patients with renal impairment (especially if receiving streptomycin) should be closely monitored. Tenofovir has no clinically significant drug interactions, with the exception of didanosine and atazanavir, which will require dosage modifications to be made [105]. AIDS patients coinfecting with hepatitis B virus/hepatitis C virus (HBV/HCV) are likely to be treated with tenofovir and lamivudine or emtricitabine.

6.6.3

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs)

The NNRTIs and PIs are extensively metabolized by the cytochrome P450 and P-glycoprotein systems (summarized in Table 6.2). When drugs metabolized by the same pathways are administered concomitantly, pharmacokinetic drug interactions commonly result. Furthermore, ritonavir is both an inhibitor and substrate of the drug transporter P-glycoprotein [2], thus increasing the potential for drug interactions.

6.6.3.1 Oral Bioavailability of Delavirdine and PIs

The absorption of delavirdine and some PIs is affected by gastric pH and/or simultaneous food intake; typically, a reduction in gastric acidity (pH >3) decreases the absorption of delavirdine. Indinavir is extensively (80%) absorbed from an empty stomach, and its bioavailability is decreased when administered with a fatty meal [106]. The addition of ritonavir to indinavir increases bioavailability, regardless of the stomach content [107]. The absorption of atazanavir (another PI) is dependent on gastrointestinal pH [94]. The concomitant administration of didanosine 200 mg

Table 6.2 Pharmacokinetics of antiretroviral drugs.

Drug name	CYP			P-glycoprotein			Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer	Inhibitor		
Nucleoside reverse transcriptase inhibitors (NRTIs)								
Zidovudine							Glucuronidation,	34–38%
Stavudine								5%
Lamivudine							5–10% metabolized to inactive <i>trans</i> -sulfoxide metabolite	<36%
Didanosine								5%
Zalcitabine								5%
Abacavir							Alcohol dehydrogenase, glucuronosyltransferase	50%
Nucleotide reverse transcriptase inhibitors (NRTIs)								
Tenofovir								
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)								
Efavirenz [92]	3A4, 2B6	3A4	2C9/19, 3A4	Yes				>99%, albumin
Nevirapine [92]	3A4, 2B6	3A4, 2B6		Yes				60%, albumin
Delavirdine [92]	3A4, 2D6, 2C9/19		3A4, 2C9, 2C19, 2D6	Yes				98%, albumin
Protease inhibitors (PIs)								
Amprnavir [93]	3A4, 2D6		3A4	Yes				90% α -acid glycoprotein

(Continued)

Table 6.2 (Continued)

Drug name	CYP			P-glycoprotein			Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer	Inhibitor		
Atazanavir [94]	3A4		3A, GT	Yes				89%, α -acid glycoprotein 86%, albumin
Darunavir [95]	3A4		3A4	Yes			Hydroxylation, hydrolysis	95% α -acid glycoprotein
Indinavir [93]	3A4, GT		3A4	Yes				60%, α -acid glycoprotein
Lopinavir [93]	3A4	GT	3A4, 2D6	Yes				98–99% α -acid glycoprotein
Nelfinavir [93]	3A4, 2C9, 2C19, 2D6	GT	3A4	Yes				>98%, α -acid glycoprotein
Ritonavir [93]	3A4, 2D6	GT, 1A2, 3A, 2C9	3A4, 2D6	Yes		Yes		98–99%, α -acid glycoprotein
Saquinavir [93, 96]	3A4		3A4	Yes				98%, α -acid glycoprotein
Tipranavir [97]	3A4	3A4		Yes	Yes		Glucuronidation	>99%, α -acid glycoprotein
Fusion inhibitors Enfuvirtide [98–100]							NADPH hydrolysis	92%, albumin

Abbreviations: CYP: cytochrome p450 isoenzyme; GT: glucuronyl transferase; NADPH: nicotinamide adenine dinucleotide phosphate.

with atazanavir 400 mg on an empty stomach reduces atazanavir absorption by 90% (the didanosine absorption remains unchanged) [108]. The systemic absorption of darunavir is increased by 30% when taken with a meal, but acid-reducing agents have no adverse effect on bioavailability [109].

6.6.3.2 CYP Interactions in PIs

In the plasma, *darunavir* is approximately 95% bound to proteins (especially α 1-acid glycoprotein), and is extensively and almost exclusively metabolized by CYP3A4. The coadministration of darunavir with small doses of ritonavir results in an increase in the bioavailability of the former, from 37% to 82% [95].

Tipranavir induces both CYP and P-glycoprotein (see Table 6.2), and although ritonavir is a P-glycoprotein inhibitor, *in vivo* data suggest that the net effect of tipranavir/ritonavir 500 mg/200 mg is P-glycoprotein induction [110]. Tipranavir may therefore potentially decrease the absorption of concomitantly administered drugs which are substrates for P-glycoprotein [97]. The potent CYP3A4 inhibitory effects of ritonavir appear to outweigh the inducing effects of tipranavir.

6.7

Treatment Issues in Coinfection

6.7.1

Shared Toxicities

Shared drug toxicities, although clinically relevant, are beyond the scope of this chapter. Rather, the reader should consult recent reviews on this subject [3, 111].

6.7.2

TB/Antiretroviral Drug Interactions

The most significant drug–drug interaction is between rifamycins and antiretrovirals. These interactions are important, as rifamycins form the backbone of current antitubercular treatment in susceptible *M. tuberculosis* infection. An understanding of these interactions is critical to appropriate management.

6.7.2.1 Rifamycins

The rifamycins induce the cytochrome P450 enzyme system in the liver and intestinal wall, leading to a decrease in the serum half-life and in the concentrations of drugs metabolized by that system. The rifamycins vary in their ability to induce the CYP450 3A4 enzymes, with rifampin being the most potent and rifabutin the least active. In the developed world, rifabutin is the preferred rifamycin when coadministered with antiretroviral therapy; however, its high cost has hitherto precluded its use in resource-limited settings where the burden of TB and AIDS is highest. As a result, the most important drug–drug interactions in the developing world are between rifampin and antiretrovirals, especially the NNRTIs and the PIs. The autoinduction

of rifampin's metabolism after repeated doses also merits attention; a pharmacokinetic study of hospitalized TB patients showed a high prevalence of low rifampin levels which could, in part, be due to autoinduction, although this might also be related to other factors such as alcohol use, gender, and drug formulation [112]. Some of these results are explained in Table 6.3, which summarizes the details of cytochrome P450 substrates and inducers.

6.7.2.1.1 Rifampin + NRTI Although there is some evidence to show that reduced levels of NRTIs such as zidovudine are observed when coadministered with rifampin as a result of rifampin inducing the glucuronidation of zidovudine [113], there is no evidence of a decrease in the intracellular concentration of the active form of the drug [114]. This implies that the efficacy of the drug is likely to be unaffected and can be safely coadministered.

6.7.2.1.2 Rifampin + NNRTI

Efavirenz There is often conflicting or lack of evidence over whether dose increments are required when this drug is given concomitantly with rifampin, although it is generally considered to be an adequate choice of antiretroviral. As rifampin is a potent inducer of the cytochrome P450 system, it leads to a decrease in the plasma levels of NNRTIs. Pharmacokinetic studies have indeed reported that rifampin decreases plasma levels. In one study, efavirenz levels were decreased by 13–25%; this was a modest decrease when compared to a 40% reduction of nevirapine and 90% with delavirdine [115], and so efavirenz is preferred for coadministration with rifampin. As a result of these pharmacokinetic studies, the CDC guidelines recommend that rifampin and delavirdine should not be used together [116]. In another study, increasing the efavirenz dose from 600 mg to 800 mg increased the plasma levels close to those observed in patients receiving 600 mg daily, without rifampin [117]. As a result of these studies, some advise that the efavirenz dose should be increased from 600 mg to 800 mg when coadministered with rifampin [118]. However, further evidence shows that trough levels are not associated with a poor clinical outcome. Several studies, using either 600 mg or 800 mg, showed no association between trough levels of efavirenz and clinical outcome, which indicated that a standard dose of 600 mg might be sufficient [119]. Furthermore, an increased dose may increase the side effects, especially in patients weighing <55 kg, and also in some black and Asian patients who have greater genetic predisposition to higher plasma levels and adverse effects. This is related to a polymorphism in CYP 2B6 enzyme in these patients, which is associated with elevated efavirenz concentrations [120, 121].

Nevirapine This is the most widely used NNRTI in resource-poor settings, as it is cheaper than efavirenz and can be used in women of childbearing age as it is not known to be teratogenic. However, it does interact with the CYP450 system, both as a substrate and inducer. Studies have shown a greater reduction in plasma levels when combined with rifampin compared to efavirenz [122]. The standard dose is an initial lead-in dose of 200 mg daily for two weeks to

Table 6.3 Substrates, inhibitors and inducers of cytochrome p450 enzymes (CYP), and P-glycoprotein (PGP).

	Substrate	Inhibitor	Inducer
CYP1A2	Acetaminophen	Erythromycin	Cigarette smoke
	Caffeine	Cimetidine	Phenobarbital
	Clozapine	Ciprofloxacin	Phenytoin
	Phenacetin	Ritonavir	Rifampin
	R-Warfarin	Erythromycin	Carbamazepine
CYP2C9/10	Theophylline	Amiodarone	Rifampin
	Dapsone	SMX/TMP	Phenobarbital
	Phenytoin	Isoniazid	Phenytoin
	S-Warfarin	Metronidazole	
CYP2C19	Tolbutamide	Fluconazole	
	Benzodiazepine	Cimetidine	Rifampin
	Proton-pump inhibitors	Diazepam	Phenobarbital
		Fluoxetine	Phenytoin
CYP2D6		Omeprazole	
		Isoniazid	
	Amiodarone	Amiodarone	
	Amitriptyline	Cimetidine	
	Clomipramine	Fluoxetine	
	Clozapine	Fluphenazine	
	Codeine	Haloperidol	
	Desipramine	Quinidine	
	Fluoxetine	Ritonavir	
	Haloperidol		
	Imipramine		
	Metoprolol		
	Propranolol		
	Risperidone		
Timolol			
CYP2E1	Halothane		Ethanol
	Acetaminophen (paracetamol)		Isoniazid
CYP3A4	Carbamazepine	Amiodarone	Phenobarbital
	Cyclosporine	Cimetidine	Carbamazepine
			Dexamethasone
			Phenobarbital
	Dapsone	Clarithromycin	Phenytoin
	Diltiazem	Erythromycin	Rifampin
	Erythromycin	Fluconazole	
	Lidocaine	Fluoxetine	
	Midazolam	Grapefruit juice	
		Indinavir	
	Nifedipine	Itraconazole	
Quinidine	Ketonazole		
Simvastatin	Ritonavir		
Tacrolimus	Sertraline		

(Continued)

Table 6.3 (Continued)

	Substrate	Inhibitor	Inducer
PGP	Tamoxifen		
	Testosterone		
	Valproic acid		
	Verapamil		
	Quinolones	Cyclosporine	Rifampin
	Itraconazole	Tacrolimus	
	Digoxin		

Abbreviations: SMX-TMP: trimethoprim-sulfamethoxazole.

allow for autoinduction of the CYP450 system by nevirapine, followed by 200 mg every 12 h. In one study, this reduced bioavailability could be overcome by increasing the dose to 300 mg every 12 h, with no short-term increase in adverse events [123]. Nevirapine has a higher risk, compared to efavirenz, of hepatotoxicity. Despite these findings, several studies from a variety of settings including Spain, Thailand and South Africa, have shown that nevirapine can be used safely and effectively with rifampin, with no difference in virological and immunological outcomes despite the plasma levels of nevirapine being reduced by up to 42% in patients coadministered rifampin [122, 123]. A small study in Thailand showed comparable short-term outcomes in both groups of nevirapine and nevirapine + rifampin. In this study, the nevirapine levels were reduced by 17% with rifampin coadministration, although the majority of the trough plasma nevirapine levels were higher than the recommended trough nevirapine level. In addition, there was no increase in adverse side effects of skin rash and hepatotoxicity when nevirapine was coadministered with rifampin [123]. The largest of these studies, conducted in South Africa, showed good virological outcomes with 80% of patients in the nevirapine + rifampin group being virologically suppressed at 18 months [122]. However, the study also showed there to be a higher probability of virological failure in the first two years of therapy in this group compared to patients receiving an efavirenz-based regimen. A possible reason for these contradictory findings was suggested as being due to a drug interaction between rifampin and the lead-in dosing phase of nevirapine which could, in theory, lead to a further induction by nevirapine of a system already induced by rifampin. In general, these studies have demonstrated good clinical outcomes despite a reduced bioavailability of nevirapine by rifampin coadministration; this suggests that it may be unnecessary to increase the standard nevirapine dose.

6.7.2.1.3 Rifampin + PI The induction of the CYP450 system by rifampin results in markedly decreased levels of fos-amprenavir, atazanavir, indinavir, nelfinavir, saquinavir and lopinavir, ranging from 82% to 95% [43, 116] (see Table 6.3). As a result it is not recommended that rifampin is used with any of these drugs. Ritonavir, on the other hand, can be given with rifampin, either alone or in combination with another PI such as lopinavir. As ritonavir is an inhibitor of the

CYP450 system it counteracts the effect of rifampin; this is of utility when the drug is combined with lopinavir or saquinavir [124].

6.7.2.1.4 Rifabutin + NNRTI In a study of healthy volunteers, rifabutin 300 mg daily appeared to have little or no effect on efavirenz concentrations, but the rifabutin concentrations were reduced by efavirenz. As a result, the guidelines suggest increasing the rifabutin dose to 450 mg or 600 mg to compensate for this interaction, with the efavirenz dose unchanged [125]. There does not appear to be any significant interaction between rifabutin and nevirapine, and the available data suggest that the two drugs can be safely coadministered, without any need to change the dose of either drug [126].

6.7.2.1.5 Rifabutin + NRTI There does not appear to be any significant interaction between NRTI and rifabutin.

6.7.2.1.6 Rifabutin + PI The coadministration of rifabutin and PIs can lead to a reduction in PI exposure, as well as a significantly increased rifabutin concentration with an associated risk of uveitis due to toxicity [127]. Current guidelines therefore advise a reduction in the rifabutin dose by up to 75% when coadministered with a PI [128]

6.8

Drug–Disease Interactions

There is conflicting evidence regarding the effect of HIV infection on the absorption of antitubercular drugs, due to the virus itself, to HIV enteropathy, or to opportunistic infections affecting the gastrointestinal tract. A study conducted in South Africa showed 39% and 27% reductions in rifampin and ethambutol, respectively, in patients with HIV infection, none of whom had diarrhea [112]. However, another study showed no evidence that infection with HIV reduces the plasma concentrations of anti-TB drugs [129]. Results from a study in Nairobi showed that HIV infection and diarrhea did not affect the pharmacokinetics of TB drugs [130]. Data from a study in India showed an association between malabsorption of TB drugs and patients with advanced AIDS with and without diarrhea, with a low CD4 count and gastrointestinal disturbance increasing the likelihood of malabsorption [131]. Data are available which suggest that rifabutin is less frequently malabsorbed when compared to rifampin [132]. Fluoroquinolones such as ciprofloxacin appear to be well absorbed in the presence of HIV, regardless of the CD4 cell count [133].

6.8.1

TB Drugs in Development, and Potential Interactions

Moxifloxacin is unique among fluoroquinolones in that its bioavailability is not affected by the concurrent administration of ranitidine (a histamine H₂-receptor

antagonist), it has minimal renal elimination, and it is almost entirely removed in the feces (as sulfate and glucuronide conjugates) [134]. However, the absorption of moxifloxacin is similar to that of other fluoroquinolones, which is impaired by concomitant administration of aluminum- and magnesium-containing antacids. The administration of these agents should be staggered by an interval of 2 h before or 4 h after taking the antacid [135].

6.8.2

AIDS Drugs in Development, and Potential Interactions

Maraviroc, a CCR5 receptor antagonist, is metabolized by the CYP3A4 isoenzymes. The coadministration of maraviroc with rifampin has been shown to result in a reduction in the plasma concentration of maraviroc by as much as 70% [136]. As a result, the current CDC guidelines recommend an increase in the dose of this antiretroviral to 600 mg twice daily when administered with rifampin. Maraviroc could also potentially interact with PIs and NNRTIs [43]. Indeed, in one study it was shown that the PI caused a significant increase in the plasma level of maraviroc, whereas efavirenz was shown to reduce maraviroc exposure by up to 50% [136].

Raltegravir is neither a potent inhibitor nor inducer of CYP 3A4 [137], and is predominantly metabolized by glucuronidation, specifically by the enzyme UDPGT 1A1[138].

6.8.3

Other Interactions of Note

A discussion of all potential drug–drug and drug–disease interactions in HIV-infected patients receiving treatment for TB, AIDS and comorbid illnesses is beyond the scope of this chapter. However, some common comorbid diseases requiring therapy will be described at this point; these therapies include oral hypoglycemic agents, anticonvulsants, and anticoagulants. For example, beta-lactams can cause changes to the gastrointestinal flora, leading to an alteration of those drugs that are dependent on enterohepatic recirculation. An alteration in the gut flora that synthesize vitamin K, thus reducing endogenous vitamin K production, can augment the effect of warfarin-mediated elevations of the concentrations of statins (e.g., lovastatin and simvastatin), and the development of rhabdomyolysis secondary to CYP3A4 inhibition has also occurred. Fluoroquinolones have also been associated with fatalities secondary to hypoglycemia in patients receiving medication to manage diabetes mellitus [139], in addition to drug- and dose-dependent prolongations of the QTc interval.

6.8.3.1 Antituberculosis Drugs and Oral Hypoglycemic Agents

There is increasing evidence to suggest that diabetes mellitus (DM) significantly increases the risk of TB. A recent meta-analysis of studies assessing the association of TB and DM showed an increase of at least threefold in the risk of active TB in people with DM [140]. Typically, after two months, the results of sputum

microscopic examinations were more often positive in diabetic patients (18.1% versus 10.0%); after six months, 22.2% of cultured sputum specimens from diabetic patients were positive for *M. tuberculosis* (adjusted odds ratio, 7.65; $p = 0.004$). The maximum plasma concentration of rifampin was above the target concentration of 8 mg l^{-1} [33] in 6% of patients with TB who had DM, compared to 47% of patients without DM. These pharmacokinetic differences might lead to an easier acquisition of drug resistance, and might help to explain the inferior bacteriological response in diabetic patients with TB [141]. Thus, it is important not only to understand any possible interactions between drugs for these two conditions, but also to tease out any interactions that are clinically significant, especially as the prevalence of DM in TB-endemic areas is rising [142]. As the oral hypoglycemic agent gliclazide is metabolized by the CYP450 2C9 isoenzymes – which are induced by rifampin – there is a clear potential for an interaction between the two drugs. A recent case study suggested such clinical significance, with a report of an increased gliclazide requirement with rifampin coadministration in a patient with type 2 DM [143]. A subsequent pharmacokinetic study in healthy volunteers showed a statistically significant reduction in the blood glucose-lowering effect of gliclazide with rifampin coadministration, that was thought to be clinically significant [144]. The results of these studies suggested that a close monitoring of blood glucose levels, with a possible adjustment of the gliclazide dose, might be required. Reports also exist of interactions between the older sulfonylureas, chlorpropamide [145] and tolbutamide, and rifampin [146, 147]. There is also evidence that rifampin significantly reduces the blood glucose-lowering effect of glibenclamide [148], repaglinide [149], and glipizide [150]. However, rifampin has not been shown to have any significant effect on the blood glucose-lowering properties of a newer sulfonylurea drug, glimepiride [31].

6.8.3.2 Antituberculosis Agents and Prednisolone

There is some evidence favoring the use of adjunctive corticosteroids in some forms of extrapulmonary TB. A recent Cochrane review concluded that steroids should be used routinely in the management of TB meningitis in HIV-negative patients. However, whilst there is some evidence of benefit in HIV-infected patients, the results are inconclusive [151]. Some data are available supporting the use of adjunctive prednisolone in TB pericarditis [152]; notably, these results highlight the need to understand the interaction of prednisolone with anti-TB drugs. The data have shown that the bioavailability of total and free prednisolone is reduced when coadministered with rifampin [153, 154], which suggests a need for dose adjustment when the two drugs are coadministered. One study conducted in India showed that the coadministration of isoniazid with prednisolone resulted in an increased renal clearance of isoniazid, regardless of the patients' acetylase status. Yet, the same study identified an increase in the acetylation rate of isoniazid in slow acetylators, which led to a decrease in isoniazid plasma concentrations. In spite of these findings, there was no difference in clinical outcome between those patients receiving prednisolone and those not, thus raising doubt over the clinical significance of these findings [155].

6.9

Conclusions

Dual AIDS-TB therapy is associated with numerous challenges, particularly drug–drug and drug–disease interactions, shared drug toxicities, IRIS, and high pill burdens. Comorbid conditions due to HIV immune suppression often necessitate additional therapy, which further exacerbates these challenges. At present, a number of drugs used to treat AIDS-TB either induce, inhibit, and/or are metabolized by CYP. In particular, rifampin (a CYP inducer) and ritonavir (a CYP inhibitor) are implicated in significant drug–drug interactions. There is a paucity of data relating to CYP metabolism and drug interactions for other drugs used to treat TB, AIDS and comorbid conditions. The emergence of metabolic syndrome and drug-resistant TB will challenge the therapeutic strategies of clinicians, particularly in Africa, China, and India. Clearly, the limitations of our current knowledge of these drug interactions require further investigation.

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Part Three
Clinical Issues

7

Clinical Issues in the Diagnosis and Management of HIV Infection

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7.1

Introduction

In 1981, when observant clinicians reported clusters of patients with cytomegalovirus disease and pneumocystis pneumonia in Los Angeles, and with Kaposi's sarcoma in New York City, this heralded the recognition of a disease characterized by extreme immune dysfunction [1, 2]. Concurrently, in the Ugandan trading communities near Lake Victoria, health workers were caring for increasing numbers of patients with diarrhea, profound wasting, oral candidiasis, and an intensely pruritic, maculopapular rash. That condition – termed “slim disease” – was initially thought to represent a different condition from the enlarging epidemic described in the United States and Europe of what is now called the acquired immunodeficiency syndrome (AIDS) [3]. However, the varying clinical presentations were not reflective of distinct diseases, but rather a shared epidemic of human immunodeficiency virus (HIV) infection occurring in different settings.

From these initial few patients, HIV has spread throughout the world, with an estimated global burden of 33.2 million infected people in 2007 [4]. While sub-Saharan Africa accounts for the majority of infections, other regions are also highly impacted, particularly areas with high rates of tuberculosis (TB). Indeed, the global intersection of these two epidemics presents tremendous new clinical challenges in diagnosis, prevention and treatment, and underscores the need for effective vaccines for both diseases. There remain significant differences in the clinical features of HIV infection between resource-rich and resource-limited settings (Table 7.1). Some of these apparent differences can be attributed to discrepancies in diagnostic and surveillance capabilities, and to the varying availability of antiretroviral therapy (ART). In addition, socioeconomic factors, concomitant TB, viral hepatitis, drug abuse, parasitic disease, and other factors can modify the clinical features of the HIV epidemic.

In this chapter we will focus on clinical issues in the diagnosis and management of HIV infection in resource-limited settings, where TB is common, and in resource-rich settings. Although emphasis will be placed on issues relevant to the care of

Table 7.1 General characteristics of the HIV/AIDS epidemic in resource-rich settings and resource-limited (notably Saharan Africa) settings.

Characteristic	Resource-rich settings	Resource-limited settings
Type of epidemic (most affected population)	Concentrated (MSM, IDU, African-Americans, immigrants from endemic countries)	Generalized (heterosexual partners, uncircumcised men)
Common presenting opportunistic conditions	<i>Pneumocystis jirovecii</i> pneumonia, cytomegalovirus retinitis, wasting syndrome [127]	Tuberculosis, cryptococcal meningitis, papular pruritic eruption
Median CD4+ cell count at time of initiation of ART (cells μl^{-1}) [128]	234	108
Cause of death	AIDS-related Non-AIDS-related (liver disease, cardiovascular disease, malignancy) [129]	AIDS-related (tuberculosis, bacterial pneumonia) [130, 131]

ART: antiretroviral therapy; MSM: men who have sex with men; IDU: injection drug user.

patients in resource-limited settings, diagnostic and treatment options in resource-rich settings with also be described, as these are rapidly becoming available throughout the world. In this chapter, we have limited our comments to HIV infection in adults and adolescents; issues pertaining to pediatric HIV infection have been well summarized in a recent review [5].

7.2

Diagnosis

7.2.1

Rationale for Testing

In addition to testing individuals with risk factors for HIV or conditions associated with HIV (such as TB), there is significant evidence supporting the routine testing of asymptomatic adults [6]. The identification of asymptomatic HIV infection can prevent the infection of others and allow treatment initiation prior to the development of AIDS.

7.2.1.1 HIV Testing for Prevention

From early in the epidemic, the development of diagnostic tests for HIV was motivated by the need to prevent new infections. HIV serology, which first became commercially available in 1985, was used to protect the blood supply through the routine testing of

blood donors [6]. Along with the use of voluntary blood donors and the addition of assays for HIV RNA to detect persons in the pre-seroconversion stage of infection, HIV testing has almost eliminated the risk of transmission via blood products. Similarly, the routine testing of pregnant women, in settings with access to obstetric and medical interventions for the prevention of mother-to-child-transmission, has dramatically reduced the perinatal acquisition of HIV. The timely identification and subsequent treatment of HIV-infected pregnant women can reduce the probability of peripartum vertical transmission from a baseline of over 20% to less than 2% [7]. The early identification of HIV-infected mothers also allows the institution of interventions to prevent breast-milk transmission of HIV [8–10], including replacement feeding [11], which are critical to reducing infant HIV infection.

Routine HIV counseling and testing to prevent the sexual transmission of HIV appears to have a more modest impact [12]. In a randomized trial of heterosexual patients utilizing sexually transmitted infection (STI) clinics in the United States, those receiving individualized HIV counseling and testing were more likely to use condoms than patients receiving only didactic messages typical of current care. However, the difference in self-reported condom use was small: 78% in the enhanced counseling and testing arm versus 73% in the control arm at six months after the intervention [13]. This benefit has not been seen in other studies. In a meta-analysis of 27 published studies on HIV counseling and testing – conducted largely in resource-rich countries – no change in risk behavior was observed for individuals who tested negative for HIV. However, HIV-infected patients, and particularly those in serodiscordant relationships, did adopt safer sex practices after counseling and testing [14]. This finding suggests that the value of routine HIV testing lies in identifying those individuals with undiagnosed HIV infection who then reduce behaviors that lead to transmission of the virus.

Research teams working in resource-limited settings have found similar results. A large randomized trial involving participants from East Africa and Caribbean showed that HIV testing, when paired with counseling, reduced risk behavior; the effect was driven by changes in practices by those testing positive for HIV and those receiving counseling as a couple [15]. In Zimbabwe, a prospective observational study demonstrated increased condom use by women testing HIV-positive; unfortunately, however, those participants who tested negative for HIV demonstrated increased risk behavior [16].

One limitation of HIV testing for prevention is that many new transmission events occur during the first several weeks after acquisition, when the virus load is high [17]. Because patients in this stage of infection are sometimes in the “window” or pre-seroconversion phase before the development of HIV antibodies, they may not be identified by routine testing during their period of peak infectiousness [12]. Thus, new strategies to identify patients with acute HIV infection are needed.

7.2.1.2 Earlier Entry to Care

Worldwide, patients continue to present for care with opportunistic complications of advanced HIV infection. A late diagnosis of HIV infection leads to increased mortality. Patients initiating highly-active antiretroviral therapy (HAART) in sub-

Saharan Africa with CD4+ cell counts of less than 50 cells μl^{-1} or World Health Organization (WHO) stage 4 disease (see below) were more than twice as likely to die as those with CD4+ cell counts greater than 50 cells μl^{-1} or asymptomatic disease [18]. Furthermore, patients with pretreatment CD4 cell counts $>350 \mu\text{l}^{-1}$ are the most likely to have recovery to near-normal counts after treatment with antiretroviral medications [19]. Earlier diagnosis and therapy may prevent mortality and help to avoid immune reconstitution inflammatory syndrome. Routine HIV testing and subsequent linkage to care has been found to be cost-effective, even in settings with an HIV prevalence of less than 1% [20, 21].

Newly identified HIV-infected patients who do not meet criteria to begin HAART immediately (see below) are still likely to benefit from early diagnosis. The period prior to the initiation of HAART allows for targeted vaccinations and interventions to modify comorbid conditions that may complicate therapy, such as substance abuse, mental illness, obesity, hypercholesterolemia, smoking, and viral hepatitis. Importantly, this period may also permit the identification and treatment of concomitant TB without the drug interactions and pill-burden that complicate simultaneous treatment for TB and HIV. In addition, efforts to assess and support adherence to treatment are crucial before the initiation of ART.

7.2.2

Recommendations for Testing

Guidelines for HIV testing vary in different countries and regions of the world [22]. There is consensus that pregnant women and patients with a STI, TB, or a possible opportunistic condition, should be offered testing. However, there are differences in recommendations for the HIV screening of asymptomatic persons. Stigma and discrimination against HIV-infected individuals led to the requirement in many settings for written informed consent in conjunction with comprehensive counseling prior to HIV testing. Unfortunately, this requirement has proven to be a barrier to the expansion of HIV testing. HIV testing and detection rates have increased in settings where formal written consent has been removed and when testing is performed unless the patient declines (opt-out testing) [23–25]. In the United States, the Centers for Disease Control and Prevention (CDC) recommended in 2006 that screening for HIV infection should be carried out in all patients aged 13 to 64 years; the testing should be voluntary; and patients should be informed orally or in writing that they will be tested, unless they decline [6]. Since that declaration, many organizations in the United States [26] and around the world have recommended a similar approach to achieve an expansion of HIV testing (see Table 7.2).

7.3

Methods of Testing

A wide array of diagnostic tests for HIV has been developed. The vast majority of HIV infections are diagnosed via serologic means, principally using enzyme-linked

Table 7.2 Summary of selected guidelines for HIV screening of asymptomatic persons.

Authority	Population	Consent	Frequency
U.S. Centers for Disease Control and Prevention (CDC) [6]	All patients aged 13–64 years	Opt-out	At least once. Yearly for those at high-risk ^a
UNAIDS/WHO [34, 132]	In generalized epidemics, ^b where adequate resources are available, all adults and adolescents should be offered HIV testing In concentrated or low-level epidemics, ^b HIV testing should be targeted to persons with symptoms or signs of HIV infection	Verbal communication is normally adequate for the purpose of obtaining informed consent; areas that require written consent should review this policy	Consider repeat testing based on risk
United Kingdom National Guidelines for HIV Testing [133]	Routine testing of high-risk patients ^c ; HIV testing of the general adult population should be considered in settings where the HIV prevalence exceeds 2 per 1000	Written consent usually not necessary	Repeat yearly for MSM and IDU, and for all individuals who have a possible exposure
Botswana National HIV/AIDS Guidelines [44]	All age groups, “universal and routine”	Opt-out	Not addressed

HIV testing should also be carried out in all pregnant women, in individuals with known risk factors for HIV infection, and in patients with symptoms suggestive of HIV infection or an opportunistic condition. MSM: men who have sex with men; IDU: injection drug users.

^a“High-risk” defined as IDU and their sex partners, individuals engaging in transactional sex, sex partners of HIV-infected persons, or individuals who themselves or whose sex partners have had more than one sex partner since their last HIV test.

^bA “generalized epidemic” is defined as a region with an HIV prevalence >1% in pregnant women. A “concentrated epidemic” is defined as a region with an HIV prevalence of >5% in at least one defined subpopulation, but below 1% in pregnant women in urban areas. A “low-level epidemic” is defined as a region where HIV prevalence has not exceeded 5% in any defined subpopulation.

^cPersons at risk include MSM, IDU, all patients with a sexually transmitted infection, sexual contacts of known HIV-infected patients, individuals from countries with a high HIV prevalence (>1%), and individuals who have had sexual contact with persons from high-prevalence areas.

immunosorbent assays (ELISA) for the detection of antibodies to HIV. Traditionally, these sensitive assays have been confirmed by a more specific test, the Western blot. However in resource-limited settings, where there is a high pre-test probability of HIV infection, a second positive ELISA from a different manufacturer is sometimes used

as a confirmatory test. Another testing algorithm in resource-limited settings is the performance of a rapid HIV test with confirmation of a positive result by an ELISA. However, these protocols may not sufficiently exclude false-positive results because a crossreacting antibody can produce a positive result on different test kits.

7.3.1

ELISA

The first laboratory screening test for HIV infection was an ELISA using antigens from disrupted cultured virus that bound HIV-specific antibodies in patient samples [27]. Although there have been substantial improvements in the sensitivity and specificity of the ELISA test in subsequent generations of this assay, the basic approach remains the same. Antibodies in the patient's serum are allowed to bind to HIV antigens that are fixed to a support matrix (e.g., a microtiter plate well or bead); the bound antibody is then detected by adding antibody to human immunoglobulin, conjugated to a chromogenic substrate such as horseradish peroxidase [28]. The specific antigens used may vary between manufacturers. The sensitivity of these assays for established infection is excellent. However, given the reliance on antibody formation, the window period before HIV infection could be detected using second-generation assays was 6–12 weeks. The use of the “sandwich ELISA,” in which multivalent HIV-specific antibodies are “sandwiched” between fixed antigen and reagent-bound antigen, has significantly shortened the window period. These third-generation assays can detect IgM antibody and are reactive in a majority of patients 3–4 weeks after exposure [29]. Most of the third-generation assays can detect HIV-1, HIV-2, and HIV-O.

Fourth-generation assays have shortened the window period further (see Figure 7.1). These tests combine a sandwich antibody ELISA with an ELISA to detect HIV p24 antigen. The core protein p24 surrounds the viral RNA, and its presence in the blood is a marker for viral replication. A surge in p24 antigenemia occurs early in infection, prior to the development of HIV-specific antibodies. Levels of p24 antigen decrease later in infection due to lower amounts of circulating virus and binding by

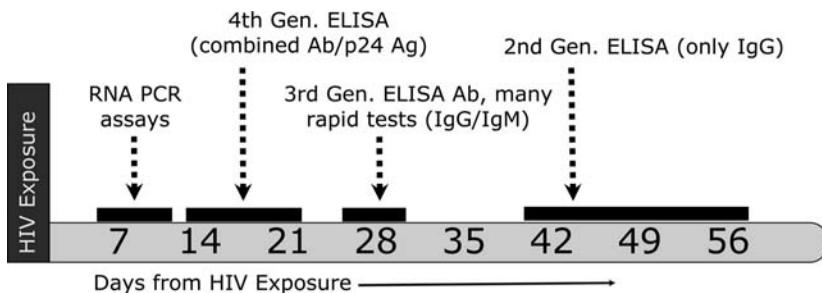


Figure 7.1 Time to first detection of HIV infection, using commercially available assays. Ab: antibody; Ag: antigen; Gen: generation; PCR: polymerase chain reaction. Adapted with permission from Ref. [134].

specific antibodies. The fourth-generation assays rely on p24 antigen detection for diagnosing early infection, generally within two weeks of exposure, and antibody detection for diagnosing established infection. The sensitivity of the fourth-generation assays approaches 100%, with specificity exceeding 99.5% [30].

The ability to test many samples concurrently in an automated fashion makes an ELISA ideal for central high-volume facilities, such as blood donor centers and hospitals. However, the need for laboratory technology and delay in processing makes ELISA testing difficult in many outpatient and resource-limited settings.

7.3.2

Rapid Tests

Rapid HIV tests are important tools for prompt detection of HIV in women presenting in labor, for community-based screening initiatives, screening in STI clinics, or other urgent-care settings. Rapid tests are also useful in areas where there is no diagnostic laboratory capable of performing a standard ELISA, such as in resource-limited settings. These tests can be performed with a fingerstick of blood or an oral fluid swab, and results are usually available in less than 20 minutes. The immunochromatographic assays rely on reagents impregnated on diffusion paper to produce a color reaction, and so do not depend on specialized laboratory personnel. In controlled settings, the performance of these tests has been comparable to standard ELISA, with sensitivity and specificity both exceeding 99%. However, in an urban emergency department the specificity was considerably lower, at 96.9%[31]. In this low-prevalence area, less than 20% of patients with reactive tests by a rapid method were found to have HIV infection after confirmatory testing. This finding highlights the importance of confirming the results of a rapid test with a more specific test, such as Western blot. There have also been rare reports of false-negative rapid tests [32].

7.3.3

Western Blot

Owing to its excellent specificity, the Western blot is considered the “gold standard” for the confirmation of a reactive screening ELISA or rapid test.

The Western blot utilizes the differential electrophoretic migration of HIV viral proteins on a polyacrylamide gel to identify antibodies to specific proteins in a patient’s serum. The patient’s antibodies bind to migrated viral proteins, thereby forming bands at specific locations. Although diagnostic criteria vary somewhat between authorities, a positive result requires the reactivity of at least two of the following bands: p24, gp41, or gp120/160. A negative result is defined as no reactivity to any of the bands. As many as 15% of HIV-uninfected persons will have an indeterminate Western blot, with reactive bands not meeting the criteria for a positive test [33]. Negative or indeterminate results can occur during early HIV infection. A false-negative test result may also occur when a patient is infected with HIV-2, unless an HIV-2-specific Western blot is used.

7.3.4

Nucleic Acid Amplification

Nucleic acid-based molecular diagnostic assays, such as reverse-transcriptase polymerase chain reaction (PCR), can be used to quantify the concentration of HIV RNA (the “virus load”). These tests provide prognostic information in HIV-infected patients who are not receiving therapy, and are useful when monitoring the response to treatment. Because laboratory assay contamination can sometimes lead to false-positive results, nucleic acid-based tests are generally not used for diagnosis. However, there are three settings in which nucleic acid-based assays may be helpful in the diagnosis of HIV infection:

- In early HIV infection, viral RNA is detectable in the blood approximately one week after exposure, which is generally several weeks prior to the development of HIV-specific antibodies and days prior to detectable levels of p24 antigen. For this reason, HIV RNA testing is useful in diagnosis of acute HIV infection in symptomatic individuals and in blood donors. Patients with positive HIV RNA results should undergo subsequent antibody testing to confirm seroconversion.
- Infants exposed to HIV during pregnancy may have their mother’s HIV-specific antibodies for up to the first 18 months of life; consequently, serologic techniques to diagnose HIV infection may not be reliable during this time period. HIV DNA PCR testing to detect cell-associated viral nucleic acid is useful in the diagnosis of infants with vertical transmission.
- HIV RNA testing can be used to clarify the results of an indeterminate Western blot.

7.4

Management of the Newly Diagnosed HIV-Infected Patient

Today, there are more than 33 million HIV-infected people throughout the world [34]. In 2007, there were an estimated 2.5 million new infections [4]. Newly diagnosed patients must undergo a thorough and efficient evaluation for the stage of disease, other active or latent infections (such as TB), barriers to care, and the presence of comorbid disease. In addition (whilst not the focus of this chapter), patients require appropriate psychosocial support and counseling directed at addressing concerns about their prognosis, the availability of treatment, the disclosure of HIV infection, and the prevention of secondary infections. There are excellent guidelines for the management of newly diagnosed HIV infection in resource-rich settings [35–38]; however, such guidelines must be adapted for use in resource-limited settings in sub-Saharan Africa and Asia.

7.4.1

Assessment of Baseline HIV Parameters

Clinical staging provides clinicians with useful information, particularly when a CD4+ cell count measurement is not available, or is delayed. Determination of the

patient's clinical stage of HIV guides the urgency of treatment initiation and predicts the risk of active opportunistic infection. Although several staging systems have been proposed, the WHO system is the most commonly used and is broadly applicable (see Table 7.3).

In addition to clinical staging, measurement of the CD4+ cell count provides prognostic information regarding the risk of developing an AIDS-related condition. The CD4+ cell count is used to guide decisions regarding when to initiate prophylaxis against opportunistic infections, and when to start ART (see below). Because the CD4+ cell count exhibits significant variability, where resources permit it is reasonable to repeat the test to confirm that the result is reproducible, prior to making a decision regarding treatment.

The plasma virus load independently predicts the risk of opportunistic infection, HIV-related complications, and death [39–42]. The virus load also predicts the rate of decline in CD4+ cell count in patients with untreated HIV infection [43]. Given scarce resources, some programs have decided to forgo baseline virus load measurement [44], although this practice comes at the cost of losing potentially useful prognostic information.

In resource-rich settings, additional specialized HIV testing is recommended to guide the medication choice. Well-defined viral mutations predict resistance to different classes of anti-HIV drugs; such mutations may develop in patients who are receiving therapy, or may be transmitted to newly infected individuals (the latter is known as “primary resistance”). Baseline genotypic resistance testing has been found to be cost-effective in settings where rates of primary resistance exceed 1% [45]. An early identification of resistance can prevent the development of further resistance and progressive HIV disease by allowing providers to avoid the use of noneffective antiretroviral agents. Testing for the HLA-B*5701 allele has also been proposed, since expression of this HLA class I allele is associated with the greatest risk of a hypersensitivity reaction (HSR) [46] to abacavir, a frequently used drug in resource-rich settings. The cost-effectiveness of universal testing depends on the prevalence of the allele, the cost of the test, and the relative cost and efficacy of abacavir compared to alternative agents, such as tenofovir [47]. The frequency of this allele in populations living in most resource-limited settings is not known.

7.4.2

Concurrent Infection

7.4.2.1 Tuberculosis

Tuberculosis poses significant challenges to HIV-infected patients and the health systems that support them. Clinical issues related to tuberculosis are addressed elsewhere in this volume, but several aspects deserve emphasis. Distinguishing between latent and active TB may be difficult in patients with advanced AIDS. The overlap between the symptoms of TB and those of advanced AIDS makes diagnosis challenging. In addition, current diagnostic methods, such as sputum examination for acid-fast bacilli and chest radiography, have low sensitivity, particularly in patients with advanced AIDS who frequently have extrapulmonary or disseminated infection.

Table 7.3 World Health Organization (WHO) clinical staging of HIV disease in adults and adolescents [58].

WHO Clinical Stage	Symptoms
Stage 1	Asymptomatic Persistent generalized lymphadenopathy
Stage 2	Unexplained moderate weight loss (<10% of body weight) Recurrent respiratory tract infections (otitis media, bronchitis, sinusitis, tonsillitis) Herpes zoster Angular cheilitis Recurrent oral ulceration Papular pruritic eruptions Seborrheic dermatitis Fungal nail infections
Stage 3	Unexplained severe weight loss (>10% of body weight) Unexplained chronic diarrhea for longer than one month Unexplained persistent fever Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis (current) Severe presumed bacterial infections (e.g., pneumonia, meningitis, bacteremia, bone or joint infection, empyema) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anemia (hemoglobin <8 g dl ⁻¹), neutropenia (<0.5 10 ⁹ l ⁻¹ , or thrombocytopenia (<50 × 10 ⁹ l ⁻¹)
Stage 4	HIV wasting syndrome <i>Pneumocystis</i> pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection (>1 month or visceral) Esophageal candidiasis (or candidiasis of the trachea, bronchi, or lungs) Extrapulmonary tuberculosis Kaposi's sarcoma Cytomegalovirus infection (organ involvement) Central nervous system toxoplasmosis HIV encephalopathy Extrapulmonary cryptococcosis, including meningitis Disseminated nontuberculous mycobacterial infection Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis Chronic isopsoriasis Disseminated mycosis (coccidioidomycosis or histoplasmosis) Recurrent nontyphoidal <i>Salmonella</i> bacteremia Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumors Invasive cervical carcinoma Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy or cardiomyopathy

The sensitivity of smear examination may be lowered further when fluorescent microscopy or optimal specimen preparation and examination are not available [48]. In a study of patients starting HAART in South Africa, only 0.7% had a positive sputum AFB smear, whereas 13.6% had a positive sputum culture for TB [49]. Further studies and development of diagnostic tests applicable to resource-limited settings are clearly needed.

Treatment of latent TB infection (LTBI) has been shown to reduce the development of active TB disease. A diagnosis of LTBI is usually made by tuberculin skin testing, but the reliability of this test is compromised by anergy and by reactivity caused by bacille Calmette-Guérin (BCG) vaccination or exposure to nontuberculous mycobacteria. In settings of high TB transmission where it is not possible to test for LTBI, the WHO recommends that HIV-infected patients receive preventive therapy, unless there is evidence of active TB or another contraindication [50]. However, this strategy has not been implemented widely.

7.4.2.2 Sexually Transmitted Infections

A high prevalence of concurrent STIs has been observed in persons testing positive for HIV. Treatment of these infections can decrease viral shedding and may reduce the risk of secondary HIV infection to sexual partners [51]. Patients should be evaluated for syphilis, gonorrhea, and chlamydia as part of their initial evaluation [38, 50]. HIV-infected patients may be infected with oncogenic strains of human papilloma virus (HPV), which are associated with the development of cervical cancer and other malignancies. Screening for cervical (and perhaps anal) dysplasia should be performed at the time of HIV diagnosis, and regularly thereafter [50, 52].

7.4.2.3 Viral Hepatitis

Worldwide, coinfection of HIV and viral hepatitis accounts for significant morbidity and mortality [53]. An estimated two to four million people are chronically infected with hepatitis B virus (HBV) and HIV, and another four to five million are coinfecting with hepatitis C virus (HCV) and HIV [54]. Concurrent viral hepatitis has several important implications for patients with HIV. Patients with viral hepatitis are more likely to experience clinically significant hepatotoxicity during treatment with HAART [55]; however, the vast majority of coinfecting patients tolerate HAART, and treatment should not be withheld [56]. The risk of hepatotoxicity is greatest with the use of nevirapine [57] and some protease inhibitors such as tipranavir or the seldom-used full-dose ritonavir. When equally effective and tolerable therapy is available, it may be reasonable to avoid these agents [58].

All patients with HIV should be screened for HBV; rates of coinfection are particularly high in endemic areas such as sub-Saharan Africa [59] and Southeast Asia. Those patients who are uninfected and do not have evidence of immunity should be vaccinated to prevent the acquisition of HBV [50]. Treatment of HBV should be considered in patients who are coinfecting with HIV and HBV. Several antiretroviral agents – lamivudine, emtricitabine, and tenofovir – have excellent activity against HBV. However, a single mutation, M204I, in HBV leads to resistance

of the virus to lamivudine and emtricitabine [53]. More than 90% of patients treated with lamivudine as the sole HBV-active agent will develop resistance over four years [60]. Combination therapy may forestall or prevent the development of resistance, leading to the recommendation that HIV/HBV coinfecting patients who need therapy should receive tenofovir with either lamivudine or emtricitabine [36, 53, 58]. Tenofovir is not widely available in resource-limited settings; however, obtaining access to this drug for HIV/HBV coinfecting patients should be a priority. The cessation or interruption of HBV-active HAART in coinfecting patients is potentially dangerous, and has led to severe HBV flares [61].

The choice of ART in HIV-infected patients is also influenced by coinfection with HCV, particularly in those who require treatment for HCV. Pegylated-interferon plus ribavirin, the most effective current treatment for HCV, has several important drug interactions. Ribavirin should not be combined with didanosine because of a high incidence of mitochondrial toxicity, which may manifest as pancreatitis or lactic acidosis. Zidovudine-related anemia can also be exacerbated by the concurrent use of ribavirin. Finally, some studies have suggested that HCV treatment efficacy is diminished when coadministered with abacavir, whereas other studies have not demonstrated this interaction [62–64]. In summary, clinicians contemplating the treatment of HCV in a coinfecting patient must avoid using didanosine, and should consider alternative options to zidovudine and possibly abacavir [65].

7.4.2.4 Other Infections

After tuberculosis, *Cryptococcus neoformans* accounts for the greatest number of AIDS-related deaths in several HIV-infected populations [66–68]. Unrecognized cryptococcal infection and cryptococcal immune-reconstitution inflammatory syndrome (IRIS; see below) contribute substantially to early mortality after the initiation of HAART [69, 70]. Serum cryptococcal antigen (CrAg) testing is inexpensive, sensitive, and specific [71], and CrAg may be detectable in serum prior to the development of symptomatic disease. In a study of HIV-infected patients in Uganda with a CD4+ cell count of less than $100 \mu\text{l}^{-1}$ and no symptoms of cryptococcal infection, 5.8% of patients had a positive serum CrAg test [70]. In a second study conducted in Uganda, cryptococcal antigenemia preceded symptoms by a median of 22 days (and by >100 days in 11% of patients) [66]. Due to the low prevalence of cryptococcal disease in the United States, routine screening with CrAg is not recommended [72]. However, the screening of patients with CrAg with advanced HIV infection may be beneficial in some high-prevalence areas [73].

Cytomegalovirus (CMV) and toxoplasmosis account for significant morbidity in patients with advanced AIDS, particularly those with a CD4+ count of less than $50 \text{ cells } \mu\text{l}^{-1}$. Seropositivity for CMV is nearly ubiquitous in adult patients with HIV. Screening for CMV in newly diagnosed HIV-infected patients is recommended in the United States; those that are seronegative are counseled to avoid exposure, for example, by using condoms, practicing optimal hygiene, and avoiding CMV-positive blood products [38, 72, 74]. A dilated retinal examination to detect asymptomatic retinitis prior to the initiation of HAART is recommended for patients with CD4+ cell

count less than $50 \mu\text{l}^{-1}$ [38]. Routine screening for serologic evidence of exposure to *Toxoplasma gondii* is also recommended at the time of HIV diagnosis. Those patients who are seropositive for *Toxoplasma* should receive prophylaxis if their CD4+ count is $<100 \text{ cells } \mu\text{l}^{-1}$ (see Table 7.4). Patients who are *Toxoplasma* seronegative should be counseled to avoid ingesting undercooked meats, and to follow strict hand hygiene after gardening, changing cat litter, or handling raw meat [72].

Table 7.4 Recommendations for prophylaxis in patients with HIV infection [35, 38, 50, 72].

Infective agent	CD4+ count (cells μl^{-1})	Prophylaxis	Notes	Source (see references below)
<i>Mycobacterium tuberculosis</i>	In patients with positive tuberculin skin test ^a , regardless of CD4 cell count	Isoniazid preventive therapy (IPT)	IPT should only be given if active TB can be excluded.	WHO and U.S. guidelines
<i>Plasmodium</i> sp.	All	Insecticide-treated nets, intermittent preventive therapy in pregnancy, cotrimoxazole 160/800 mg (1 DS tab) daily in patients with CD4+ cell count $<350 \mu\text{l}^{-1}$	In malaria-endemic areas	WHO guidelines
Severe bacterial infection	<350 (in resource-limited settings)	Cotrimoxazole 160/800 mg (1 DS tab) daily	In regions with high burden of bacterial infection	WHO guidelines
<i>Pneumocystis jirovecii</i> (PCP)	<200	Cotrimoxazole 160/800 mg (1 DS tab) daily	Other indications for PCP prophylaxis include presence of oropharyngeal candidiasis, CD4+ cell % <14	WHO and U.S. guidelines
<i>Toxoplasma gondii</i>	<100	Cotrimoxazole 160/800 mg (1 DS) daily		WHO and U.S. guidelines
<i>Mycobacterium avium</i> complex	<50	Azithromycin 1200 mg weekly or clarithromycin 500 mg twice daily	Azithromycin has fewer interactions with other drugs than clarithromycin	U.S. guidelines

^aSee Section 7.4.2.1 for discussion on TB preventive therapy in HIV-infected patients who live in settings where tuberculin-skin testing is not available.

7.4.3

Comorbid Conditions

An initial evaluation of a patient with HIV should include an assessment for concurrent medical conditions that could complicate HIV treatment [38]. Women of child-bearing potential should be asked whether they are having sexual intercourse that could lead to pregnancy, as well as the time of their last menstrual period, because pregnancy will affect the choice and timing of ART. Efavirenz should not be used during pregnancy because of potential teratogenicity. Pregnant women should receive ART regardless of their CD4 + cell count in order to reduce the likelihood of transmission of HIV to their infant.

Patients should also be assessed for underlying glucose intolerance, dyslipidemia and central obesity, because several antiretroviral medications can exacerbate these problems. In addition, all patients with HIV infection should undergo an evaluation for cardiovascular risk factors. Untreated HIV infection may increase the risk of cardiovascular events, and certain classes of antiretroviral agents may raise the likelihood of myocardial infarction [75].

Newly diagnosed patients should be evaluated for symptoms and signs of peripheral neuropathy and neurocognitive abnormalities; the former would mitigate against the use of didanosine or stavudine, while the latter might lead one to choose drugs with a better central nervous system penetration [76], although this last point is controversial [77].

Screening for underlying renal impairment bears particular emphasis. Renal insufficiency and proteinuria are associated with an increased risk of death and development of AIDS-defining illness [78, 79]. Renal disease is common in HIV-infected populations, and appears to be more prevalent in sub-Saharan Africa. In Zambia, one-third of patients presenting for the initiation of HAART had renal impairment, and these patients had increased mortality compared with individuals with normal renal function [80]. Screening by serum creatinine alone may overestimate renal function, particularly in populations with a low muscle mass. Where feasible, creatinine clearance should be estimated and urinary protein measured. Patients with HIV-associated nephropathy should be treated with ART, regardless of their CD4 + cell count. Underlying renal disease may also affect the choice of regimen. For example, tenofovir should be avoided or used with caution in patients with underlying renal impairment, while several other antiretroviral medications must be dose-reduced in patients with renal dysfunction.

7.4.4

Adherence Assessment

The long-term effectiveness of HAART depends on a patient's ability to take therapy for many years; indeed, current regimens require lifelong adherence. Clinicians are generally poor at predicting a patient's likelihood of adhering to antiretroviral medication. However, interventions to diagnose and ameliorate known barriers to adherence – such as depression, substance abuse, poor social support, reluctance to

disclose HIV status, and distrust of medical system – may be beneficial [81]. The integration of a family member or friend as a treatment supporter has been associated with improved adherence [82]. In resource-limited settings, structural barriers to care – such as distance to the clinic, financial constraints, and insecurities of drug supply – seem to be more important than behavioral issues [83]. In addition to working to improve health systems, counseling patients and empowering them to develop strategies to overcome these barriers may help to promote steady adherence in unstable settings.

7.4.5

Prophylaxis

The CD4+ cell count is a useful method to assess a patient's risk of opportunistic infections and to guide preventive therapy (see Table 7.4). Some conditions, such as malaria and tuberculosis, affect patients in all CD4+ cell count strata [84], so clinicians must be particularly vigilant for these afflictions in endemic areas.

7.5

Antiretroviral Therapy

Waves of optimism and discouragement characterized efforts at ART during the late 1980s and early 1990s. For example, zidovudine therapy delayed progression to AIDS or death, but its early use in asymptomatic disease did not prolong survival compared with delayed initiation [85]. A breakthrough in the therapy of HIV infection came in 1995 with the development and concurrent use of combinations of antiretroviral medications – termed HAART – which led to the sustained suppression of HIV replication and thereby prevented the development of viral resistance. Patients initiating therapy in resource-rich settings after 1996 have had markedly fewer AIDS-related infections and deaths [86, 87], and the life expectancy of HIV-infected patients is now measured in decades [88]. Today, the use of HAART is expanding in resource-limited settings; where it has been available, its impact has been no less remarkable [89, 90].

By 2008, over 25 antiretroviral medications had been developed for clinical use, encompassing six different classes. In the following sections we will summarize the current theory and practice of the use of ART, recognizing that future investigations are expected to modify these recommendations.

7.5.1

When to Start

7.5.1.1 Asymptomatic Patients

The goal of treating asymptomatic HIV-infected patients is to prevent AIDS-related illness and mortality. It is clear that patients who have experienced an AIDS-related illness should initiate therapy, regardless of their CD4+ cell count. Results from

outpatient cohort studies in resource-rich settings suggest that patients who initiate therapy prior to their CD4 + count falling below 200 cells μl^{-1} have fewer AIDS-related events and a reduced mortality [91, 92].

The appropriate time to start HAART above a CD4 + count of 200 cells μl^{-1} is not certain. In the SMART study of continuous versus CD4 + cell count-guided intermittent therapy, those subjects not receiving ART who were randomized to start treatment immediately had a lower rate of opportunistic infections or death than those who started ART when their CD4 + count was less than 250 cells μl^{-1} . This finding suggests that HAART should not be deferred until the CD4 + cell count drops below 250 μl^{-1} [93]. In a United States clinic population, those patients who initiated therapy prior to their CD4 + cell count falling below 500 μl^{-1} seemed to have a decreased mortality, although the difference did not meet statistical significance [91]. In a Baltimore cohort, those patients starting therapy with CD4 + cell counts greater than 350 μl^{-1} were more likely to achieve near-normal CD4 + cell counts than individuals who started treatment at lower counts [19]. In addition, there is evidence that uncontrolled HIV replication may increase the risk of malignancy, cardiovascular, renal and hepatic disease – conditions previously not felt to be HIV-related [94, 95], and which may make concurrent treatment for TB and other comorbidities more challenging.

In resource-rich countries, several professional organizations recommend initiating HAART in patients whose CD4 + cell count is less than 350 μl^{-1} [35, 36, 96]. In addition, guidelines suggest considering therapy in some patients whose CD4 + cell count is greater than 350 μl^{-1} , such as in individuals at increased risk for progression (e.g., high HIV RNA level or rapidly falling CD4 + cell count) or in patients with comorbidities that may be worsened by HIV replication (e.g., viral hepatitis, cardiovascular disease, HIV-associated nephropathy).

In most resource-limited settings, ART is initiated when CD4 + cell counts fall below 200 μl^{-1} [58]. However, the high prevalence of TB and other infections in resource-limited settings may lead to substantial morbidity and mortality in those patients whose CD4 + cell count is above this threshold. There are strong reasons to consider earlier initiation of ART in these areas. Data from the Cote d'Ivoire have indicated that HIV-infected patients have high rates of bacterial infection, despite cotrimoxazole prophylaxis; in fact, serious bacterial infections were the most common cause of admission to the hospital [97]. Additional studies from the Cote d'Ivoire found high rates of infection – including severe malaria and bacterial infection – even in HIV-infected patients with CD4 + cell counts greater than 350 μl^{-1} [98]. An earlier initiation of HAART may also decrease the burden of TB in HIV-infected patients. In a South African cohort, the use of HAART was associated with a lower incidence of TB in patients whose CD4 + cell count was less than 350 μl^{-1} [84]. By reducing the incidence of active TB, the more widespread use of HAART may help to curb national TB epidemics [99].

7.5.1.2 Patients with Tuberculosis

In many resource-limited settings, patients require concurrent therapy for HIV and TB, which results in numerous challenges; these include drug interactions between

antituberculous and antiretroviral agents, overlapping toxicities of the drugs, and the potential for IRIS. These topics are reviewed in greater detail elsewhere in this volume.

The optimal timing of when to initiate HAART in an HIV-infected patient with TB is not known, although hopefully several ongoing trials will provide information regarding this decision. Until the results of these studies are known, there is evidence supporting the initiation of HAART as soon as possible in patients with advanced immunosuppression [36, 58, 100, 101], in some instances as early as two weeks after starting antituberculosis therapy. Patients with high CD4 + cell counts may be able to safely defer HAART until completion of the intensive phase of antituberculosis treatment [36, 58].

7.5.1.3 Patients with an Opportunistic Infection

Clinicians commonly face a decision about when an HIV-infected patient with an opportunistic infection (OI) should initiate HAART. A delayed initiation may result in progression of the underlying HIV disease, whereas an early initiation may lead to a higher rate of IRIS. In a recent multicenter trial, patients with an acute OI were randomized to receive either early or delayed ART. Patients in the early therapy group initiated HAART at a median of 12 days after starting OI therapy, whereas those in the deferred group started ART at a median of 45 days. By 48 weeks, fewer subjects in the immediate-treatment group had died or had a new serious OI, compared to subjects in the deferred-treatment group (14% versus 24%, respectively). IRIS was not more common in patients receiving early therapy. Of note, *Pneumocystis jirovecii* pneumonia (PCP) accounted for the majority of the opportunistic infections, and adjunctive corticosteroids used in the treatment of PCP may have prevented IRIS. Conditions in which IRIS is potentially more morbid (e.g., cerebral toxoplasmosis, CMV retinitis, cryptococcal meningitis) accounted for a minority of cases, and caution should be applied when extrapolating the findings of this study to patients with these diseases. An observational study from Botswana found that patients on HAART at the time of admission of cryptococcal meningitis were more likely to survive to discharge [102], suggesting the benefit of ART in this condition.

7.5.1.4 Primary HIV Infection

It remains unknown whether the treatment of early or acute HIV infection confers any clinical benefit to patients. Therapy during this stage has the potential to prevent the profound loss of memory CD4 + cells that occurs within weeks of infection with HIV [103]. Patients treated during acute infection seem to retain anti-HIV immune responses that are associated with long-term non-progression [104]. Small observational studies have noted an improved control of virus load in patients treated early who subsequently interrupted therapy, compared to historical controls [104]. However, this effect was not durable and consequently is of uncertain clinical significance [105, 106]. Possible benefits of early ART need to be balanced against potential toxicity, increased costs, and the risk of drug resistance associated with a longer duration of therapy. Several randomized trials evaluating the early treatment of HIV are in progress and should clarify this important issue.

7.5.2

Choice of Initial Therapy

The first-line therapy for HIV infection consists of a combination of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus either a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI) (see Table 7.5). Whereas in resource-rich settings there are many different regimen choices, in resource-limited settings most patients currently initiate therapy with one of a limited number of regimens, determined by a national program. In the aggregate, patients seem to achieve comparable outcomes whether treated with an individualized or programmatic approach [107].

In settings in which multiple options are available, the clinician should choose an effective regimen which is convenient – both in terms of the dosing schedule and pill burden – and has a low likelihood of causing short- and long-term side effects.

7.5.2.1 Backbone: Nucleoside Reverse Transcriptase Inhibitors

Two NRTIs are used in most initial regimens. The selection of NRTI is based on potency, toxicity, dosing frequency, and availability of coformulation. In resource-rich settings, tenofovir plus emtricitabine (coformulated) or abacavir plus lamivudine (coformulated) are commonly used first NRTI combinations. Although tenofovir is effective and generally well tolerated, there are specific situations in which it must be used with caution. Renal impairment has been reported with tenofovir use, and the drug should be used cautiously in patients with risk factors for kidney disease, such as pre-existing renal insufficiency or the concomitant use of nephrotoxic agents [108–111]. In addition, tenofovir is normally not used in pregnant women because of uncertainty regarding its effect on fetal bone metabolism [112].

The main adverse event associated with abacavir use is the development of a potentially life-threatening hypersensitivity reaction. Screening for HLA-B*57:01 and avoiding the use of this drug in those individuals who are positive for this allele can eliminate the risk of this event (as discussed previously). More recently, concerns have been raised regarding the potency and toxicity of abacavir. In a randomized trial, patients taking abacavir and lamivudine with virus loads greater than 100 000 copies ml⁻¹ were less likely to achieve sustained HIV suppression than were patients receiving tenofovir/emtricitabine (with either efavirenz or ritonavir-boosted atazanavir) [113]. In addition, two observational cohorts have linked abacavir to an increased risk of myocardial infarction, particularly in patients with other risk factors for cardiovascular disease [114, 115]. Abacavir/lamivudine has been moved from a preferred to an alternative NRTI-component by one U.S. guidelines committee, because of concerns regarding its potency and toxicity [116].

In many resource-limited settings, the combination of stavudine plus lamivudine is used in first-line regimens. This combination is rarely used in resource-rich settings however, due to concerns regarding stavudine-related toxicities, such as lactic acidosis, lipodystrophy, and neuropathy. Zidovudine plus lamivudine (coformulated) or didanosine plus either lamivudine or emtricitabine are other alternative NRTI pairings. Zidovudine plus lamivudine is widely used in pregnant women, a

Table 7.5 Potential options for initial antiretroviral therapy [35, 58, 96, 116].

	Considerations	Potential toxicity
Nucleoside Reverse Transcriptase Inhibitor (NRTI) backbone		
Tenofovir with emtricitabine	Once-daily, coformulated pill. Preferred NRTI component in resource-rich settings	Can cause nephrotoxicity; use with caution in patients with renal disease or in patients who are taking potentially nephrotoxic agents
Abacavir with lamivudine	Limited availability in resource-limited settings Once-daily, coformulated pill. May be less effective than a tenofovir-emtricitabine-based regimen in patients with HIV RNA >100 000 copies ml ⁻¹ [113]. Considered to be an alternate NRTI component in one U.S. guideline [116]. Limited availability in resource-limited settings	Risk of hypersensitivity reaction abrogated by HLA-B5701 screening [46] Large observational studies suggest that abacavir may be associated with an increased risk of cardiovascular events [114, 135]
Zidovudine with lamivudine	Twice-daily, coformulated pill NRTI regimen of choice in pregnant women Not part of first-line regimen in resource-rich settings	Potential for severe anemia. Associated with higher rate of metabolic complications and lipodystrophy than abacavir- or tenofovir-based regimens
Anchor (either NNRTI or ritonavir-boosted PI)		
Non-nucleoside reverse transcriptase inhibitor (NNRTI)		
Efavirenz	Once-daily Available in coformulation with tenofovir and emtricitabine Teratogenic, so sexually active women of child-bearing potential should use contraception	Central nervous system toxicity can be limiting Rash can occur, often self-limited Risk of hepatotoxicity, especially in patients with viral hepatitis coinfection
Nevirapine	Twice-daily	Systemic hypersensitivity reaction may occur: women with CD4+ cell count >250 µl ⁻¹ and men with CD4+ cell count >400 µl ⁻¹ are at increased risk

(Continued)

Table 7.5 (Continued)

	Considerations	Potential toxicity
	<p>Available in coformulation with stavudine and lamivudine May be less effective in women who received single-dose nevirapine within the past 6 months for prevention-of-mother-to-child-transmission of HIV [118] Rifampin reduces levels of nevirapine, which may compromise efficacy [117] Not part of first-line regimen in resource-rich settings</p>	<p>Rash can occur, often self-limited Risk of hepatotoxicity, especially in patients with viral hepatitis coinfection</p>
	Ritonavir-boosted protease inhibitor (agents below should be given with ritonavir)	
Atazanavir	<p>Once-daily dosing</p> <p>Not coformulated with ritonavir Acid-reducing agents reduce absorption of atazanavir, and should be avoided or used with caution Limited availability in resource-limited settings</p>	<p>Associated with indirect hyperbilirubinemia, which is usually benign; occasionally patients may develop clinical jaundice May be associated with gastrointestinal side effects Less likely to cause dyslipidemia than other protease inhibitors</p>
Darunavir	<p>Once-daily Not coformulated with ritonavir Limited availability in resource-limited settings</p>	<p>Higher rate of rash with darunavir than with lopinavir [136] May cause dyslipidemia Rare reports of hepatotoxicity; monitor carefully in patients with viral hepatitis coinfection or concurrent use of other hepatotoxins</p>
Fosamprenavir	<p>Some experts reserve use of this drug for patients who have failed a previous PI-regimen because of its excellent activity in this situation</p> <p>Once- or twice-daily Not coformulated with ritonavir Similar efficacy to lopinavir/ritonavir in a randomized study [137] Limited availability in resource-limited settings</p>	<p>May be associated with gastrointestinal intolerance May cause dyslipidemia</p>
Lopinavir	<p>Once- or twice-daily Coformulated with ritonavir Often used along with AZT/3TC in pregnant women</p>	<p>May be associated with gastrointestinal intolerance May cause dyslipidemia</p>

situation in which it has a long track record of safety and efficacy. Zidovudine may cause anemia, and is associated with increased mitochondrial and metabolic toxicities compared to tenofovir- or abacavir-based regimens. With the increasing availability of tenofovir and abacavir in resource-limited settings, stavudine and zidovudine use will likely decrease.

Certain NRTI pairings should not be used because of toxicity or antagonism, notably zidovudine plus stavudine, stavudine plus didanosine, tenofovir plus didanosine, and lamivudine plus emtricitabine.

7.5.2.2 Anchor: Non-Nucleoside Reverse Transcriptase Inhibitors or Protease Inhibitors

For first-line therapy, two NRTIs should be combined with either a NNRTI or a ritonavir-boosted PI.

The two most commonly used NNRTI anchors are efavirenz and nevirapine, both of which are available in convenient three-drug, fixed-dosed formulations. The fixed dose combination of tenofovir, emtricitabine, and efavirenz is dosed as a single pill, given once daily. Stavudine, lamivudine, and nevirapine is also coformulated and dosed as one pill, given twice daily.

NNRTI-based regimens are generally well tolerated. Both, nevirapine and efavirenz, can cause rash or hepatotoxicity. Efavirenz has also been associated with teratogenicity, and should not be used in pregnant women or in sexually active women of child-bearing potential who are not using contraception. Efavirenz can cause neuropsychiatric side effects which, although usually transient, may affect the ability of some patients to tolerate this agent. Nevirapine has been associated with a severe hypersensitivity reaction in women with CD4+ cell counts greater than $250 \mu\text{l}^{-1}$ and in men with CD4+ cell counts greater than $400 \mu\text{l}^{-1}$, and should not be used in these populations. Nevirapine levels are decreased by the coadministration of rifampin. In patients who initiate ART while receiving antituberculous medications, this drug interaction may compromise the efficacy of nevirapine-based regimens [117]. A final consideration in deciding to initiate a NNRTI-containing regimen in a resource-limited setting is whether a woman has previously received single-dose nevirapine at the time of delivery to prevent mother-to-child transmission of HIV. In such individuals the rate of NNRTI-related resistance is high, and there is concern that this may compromise future effectiveness of NNRTI-based regimens, particularly if used soon after the pregnancy [118].

A ritonavir-boosted protease inhibitor, in conjunction with a NRTI backbone, is also a recommended option for initial therapy in several guidelines from resource-rich settings [35, 36]. The recommended PIs include lopinavir, atazanavir, fosamprenavir, saquinavir, and darunavir – each combined with low-dose ritonavir, which pharmacologically boosts their plasma levels. The major side effects of PI-based combinations are gastrointestinal intolerance and dyslipidemia (although the latter is not as severe with atazanavir). PI-containing regimens also generally have a greater pill burden than NNRTI-containing regimens. Finally, rifamycins – especially rifampin – interact with PIs, making the latter therapy problematic in patients receiving concurrent antituberculous therapy.

Several factors should be considered when deciding between NNRTI- or PI-based regimens for initial therapy, if both are available. In a randomized study, the rate of virologic failure was lower in patients taking an efavirenz-based regimen than in those taking a lopinavir/ritonavir-based regimen. However, in those patients who did have virologic failure, multiclass resistance developed more frequently with efavirenz-based than with lopinavir/ritonavir-based regimens [119]. Considerations that favor an efavirenz-containing regimen are: (i) a low pill burden; (ii) a convenient dosing schedule; and (iii) fewer drug interactions than some PIs. Considerations favoring a ritonavir-boosted PI combination include: (i) a lack of neuropsychiatric side effects that may occur with efavirenz; (ii) safety and efficacy of using lopinavir/ritonavir in pregnant women with a CD4 + cell count $>250 \mu\text{l}^{-1}$ (nevirapine should be avoided in such patients, as discussed above); and (iii) a greater number of viral mutations needed to compromise efficacy of the class than with NNRTIs. In settings where both options are available, the choice of a NNRTI- versus a PI-based regimen should be individualized.

7.5.3

Monitoring

Subjects receiving ART should be monitored for treatment toxicities and for their response to therapy. In practice, the extent of monitoring is greatly influenced by available resources.

Monitoring for *toxicities* includes the assessment of both clinical and laboratory adverse events. The WHO guidelines state that, in resource-limited settings, clinical monitoring should be the primary tool for monitoring patients, but that laboratory monitoring protocols should be developed on a country-wide basis [58]. Laboratory testing should generally be directed by signs and symptoms, with several important exceptions. The WHO recommends that patients receiving zidovudine should undergo routine monthly hemoglobin measurements for the first three months after treatment initiation, because of the potential for severe anemia. The routine monitoring of liver enzymes should be considered during the first three months of therapy in certain groups of patients initiating a nevirapine-based regimen, such as women with a CD4 cell count greater than $250 \mu\text{l}^{-1}$, and patients with viral hepatitis or liver disease. In those patients receiving tenofovir the measurement of renal function is advisable.

In resource-rich settings, routine laboratory monitoring is generally performed, although this practice has not been evaluated for cost-effectiveness. Guidelines published in the United States suggest checking a complete blood count, electrolytes, renal function and liver enzymes at the time of ART initiation, at 2–8 weeks after initiation, and then every 3–6 months in patients on a stable regimen [116]. Fasting glucose and lipids should be checked when the ART is started and then at least every 6–12 months. In addition to the tests outlined above, patients receiving tenofovir should undergo a yearly urinalysis; some experts have suggested also monitoring serum phosphorus levels. Laboratory tests should be performed more frequently if clinically indicated.

Monitoring for *therapy response* also differs in practice between resource-rich and resource-limited settings. In resource-rich settings, after the initiation of ART the CD4 cell count is normally monitored every 3–4 months, and the virus load is generally measured every 4–8 weeks until it is undetectable. Approximately 75% of patients will achieve a virus load less than 400 copies ml⁻¹ after a two-month period of therapy [120]. Patients with a detectable virus load after six months of therapy, or with newly detectable values after previously being undetectable, should undergo a comprehensive evaluation for adherence, resistance testing, and review of any possible drug interactions. An early identification of failure can prevent the accumulation of further resistance, and forestall clinical progression.

In resource-limited settings, the WHO suggests using clinical criteria to assess treatment response. The routine monitoring of CD4 cell count (where available) is recommended every six months, or more frequently if clinically indicated. Unfortunately, virus load testing is not available in many resource-limited settings. CD4 + cell count changes in patients receiving ART have limited utility in identifying virologic failure in individual patients [121, 122], and expansion of the availability of virus load testing in resource-limited settings is urgently needed.

To address the need for improved monitoring in resource-limited settings, new inexpensive, reliable, and rapid diagnostic techniques are being developed [123]. For example, innovative approaches permit point-of-care measurement of CD4 + cell count [124]; versions of these instruments are currently undergoing field evaluation. Novel tests to detect virologic response, either by measuring HIV RNA or p24 antigen, are being studied [125]. New methods to monitor for laboratory toxicities would also greatly improve the care of HIV-infected patients.

In addition to clinical and laboratory monitoring, patients receiving ART should undergo regular assessments of their medication adherence, as this is one of the most important predictors of virologic suppression. In a study from Uganda, treatment interruption was strongly associated with the development of drug resistance [126]. Adherence monitoring – using methods such as 30-day patient recall, pill counts and timeliness of pharmacy refills – may represent useful methods for predicting which patients are at highest risk of virologic failure, and should be integrated into all ART programs [81].

7.6 Summary and Conclusions

Starting with initial case reports in the early 1980s, HIV has spread throughout the world and has caused more than 25 millions deaths by 2007. Many of the 33.2 million people currently infected with HIV are unaware of their diagnosis. Universal HIV testing has the capacity to prevent unsuspected transmission of new infections and to identify those people in need of treatment. Newly diagnosed patients should undergo a comprehensive evaluation for medical comorbidities, along with interventions to reduce any socioeconomic and logistical barriers to care. Patients should also be assessed for active or latent tuberculosis infection, and should receive prophylactic

therapy to prevent opportunistic infections, depending on their CD4+ cell count. Antiretroviral therapy reduces AIDS-related events and mortality, and may also prevent non-AIDS-related complications that are linked with uncontrolled viral replication. First-line regimens should be chosen to maximize convenience and tolerability, and to minimize short- and long-term toxicities. Adherence remains the best predictor of long-term success, and interventions to maximize adherence are an important cornerstone of effective ART programs. The successful and durable treatment of HIV is possible in both resource-rich and resource-limited settings. An expansion of the availability of ART to all those who need it should be one of our highest priorities.

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8

HIV-Associated Tuberculosis: Clinical Challenges

Neil W. Schluger

8.1

Introduction

The immune defects associated with human immune deficiency virus (HIV) infection create intense vulnerability to disease caused by *Mycobacterium tuberculosis*. Unsurprisingly then, tuberculosis (TB) is a leading cause of morbidity and mortality in persons with HIV infection, and in regions such as sub-Saharan Africa, HIV infection is the major driver of the TB epidemic. Tuberculosis infection and active disease pose major diagnostic and treatment challenges in persons with HIV infection. These challenges are related to unusual clinical manifestations of the disease caused by immunosuppression; to abnormal immune responses which may make diagnosis difficult; to a high frequency of adverse effects from TB drugs; and to drug–drug interactions between antituberculosis medications and antiviral agents which now form the mainstay of HIV treatment. These clinical challenges will be reviewed in detail in this chapter.

8.2

Epidemiology of Tuberculosis in HIV-Infected Persons

The incidence of HIV infection among persons with TB varies worldwide. As noted above, HIV infection among persons with TB is a major feature of the TB epidemic in sub-Saharan Africa where, in many countries, approximately half of all persons with TB also have HIV infection. According to the most recent report from the World Health Organization, between 30% and 50% of all TB patients in South Africa, Swaziland, Zimbabwe, Zambia, and Botswana are infected with HIV, and the rates are only slightly lower in several other countries in sub-Saharan Africa [1]. In the two countries that together account for nearly 40% of all TB cases in the world – India and China – the rates of HIV coinfection appear to be lower. A recent survey from eight states in India indicated a range of 1.0% to 13.8% for HIV coinfection among persons with TB [2]. In China, reliable estimates of the prevalence of HIV coinfection

among tuberculosis patients are extremely difficult to come by. The most recent WHO Global Report on Tuberculosis estimated that 0.32% of TB cases in China occur in HIV-infected persons, although the reliability of this estimate is difficult to judge [1].

In developed countries, the prevalence of HIV coinfection among persons with TB is substantially less than it is in high-burden African nations. In the United States, for example, rates of HIV infection among TB patients have declined from about 15% a decade ago to almost 0% at present, although among those aged 25–44 years approximately 15% still have HIV infection [3]. In France, the WHO estimates that 6.7% of newly diagnosed TB cases will occur in patients with HIV infection, with rates of 1.82% in Germany, 7.25% in Italy, and 3.58% in the United Kingdom [1].

Despite the variance in rates of HIV coinfection among patients with TB, it is clear that this represents a major global challenge not only to TB control programs but also to the lives of millions of individuals worldwide.

8.3

Clinical Issues in the Care of HIV-Infected Patients with Tuberculosis

8.3.1

Latent Tuberculosis Infection

It is generally estimated that almost one third of the world's population – some two billion people – have latent TB infection (LTBI) and that, in otherwise healthy people, latent infection carries with it an approximate 10% risk of progression to active TB. However, in patients with HIV infection, the results of some studies reported prior to the advent of highly active antiretroviral therapy (HAART) indicated that the risk of progression to active TB disease was as high as 10% annually [4–6]. It was clear from several of these studies that essentially all HIV-infected patients with LTBI would progress to active TB if they did not die first from some other cause. In view of this it is not surprising that, in some regions such as sub-Saharan Africa, TB is the most common pulmonary complication of HIV infection. Although, in the era of HAART the risk of progression from latent to active TB is probably lower for HIV-infected patients than previously observed, it still remains considerably higher than in immunocompetent persons.

The treatment of latent infection (see below) is effective in reducing morbidity from TB in patients with HIV. Of course, the institution of treatment for latent infection rests on an accurate diagnosis of latent infection in the first place, and HIV infection poses challenges in this regard.

Latent infection with *M. tuberculosis* generally results from the inhalation of viable bacteria that have been expelled into the atmosphere by somebody with active pulmonary TB. The organisms are engulfed by macrophages and a characteristic immune response results. Although there are likely some (or perhaps many) instances in which the inhaled organisms are completely eliminated by an innate immune response, in many persons the immune response will result in a containment of the

infection through granuloma formation. Within granulomas, the organisms can remain non-growing, but viable, for many years.

Competent and effective granuloma formation depends on a variety of host factors, but in recent years the important role of tumor necrosis factor (TNF) has been highlighted. Other cytokines such as interferon-gamma (IFN- γ) are also likely to play a significant role [7–10]. Obviously, immune derangements associated with the advanced stages of HIV infection will have a significant impact on the ability to effectively contain mycobacteria within well-formed and well-functioning granuloma. A detailed discussion of the host immune responses in TB, and their derangements in the disease condition, is beyond the scope of this chapter, but is provided elsewhere in the book.

8.3.1.1 LTBI Diagnosis

8.3.1.1.1 The Tuberculin Skin Test The diagnosis of LTBI has been accomplished for the past 100 years by using the tuberculin skin test (TST), which is a modification of the original skin test described by Charles Mantoux in 1908 [11]. In this test, purified protein derivative (PPD), a mixture of some 200 proteins produced by *M. tuberculosis*, is injected intradermally. At 48–72 h later, the site of the injection is assessed for the characteristic induration that characterizes a positive test, which is a manifestation of a type IV (cell-mediated) delayed-type hypersensitivity (DTH) reaction. The type IV DTH response results from an interaction between antigen (and antigen-presenting cells) and cell-mediated immune mechanisms. Primed memory T cells recognize the mycobacterial antigens which are presented in the context of major histocompatibility complex (MHC) class II molecules, and they begin to undergo stimulation and proliferation. The stimulated T cells release a number of cytokines (most prominently IFN- γ) that further attract and stimulate tissue macrophages, which themselves will release TNF. The results is a characteristic erythema and induration.

In patients with HIV infection there is abundant evidence that DTH responses are severely impaired as the T-cell number and function decline, and this will have significant implications for the ability to diagnose TB infection via a skin test. *Cutaneous anergy* in patients with HIV infection is extremely common, and becomes more common as the T-cell numbers fall. In several studies, anergy to common skin-test antigens such as mumps, tetanus, or candida was found in as many as 30% of patients, especially when those with CD4 + T-cell counts less than $400 \mu\text{l}^{-1}$ were examined. Among patients with active TB and apparently competent immune systems, about 80% will have positive TSTs, but in patients with advanced HIV infection this percentage will drop sharply, to 35% or less [12–14]. Because of this, some public health authorities had previously recommended that HIV-infected patients should routinely undergo anergy panel testing (using mumps, candida, and tetanus antigens) as a positive control when tuberculin skin testing was performed. This recommendation was based on observations in the early years of the AIDS epidemic in the United States which suggested that anergic HIV-infected intravenous drug users had almost the same risk of developing active TB as did TST-positive patients with AIDS. However, the findings of more recent epidemiologic

studies in the United States and other established market economy countries do not support this observation, and there is currently no recommendation for routinely including anergy testing along with the TST.

Despite the obvious limitations in the use of skin testing to diagnose LTBI in patients coinfecting with HIV, this tool remains the most widely available and the one with which there is the greatest experience. In order to compensate partially for the decreased sensitivity of the test in these patients, a cut-off value of 5 mm of induration is generally used as the criterion for a positive test for LTBI in HIV-infected patients, as opposed to 10 mm in most other patients. This recommendation has been adopted by the United States Centers for Disease Control and Prevention, the American Thoracic Society, the Infectious Diseases Society of America, and the British Thoracic Society, among others [15–18].

8.3.1.1.2 The Interferon-Gamma Release Assay In recent years, new tools for the diagnosis of TB infection have been developed, and are now achieving wider clinical use. These tests, which are known generally as interferon-gamma release assays (IGRA), were developed using a recent understanding of both the genetics of *M. tuberculosis* and other mycobacterial species and the human host response that underlies the hypersensitivity reaction associated with a TST [19].

IGRA, as their name implies, rely on the detection of *in vitro* IFN- γ production by peripheral blood mononuclear cells after stimulation with highly specific mycobacterial antigens. Two IGRA assays are now commercially available in many parts of the world. The Quantiferon Gold test (Cellestis, Inc., Melbourne, Australia), measures IFN- γ production in serum via an ELISA, while the T.SPOT.TB test (Oxford Immunotec, Oxford, England) uses the ELISPOT technique to measure IFN- γ -producing cells. Both systems employ the mycobacterial antigens CFP-10 and ESAT-6, which are found in members of the *M. tuberculosis* complex of organisms (*M. tuberculosis*, *M. microti*, *M. africanum*, and *M. bovis*). However, these antigens are not found in any of the Bacille Calmette-Guérin (BCG) vaccine strains of *M. bovis*. Thus, these tests offer a great advantage over skin testing with PPD, namely that the results should not be confounded by prior vaccination with BCG nor by exposure to environmental (nontuberculous) mycobacteria.

At present, few data are available regarding the performance of IGRA in persons with HIV infection. Mandalakas and colleagues in South Africa reported that, in a small cohort of adults and children, the TST and IGRA results were frequently discordant [20]. Karam and colleagues found that IGRA ELISPOT results were more often positive than TST in a cohort of 253 HIV-infected persons in Senegal [21]. Raby and coworkers examined a cohort of 112 patients with active TB (as a surrogate “gold standard” for infection), and found that the Quantiferon assay was positive in 84% of HIV-negative patients and 69% of HIV-positive patients, as compared to TST positivity rates in HIV-positive patients of 41% if a 10 mm cut-off was used, and 55% if a 5 mm cut-off was used [22]. These data were consistent with IGRA studies in a variety of settings, which indicated that the tests may be more sensitive overall than TSTs. At present however, no national guidelines – among the many that have been developed – recommend routinely replacing TST with IGRA for the diagnosis of LTBI

in patients with HIV infection. However, as additional data become available regarding the performance of IGRA in HIV-infected persons, this situation seems likely to change.

In any patient, active TB must be excluded before a diagnosis of LTBI can be made. In HIV-infected patients, this may be more considerably more challenging, as the chest radiographs may be normal (or minimally abnormal) even in the setting of sputum culture-positive TB. Careful symptom screening should be carried out in addition to chest radiography, and active TB must be rigorously excluded before any treatment for latent infection is prescribed.

8.3.1.2 LTBI Treatment

The treatment of LTBI in patients with HIV infection is unequivocally effective in preventing cases of active TB [23, 24], a point which was established clearly in a series of (somewhat controversial) randomized trials conducted several years ago [25]. The current recommendations from the United States Centers for Disease Control and Prevention/American Thoracic Society/Infectious Diseases Society of America recommend a nine-month treatment with isoniazid (INH) as the first-line regimen for HIV-infected persons with LTBI. The nine-month recommendation is something of an extrapolation, as clinical trials evaluated both 12- and six-month regimens, but not specifically nine-month regimens.

8.3.1.2.1 Two-Month Regimens Several studies have been conducted to evaluate the efficacy of a two-month regimen of rifampin and pyrazinamide in the treatment of LTBI in HIV-infected persons [26, 27]. These results of these controlled trials, which were conducted in populations in Haiti, Mexico, the United States, Brazil, Spain, Zambia, and Hong Kong, indicated that the rifampin/pyrazinamide (2RZ) regimen was as effective as INH in preventing progression from latent infection to active disease. Soon after the results of several such studies had been reported, a number of national TB control programs adopted the 2RZ regimen as an acceptable alternative to nine months of INH in patients with HIV infection. Shortly after these recommendations were made, case reports of severe and often fatal infection began to appear [28, 29]. These experiences, together with meta-analyses of the data from previous trials with 2RZ in the treatment of LTBI, led to the withdrawal of this regimen as a recommended strategy for the treatment of latent infection in HIV and non-HIV-infected persons.

8.3.1.2.2 Three-Month Regimens In the United Kingdom, a three-month regimen of INH and rifampin has long been recommended for the treatment of LTBI in the general population. One study in Uganda included this regimen in a trial in HIV-infected persons, and found that the beneficial effect lasted for up to three years [30]. Few other data are available concerning the use of this approach in HIV-infected persons, however.

8.3.1.2.3 Four-Month Regimens An alternative regimen for treatment of LTBI is a four-month period of rifampin (4R) [31]. Although this regimen has not been widely

evaluated for efficacy, in general it appears to be safe and well tolerated by most patients. However, it should be used with caution in patients with HIV infection, as several studies have described the development of rifampin mono-resistance in patients with advanced HIV infection [32–36]. Although the risk of this has only been described in patients treated with highly intermittent dosing schedules in the context of active disease and not latent infection, it seems prudent to avoid this regimen generally in AIDS patients, given the availability of other effective regimens and the disastrous consequences of the development of rifampin-resistant tuberculosis both for individual patients, and in TB control programs generally.

8.3.1.2.4 Isoniazid More recently, a bolder approach to preventing TB among persons with HIV infection in high-burden countries has been investigated. In this approach, Allison Grant and colleagues evaluated a program in which INH was given to HIV-infected patients in South Africa on a community-wide basis, without skin testing to specifically identify those persons with LTBI [37]. Their assumption was that the rate of LTBI was sufficiently high to warrant the administration of INH across a population. Prior to INH administration, all persons in the study were carefully screened and evaluated for active TB – a necessary but potentially expensive component of such a program. After excluding from the trial (to the best of their ability) those persons with active TB, INH was given on a population basis using a complicated but rigorous study design. The widespread administration of INH to a population using this approach was associated with a lower rate of active TB, and indicates the promise of a large-scale effort at TB prevention in high-burden countries. The experiment should be repeated on a larger scale, however. A warning has also been issued, based on mathematical modeling studies, that the administration of INH on a massive scale could result in the generation of a significant increase in drug-resistant tuberculosis [38].

8.3.2

Active Tuberculosis

8.3.2.1 Active Tuberculosis Diagnosis

The diagnosis of TB generally relies on a clinical assessment, chest radiography, sputum smear examination, and sputum culture. In regions where all of these modalities are available, 85–95% of all cases will be confirmed by culture results. However, in many resource-poor countries there is no widespread availability of sputum culture and chest radiography, owing to the high costs associated with these technologies. This creates particular problems in patients with HIV infection, in whom the diagnosis of active TB can be much more subtle [39].

8.3.2.1.1 Clinical Presentation The clinical presentation of TB has been well known for centuries: the triad of symptoms of fever, cough, and weight loss has been described not only by physicians, but also by writers and poets. Although this triad is reasonably sensitive, it is obviously far from specific, and its specificity is likely to be lower in patients with HIV infection, who are susceptible to an enormous range of opportunistic infections.

A study conducted by von Reyn and colleagues reported the striking finding that subclinical TB (TB without clinical symptoms or radiographic abnormalities) was detected in 29% of HIV-infected persons volunteering for a tuberculosis vaccine trial [40]. However, this was a small series and the incidence of TB with minimal symptoms was somewhat higher than in some other previously reported studies. Nonetheless, it underscores the fact that the presentation of TB in the setting of HIV infection can be quite subtle.

8.3.2.1.2 Chest Radiography Several groups have reported that the distribution of radiographic findings in patients with HIV infection and TB is quite different from that in non-HIV-infected persons [41–46]. The radiographic features of pulmonary TB are well known; typically, the disease presents in the upper lobes or in the superior segments of the lower lobes, and cavitation is often seen. Other presentations include pleural effusion with or without a parenchymal infiltrate, a military pattern (innumerable tiny 1–2 mm nodules scattered throughout the lungs, more predominantly in the lower lung fields), normal radiographs, or hilar and/or mediastinal adenopathy, usually without parenchymal infiltrates. These so-called “atypical” radiographic manifestations of TB, which had often been thought to represent primary (or recently acquired) infection, instead are now known to represent simply a different manifestation of the host–pathogen interaction than occurs in patients with intact immunity [47]. They may account for as many as one-third of all radiographic presentations, they become more common as the CD4+ T-cell numbers decline, and they highlight the subtleties of making a diagnosis of active TB in patients with HIV infection. Clinicians must be highly suspicious of TB in any HIV-infected patient – especially in a TB-endemic region – if there are minimal symptoms compatible with TB, or if the slightest abnormality is noted on chest radiography, if it is available.

8.3.2.1.3 Sputum Smear and Culture When there is clinical suspicion of TB, sputum smear examination should be performed as soon as possible. As is well known, the smear examination will be positive in about 50–70% of cases with active pulmonary TB, so even in the best of scenarios many cases would go undetected by this method. In patients with TB and HIV infection, the positivity of sputum smear examination is lower than it is in immunocompetent hosts [48]. This creates an enormous problem, especially in countries or regions where sputum culture is not routinely available. Delays in diagnosis are associated with prolonged infectiousness (even smear-negative cases have the potential to spread infection, though they are clearly less infectious than smear-positive cases) and increased morbidity and mortality in any given patient. Active TB, as is the case for many opportunistic infections, is associated with accelerated HIV replication and an increasing viral load, and a prolonged period of non-treatment will therefore accelerate the natural history of HIV infection [49, 50]. This will be especially true if antiretroviral therapy (ART) is not available. Thus, there is great urgency to improve the diagnostic yield in smear-negative cases of TB.

In many resource-constrained settings, algorithmic approaches to the diagnosis of smear-negative TB are employed [48]. In most such regions, several sputum smears are collected (minimally three) after which, if all are negative, a course of oral antibiotics should be administered for 7–14 days. The patient must then undergo

a clinical assessment and, if clinically improved, a diagnosis other than TB can be rendered. However, if no improvement has occurred after antibiotic treatment, chest radiography should be performed, if possible, and the treatment for TB then instituted. In the settings where such a treatment algorithm is employed, delays of two to four weeks in instituting therapy may be observed, and there will of course be a number of patients who are treated for TB but do not in fact have the disease.

The question then remains, can the diagnosis of active TB in patients with HIV infection be improved, using the existing technologies? The answer is certainly “yes”; for example, if broth-based culture systems such as the BACTEC MGIT were to be more widely available, the overall case detection rates would be higher. In a recent study conducted by Campos and colleagues, it was shown that the use of commercially available nucleic acid amplification (NAA) assays could improve the diagnosis of TB considerably in HIV-infected persons, with NAA having a significantly better performance than the smear [51]. This study was performed in the United States, a resource-rich environment, and it is unclear whether the technologies employed could be made available in poor countries, because of their high costs. There do appear to be cost–benefit scenarios where such an approach would make sense, though the actual cost of the tests may still be out of reach.

8.3.2.2 Active TB: Treatment

The issues involved in the treatment of active TB in patients with HIV infection are numerous and complex. Almost every aspect of treatment, including the drug regimen, the duration of therapy, and monitoring for clinical response and relapse, is controversial and presents difficult challenges for the clinician.

In many – if not most – patients with HIV infection, TB represents the index opportunistic infection that establishes a person as having full-blown AIDS. Although, the treatment of TB in patients with HIV/AIDS is effective, the mortality rate in most reported series is significantly higher in these cases than in those without HIV infection. The cause of this higher mortality is unclear. In several series, there has appeared to be an excess of early mortality and, as noted above, it has been well established that active TB increases HIV replication and thus may accelerate the natural history of the HIV infection [52, 53]. This may in part explain some of the early mortality noted in these patients.

8.3.2.2.1 The Drug Regimen The choice of a chemotherapy regimen for TB can be complicated by the simultaneous administration of ART [54, 55]. From the results of studies conducted during the pre-HAART era, it is clear that standard antituberculosis regimens are active and effective in the treatment of patients with HIV infection. No data are available, nor have studies been conducted, to suggest that additional drugs should be added to standard induction or continuation-phase chemotherapy, as had been the practice of some physicians during the earlier phases of the HIV epidemic. There are no data to support the practice, for example, of routinely adding a quinolone to the initial four-drug regimen in the intensive phase of treatment. However, in patients already receiving HAART, selection of the initial and continuation-phase therapy is much more complicated.

The simultaneous administration of antituberculosis drugs and HAART poses several challenges which may make each therapy less effective. The sheer number of medicines involved in such combined regimens is daunting, and may lead to nonadherence because of that fact alone. Also, most of the drugs involved in the treatment of HIV infection and TB can be associated with significant adverse effects. Finally, there are important drug interactions between antituberculosis drugs (notably rifampin, the most important in the regimen) and some classes of antiretroviral drugs that can limit the effectiveness of each treatment. Consequently, there may be a risk that patients receiving medications for both TB and HIV infection are, in reality, being effectively treated for neither condition [56–59].

Because of the difficulties in treating tuberculosis and HIV infection simultaneously, the preferred strategy, if possible, is to treat the tuberculosis first, for at least two months, and then to institute HAART. However, as this may not be possible in all cases, in which cases might it be desirable to treat both infections simultaneously, and what might be the best strategy [60]?

8.3.2.2.2 Drug Combinations and Interactions The major drug interactions which can cause each regimen to be ineffective are those involving rifamycins (the most potent inducers of the cytochrome P450 system in clinical use) and two of the most important classes of antiretrovirals – protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) – both of which are metabolized by the CYP3A4 hepatic isoenzyme. Of the three available rifamycins, rifampin is the most potent inducer of CYP3A4 and rifabutin the least potent, with rifapentine having intermediate activity. To complicate the situation, rifampin itself is not metabolized by CYP3A4 but rifabutin is; consequently, exposure to rifampin can be expected to increase with the concomitant administration of a PI.

Currently approved PIs include indinavir, nelfinavir, saquinavir, ritonavir, amprenavir, fosamprenavir, atazanavir, tipranavir/ritonavir and darunavir/ritonavir, administered together, and lopinavir/ritonavir in fixed combination. In theory, rifampin may reduce the serum level and area under the time–concentration curve (AUC) for any of these drugs, rendering them ineffective or less effective. However, this effect may be minimized if ritonavir is used as the PI.

Drug interaction studies have indicated that, in general, the magnitude of the effect of an interaction between rifamycins and PIs is much less if rifabutin is used rather than rifampin. Rifabutin can be used safely with a single PI, as well as with some PI combinations.

The three currently approved NNRTIs – delavirdine, nevirapine, and efavirenz – are all metabolized by the hepatic CYP3A, and their levels can be expected to decline somewhat in the presence of rifamycins. The effect of NNRTIs on rifamycin levels is less predictable, and varies with the different NNRTI used. It appears that efavirenz and rifampin can be used together safely, with good results in terms of TB and HIV treatment, although some dose adjustments to the NNRTI may be necessary. The same may be true of nevirapine, which offers certain advantages over efavirenz, including the fact that it is safe in pregnancy. Rifabutin can also be used with efavirenz, although because its dose should be increased this runs the

risk of a higher frequency of adverse effects, including uveitis. Nevirapine and rifabutin can be used together without dose adjustments for either drug.

Data are available from clinical trials that rifabutin-based regimens for the treatment of TB are safe and effective in patients receiving HAART. Recently, the Tuberculosis Trials Consortium (TBTC) published a study evaluating a regimen in which rifabutin was substituted for rifampin in HIV-infected patients receiving HAART. The exact HAART regimen was left to the discretion of the treating physician, but many patients in the trial received a PI. In general, HAART was begun after two months of antituberculosis medication had been given. The most striking finding of the study was that mortality within one year of the time of diagnosis of TB was only 3.5% in patients treated with rifabutin-containing antituberculosis regimens. This compared favorably with studies of TB treatment of AIDS patients in the pre-HAART era, in which the one-year mortality following the diagnosis of TB was as high as 20% [36]. The TBTC study provided compelling arguments both for the early introduction of HAART in HIV-infected patients with TB, and the safety and efficacy of rifabutin-containing regimens. However, the TBTC study and the implications drawn from it are somewhat limited; for example, as the study was single-arm in design it is impossible to know whether a rifampin-containing regimen would have been more effective, or less effective, than the regimen studied. A second problem is that rifabutin is expensive, unaffordable or unavailable in many of the regions where the problem of HIV-associated TB is most acute.

In general, the major preference would be to keep rifamycins in the regimens of HIV-infected TB patients because it is this class of drugs which allows short-course chemotherapy of six to nine months. Although a regimen of daily streptomycin, INH and pyrazinamide administered for nine months was tested with success 30 years ago, such a long course of injections would not be acceptable to most patients. Moreover, the injectable antituberculosis agents are associated with significant adverse effects, including renal failure and vestibular toxicity. In general, the omission of a rifamycin from the antituberculosis regimen would mean at least an 18- to 24-month period of tuberculosis treatment.

In general, the most sensible approach – if drugs are available – would appear to be that taken by the New York City Department of Health and Mental hygiene Tuberculosis Control Program, which recommends several possible therapeutic approaches:

1. A two-month treatment with INH, rifampin, pyrazinamide and ethambutol, followed by four months with INH and rifampin.
2. A two-month treatment with INH, rifabutin, pyrazinamide and ethambutol, followed by four months with INH and rifabutin.
3. A two-month treatment with INH, rifampin, pyrazinamide, capreomycin, *para*-aminosalicylic acid and ethambutol, followed by four months with INH and rifabutin.
4. A nine-month treatment with INH, pyrazinamide, and streptomycin.

It is obvious that the first two recommended regimens are vastly preferable to the second two. If clinically acceptable, it would be preferable to delay HAART until at least two months of a rifampin-based antituberculosis regimen has been completed.

8.3.2.2.3 Dosing Frequency The dosing frequency of drugs is also a matter that deserves comment. During the pre-AIDS era, several studies demonstrated the feasibility of treating TB with twice- or thrice-weekly regimens. Such regimens have two important advantages: first, they make the delivery of treatment by directly observed therapy much easier; and second they allow the resources to be spread over more patients. However, more recent studies have indicated that highly intermittent regimens are more likely to be associated with treatment failure and relapse [36]. In the setting of HIV infection, they may also be more likely to lead to the development of acquired rifampin resistance, a particularly devastating complication. Thus, when rifamycins are used in the antituberculosis regimen they should be given daily for as long as possible, and never less frequently than three times weekly. The risk of acquired rifampin resistance appears greater in persons with low CD4+ T-cell counts.

8.3.2.2.4 Duration of Therapy The duration of therapy for TB in patients with HIV infection is also a matter of some controversy. In general, for patients with drug-susceptible TB, six-month regimens in which rifampin is used throughout treatment are universally recommended. An eight-month regimen in which rifampin is used only for two months, followed by a six-month continuation phase of INH and ethambutol to complete the treatment, is clearly inferior in terms of a combined endpoint of treatment failure and relapse [61].

In recent years, a number of prospective randomized trials have highlighted situations in which a six-month regimen is associated with an unacceptably high risk of relapse. The results of the Tuberculosis Trials Consortium's Study 22 showed that the presence of extensive, bilateral cavitary TB, an initial body weight less than 90% of predicted and, most importantly, a failure to convert sputum cultures to negative after the two-month induction phase of chemotherapy, are associated with a risk of relapse in excess of 15% [62]. These caveats should be borne in mind when treating patients with HIV infection, who may be especially prone to present with advanced TB because of impaired T-cell immunity. Previous studies of human immune responses in TB have shown that a robust TH1-type immune response, impaired in patients with HIV infection, is associated with less-advanced pulmonary TB. Such responses are restored, at least to a certain degree, in patients who are receiving treatment with HAART. Thus, it would not be surprising if six-month TB treatment regimens were associated with higher failure rates in AIDS patients, particularly in those in advanced stages of their disease.

Although randomized, controlled trials of the duration of therapy in patients with TB and HIV infection have not been conducted, cohort reviews have indicated that indeed six-month treatment regimens are associated with higher rates of treatment failure and relapse. The New York City Department of Health Tuberculosis Control Program examined its experience and found that HIV-infected patients treated with nine-month antituberculosis regimens have better outcomes than patients treated for six months [63].

Although there are many HIV-infected patients in whom a six-month period of antituberculosis treatment is likely to be associated with an excellent outcome, clinicians should have a low threshold for extending therapy to nine months if there

is any evidence of extensive disease at the time of diagnosis, evidence of advanced immunosuppression, and/or a slow initial response to therapy. In such cases (with a drug-susceptible isolate, of course), a two-month regimen of four drugs, followed by an additional six months of INH and rifampin, is the most prudent course.

Drug-resistant TB in patients with HIV infection poses a great risk of morbidity and mortality. Experience in New York City and elsewhere during the early 1990s indicated that patients with HIV infection and multidrug-resistant (MDR) TB (defined as TB resistant to at least INH and rifampin) had very high mortality rates and a very short median survival, although it was shown that the prompt placement of a patient on an appropriate drug regimen could lead to an improved survival [64].

More recently, dramatic reports have emerged from South Africa regarding outbreaks and dissemination of so-called extensively drug-resistant (XDR) tuberculosis among patients with HIV infection. XDR cases are caused by strains of *M. tuberculosis* that are resistant to essentially all useful antituberculosis drugs. The first of these reports, from the Tugela Ferry region of KwaZulu Natal Province, reported near-100% mortality in HIV-infected patients with XDR-TB, where the median survival was measured not in months, but in days [65]. Several other reports have served to further dramatize the situation. Although it is possible that, as in New York City more than a decade ago, better infection control procedures can limit the spread of new infections, the paucity of available drugs bodes poorly for most of these patients [66].

8.3.2.2.5 Immune Reconstitution Inflammatory Syndrome (IRIS) A final issue of importance and controversy in the care of patients with TB and HIV infection is the immune reconstitution inflammatory syndrome (IRIS) [67–69]. For many years, so-called “paradoxical reactions” were noted in patients being treated for TB; these would typically manifest as an apparent clinical deterioration in patients who initially had shown a good response to antituberculosis therapy. Especially prevalent among these paradoxical reactions were cases involving central nervous system (CNS) tuberculomas, or cases with significant mediastinal lymphadenopathy. In these patients, after an initial period of clinical improvement, fever and local symptoms appeared, raising concerns with regards to adherence to the medical regimen, or about the development of drug resistance.

With the advent of HAART, the paradoxical reactions became much more prevalent, and have since come to be classified as IRIS. Presumably, this syndrome – as its name implies – represents the restoration of an effective immune response following antiretroviral drug treatment. When the immune system has been reconstituted, local and systemic signs of inflammation return, causing symptoms; this is especially the case if the antigenic burden, from whichever infection present, is still high.

Although a precise definition of IRIS does not exist, patients often develop fever and an apparent worsening of signs and symptoms of their disease wherever it is present. The major differential diagnostic challenge (as noted above) is to decide whether these symptoms are due to treatment failure or to immune reconstitution. In the setting of TB, this distinction can be difficult if there is no culture confirmation

or drug susceptibility testing available. When a patient is known to have a drug-susceptible isolate of *M. tuberculosis*, and to be receiving the correct drug regimen to which there is good adherence, a worsening of symptoms in the setting of concurrent HAART is likely to represent an IRIS response.

The incidence of IRIS has been reported to range from 2% to 30% among patients with opportunistic infections who are receiving HAART therapy. Although IRIS has been reported to occur from as soon as three days after the start of treatment to as long as one year after, the syndrome most often develops between six and 10 weeks after the onset of concurrent treatment for HIV infection and an opportunistic infection, when the antigenic burden from the secondary infection is still high. This timing corresponds to the results of *ex vivo* studies, which show the restoration of TH1-type T-cell function (specifically, the ability to secrete IFN- γ after stimulation with *M. tuberculosis*-specific antigens) beginning a few weeks after the institution of HAART.

The management of IRIS reactions is largely symptomatic and supportive. If the symptoms are severe, an interruption of HAART may ameliorate the situation, although obviously this strategy runs the risk of allowing the progression of HIV infection at a time when the patient may be particularly vulnerable, namely during an active opportunistic infection. There have been several reports of improvement in IRIS symptoms following the administration of systemic corticosteroids, particularly in patients with painful and uncomfortable lymphadenopathy or CNS tuberculomas. However, there are no well-defined management strategies that have been evaluated in clinical trials.

8.4

Conclusions

AIDS-associated TB represents a major and growing public health crisis in many parts of the world, but particularly in sub-Saharan Africa, where HIV is the main driver of the TB epidemic. Tuberculosis in AIDS patients presents challenges of diagnosis and treatment, and successful care of these patients requires expertise in both conditions. With careful management, many patients will have good outcomes, although the spread of drug-resistant TB in HIV-infected patients poses an enormous challenge that, at present, is not being met with success.

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9

TB/AIDS Coinfection: An Integrated Clinical and Research Response

Anne E. Goldfeld and Elizabeth L. Corbett

9.1

Introduction

Although curable, tuberculosis (TB) is estimated to be the single largest cause of death among AIDS patients globally, being responsible for at least 12% – and perhaps up to 30–50% – of all AIDS-related deaths that have occurred [1–5]. That the two diseases are inextricably intertwined has been evident from the first reports of AIDS in countries heavily burdened by TB. Increased rates of TB were, in fact, the first indicators that the AIDS disease complex had taken hold in Haiti and Democratic Republic of the Congo during the early 1980s, at the time when the first AIDS cases were being described in the United States and Europe [6, 7].

At the individual patient level, TB and HIV form a type of “disease complex,” with each pathogen manipulating the host response in such a way as to enhance the other pathogen’s ability to cause disease pathology. In most cases, TB is the first pathogen to infect the patient, with HIV infection occurring later. With progressive HIV infection and its associated immune compromise, there is an enhanced risk of reactivation of latent TB infection (LTBI), an increased likelihood of progressive TB disease from newly acquired TB infection, and an increase in recurrent TB or TB relapse (Figure 9.1). In the cases where HIV infection predates TB infection – such as in mother-to-child transmission of HIV – the generalized immune stimulation that accompanies secondary TB infection results in driving HIV replication and disease progression [8] (Figure 9.1). Thus, both infections require early and effective intervention and treatment. The major reason that the global toll of coinfection is so dramatic includes the lack of, or delayed access to, HIV treatment, inadequate TB case detection and cure in general, and the lack of screening of patients for both infections when either TB or HIV has been diagnosed. The current limitations in TB diagnostics, available TB drugs, and limited knowledge about the optimal cotherapy of those drugs already in hand, are also great impediments to reversing the humanitarian disaster of TB/HIV coinfection.

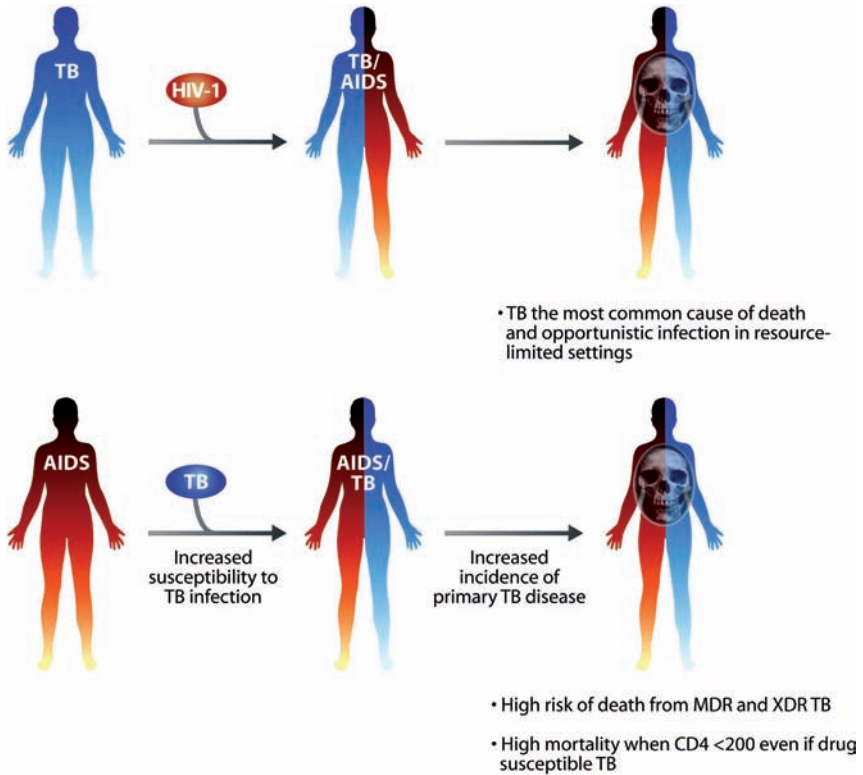


Figure 9.1 A schematic representation of the clinical impact of TB and HIV coinfection, and the impact of the order of infection.

An assessment of the trends in TB incidence between 1997 and 2000 (Table 9.1), highlights the linkage between TB and HIV infection. In sub-Saharan Africa, where an estimated 85% of the HIV-associated TB cases are located, this association is dramatically illustrated (Figure 9.2a). For example, TB notification rates have increased several fold in Botswana, Zambia, Zimbabwe, Malawi, Tanzania, and Uganda due to the increasing rate of HIV infection and the maturity of the AIDS epidemic in these countries (Figure 9.2b). Notably, there are now a number of countries reporting declining rates, such as Botswana, Zimbabwe, and Zambia (Figure 9.2b). These recent improvements may reflect changes in TB incidence secondary to falling HIV prevalence, improved TB control, or combinations of the two.

Even after the declaration of TB as a “Global Emergency” by the World Health Forum in 1993, policies to simultaneously address both diseases were slow to evolve [5]. For the most part, each disease was treated as a separate entity until the past five years, to the extent that it was exceptional for TB patients in resource-poor settings to be offered HIV testing prior to this time, even in clinics where the vast majority of TB patients were HIV-infected [9]. Conversely, AIDS patients were not routinely screened for evidence of TB, and access to antiretroviral therapy (ART) was unavailable. Recognition of the need for an integrated approach to TB/AIDS as a

Table 9.1 Regional trends, burdens of morbidity and mortality, and associations with HIV infection in the world at the start of 2000. Data from the WHO.

WHO region	Africa	Americas	Eastern Med.	Europe	South-east Asia	Western Pacific	Global
Population (millions)	640	832	485	874	1536	1688	6053
Trends in TB incidence 1997–2000 (%/year)	3.9	−4.1	−1.4	2.8	−1.3	0.0	0.4
New cases of TB, all forms							
No. of cases ($\times 10^3$)	1857	382	587	468	2986	2031	8311
Incidence (per 100 000)	290	46	121	54	194	120	137
HIV prevalence in new adult cases (%)	38	5.9	1.8	2.8	3.2	1.3	11
Attributable to HIV ($\times 10^3$)	421	12	5.2	8.2	53	13	511
Attributable to HIV (% of adult cases)	31	5.1	1.5	2.6	2.7	1.1	9
New SS+ cases of TB							
No. of cases ($\times 10^3$)	785	169	264	210	1338	913	3679
Prevalence SS+ TB (per 100 000)	185	27	103	35	209	117	122
Prevalent SS+ cases HIV+ve (%)	7.5	1.0	0.2	0.5	0.3	0.1	1.4
Infection prevalence among adults							
<i>M. tuberculosis</i> infection (%)	31	15	27	14	46	32	30
<i>M. tuberculosis</i> /HIV coinfection (%)	2.7	0.1	0.1	0.0	0.3	0.0	0.4
Deaths from TB							
Total deaths ($\times 10^3$)	482	55	135	72	727	368	1839
Deaths per 100 000 population	75	6.6	28	8.3	47	22	30
In HIV+ve adults ($\times 10^3$)	203	3.9	3.0	1.6	29	5.7	246
Adult AIDS deaths due to TB (%) ^a	12	4.1	11	10	8.1	17	11
TB deaths attributable to HIV (%)	39	6.5	2.0	2.1	3.7	1.4	12

^a‘Adult’ indicates 15–49 years old; SS + , sputum smear-positive.

The WHO African Region comprises sub-Saharan Africa and Algeria; the remaining North African countries are in the Eastern Mediterranean Region.

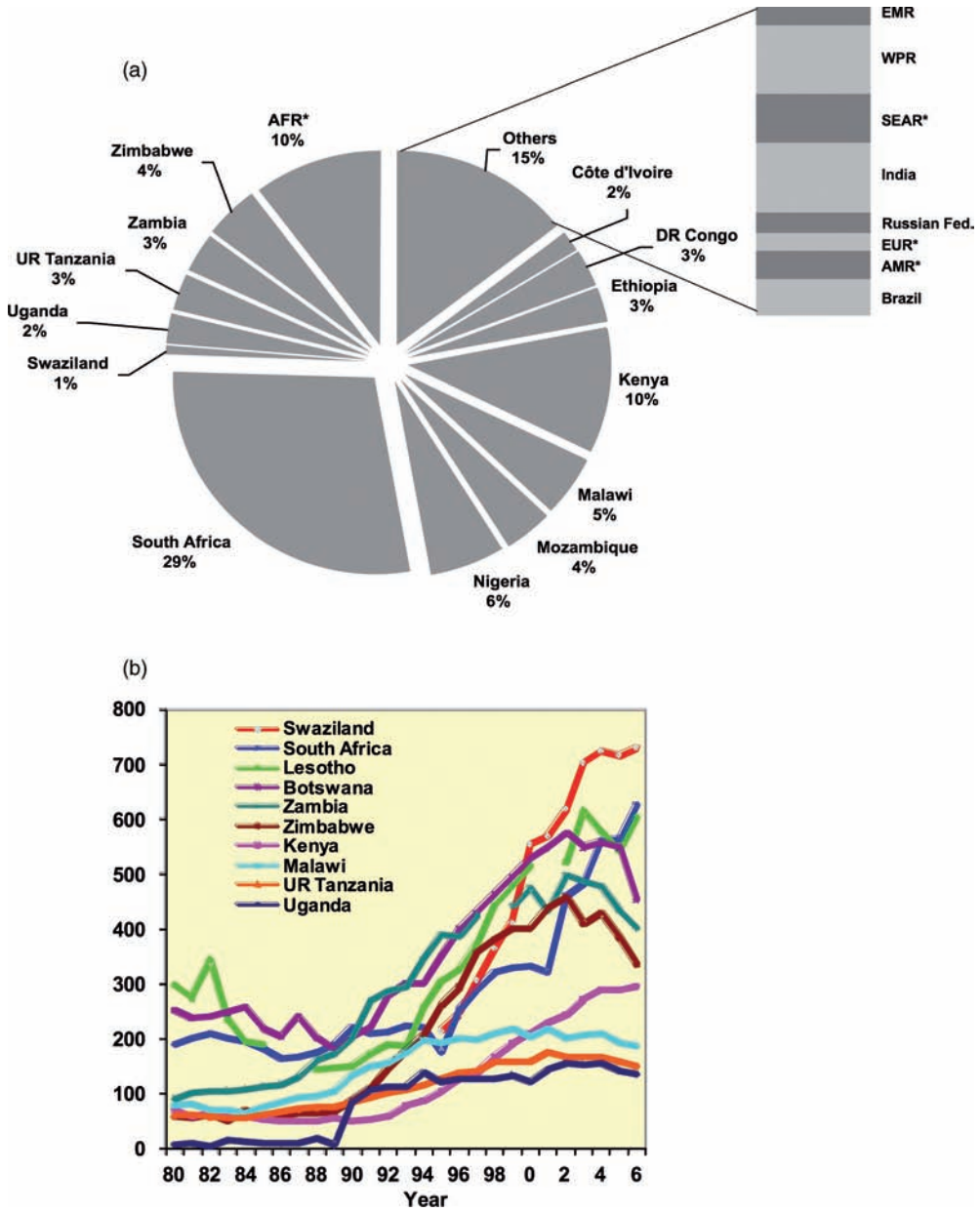


Figure 9.2 (a) Geographic distribution of HIV-positive TB cases. For each country or region, the number of TB cases arising in people with HIV is shown as a percentage of the global total of such cases. AFR* is all countries in the WHO African Region except those shown separately; AMR* excludes Brazil; EUR* excludes the Russian Federation; SEAR* excludes India. Adapted from WHO 2008 Global Tuberculosis Control Report. (b) Trends in TB patient notifications per 100 000 population in 10 countries of sub-Saharan Africa between 1980 and 2006. Data extracted from WHO 2008 Global Tuberculosis Control Report.

linked epidemic has emerged only recently. Even so, attempts to implement existing recommendations and mount an effective public health response to HIV/TB in resource-poor settings have lagged. The critical need for an integrated approach has been dramatically highlighted by the outbreak of extensively drug-resistant TB (XDR-TB) affecting a health facility in KwaZulu Natal, South Africa, which was primarily driven by poorly controlled institutional transmission of XDR-TB in a hospital ward housing highly vulnerable immune-suppressed AIDS patients [10–12].

The scaling-up of HIV care services over the past five years has made it increasingly clear that TB and AIDS infections cannot be well managed, or even well controlled, in isolation from the other. Rather, integrated interventions from the level of the individual patient to public health responses are required, as proposed in Table 9.2.

Such interventions include:

- Access to AIDS drugs and effective HIV care in resource-poor countries.
- Improved TB case detection and cure rates which will prevent further loss of TB control by decreasing the pool of infectious people and by maintaining TB drug-susceptibility patterns.
- Access to TB drug-susceptibility testing and access to quality assured second-line drugs (SLD) for multidrug-resistant TB (MDR-TB) to prevent the increased spread of MDR-TB.

Table 9.2 Integrated interventions for tuberculosis and HIV infection to reduce morbidity and mortality.

1. Improving short- and long-term outcomes for HIV-infected persons with TB	<ul style="list-style-type: none"> Universal HIV testing of all TB patients and patients suspected of TB Screening of all HIV-infected persons for TB Rifampicin-containing short-course chemotherapy for all TB patients Improved diagnosis, treatment, and follow-up for sputum smear-negative TB Early initiation of ART for HIV-infected TB patients with CD4 count $<250 \mu\text{l}^{-1}$ Cotrimoxazole prophylaxis for all HIV-infected patients with CD4 count $<200 \mu\text{l}^{-1}$ Scale up of MDR-TB diagnosis Access to MDR care
2. Preventing new and recurrent TB in persons with HIV	<ul style="list-style-type: none"> Universal knowledge of HIV status Primary isoniazid preventive therapy for all HIV-infected persons Early ART for HIV-infected TB patients with CD4 count $<250 \mu\text{l}^{-1}$ Infection control to prevent transmission of <i>M. tuberculosis</i> and MDR-TB
3. Reducing community level transmission of HIV and TB	<ul style="list-style-type: none"> Universal knowledge of HIV status Active case finding for TB Community-based approaches to enhance adherence and case detection that reach individuals who do not live near a health center Integrated TB/AIDS care to enhance adherence and case detection Universal access to ART Universal access to MDR care

Major unmet challenges of TB/HIV that need to be addressed include:

- Ensuring universal access to HIV testing and ART and to preventive therapy for opportunistic and latent TB infection.
- Ensuring access to rapid diagnosis and access to effective treatment for MDR-TB, which is nonexistent in most health facilities in resource poor settings [10]. In fact, only 49 000 people are currently accessing MDR therapy [WHO Stop TB Website, <http://www.who.int/tb/>] when the need has been estimated at 500 000 by the WHO and some estimates range to between two and four million (J.P. Cegielski, personal communication, U.S. Institute of Medicine Meeting on MDR-TB, November 5, 2008).
- Enhancing efforts focused on TB infection control [11]. AIDS patients are needlessly and excessively exposed to the risk of major morbidity and death from TB from *de novo* infection. Institutional TB transmission and shared social risk factors place certain communities or social networks at a disproportionately high risk of coming into contact with TB [4] in health and other facilities.
- Enhancing efforts to develop and scale-up effective community-based diagnosis and care to improve new TB case finding. Access to care in rural areas remains rudimentary or essentially nonexistent in most resource-poor countries, including those at the epicenter of the HIV/TB epidemic.
- Improving tools to diagnose TB disease and LTBI [13]. TB diagnosis is particularly problematic for HIV-related TB infection and disease; this contributes to delayed TB disease detection and increased mortality, and also hinders the exclusion of a diagnosis of TB in patients about to start ART or isoniazid (INH) preventive therapy [5].
- Decreasing the excessive mortality from TB, which remains extremely high among persons living with HIV. Even so, the mortality is probably grossly underestimated due to the high burden of unrecognized TB deaths [5] or deaths due to immune reconstitution syndrome [8].
- Determining how to best clinically manage the concurrent presentation of TB disease and HIV in resource-limited settings [14, 15], despite this being identified as a major priority for research almost a decade ago. This is true for both drug-sensitive and drug-resistant TB disease.
- Increasing collaboration of health systems at all levels for TB/HIV treatment and control, as HIV-related TB poses a major threat to both TB control and HIV care in even the most affluent countries [16].
- Basic research into the host immune response and into TB/HIV factors that determine disease outcome to enhance discovery of new vaccines and therapies.

In this chapter, attention will be focused on TB and HIV coinfection exclusively in resource-poor countries, where the vast majority of coinfecting individuals live. The current understanding of the major clinical, epidemiological, and public health issues concerning HIV/TB will be summarized, and the major recent developments

and persisting obstacles towards improving clinical and public health management of TB/HIV will be detailed. The current understanding of the global burden of TB/HIV and the best treatment options of HIV/TB in resource-poor settings will be described, including: (i) the clinical and health system barriers to diagnosing TB in patients who are HIV-positive; (ii) the choice of regimen and timing of ART in patients presenting with TB; (iii) the danger of unmasking paradoxical reactions both in AIDS patients with known TB and in those not known to have active TB; (iv) research into host and pathogen factors involved in disease outcome and (v) the need for, and an example of, a coordinated TB/AIDS program.

9.2

TB/HIV Epidemiology

In this section, the global, population, and clinical epidemiology of HIV-related TB, including evidence of any secondary impact on TB control at the population level, and the epidemiology of TB in the context of ART, is described.

9.2.1

Global Epidemiology of HIV/TB Coinfection and Disease: Estimates and Regional Time Trends

Global estimates of the burden of TB mortality and morbidity are based on one of the components of the “DOTS” (directly observed therapy strategy) system, which is to record and report the number of TB cases and treatment outcome. In 2008, 202 of the 220 countries in the world compiled and reported their data to WHO [17]. The reported numbers of sputum smear-positive cases provides a very specific estimate of the minimal global burden of TB, although it is certain to be an underestimate because not all patients are smear-positive. In fact, many patients with TB are never diagnosed, as shown by the high burden of undiagnosed disease detected during population-based TB prevalence surveys, and deaths from undiagnosed TB probably outweigh known TB-related deaths. A functional chain of reporting from clinic to national to international level is needed; obvious system failures include the marked year-by-year fluctuations that are reported from some countries (e.g., see Swaziland in Figure 9.2a).

In 1999, estimates of the true incidence of TB were made through a country-by-country adjustment of case-notification rates for under-reporting and under-diagnosis [18]. These were revised with special reference to HIV/TB in 2000, including estimates of coinfection (LTBI plus HIV infection) [4]. Although still imprecise, these data have provided a methodology and benchmark against which to measure progress in TB control, and also drew attention to the problem of low global “case-detection rates” or under-diagnosis of TB, as shown in Figure 9.3. Annual updates have been made since 1999 by the WHO, including global and regional estimates of HIV/TB cases and deaths. The high number of HIV-related deaths from TB, and the staggeringly high rates of HIV and TB coinfection in Southern Africa with trends at the start of the decade, are summarized in Table 9.1.

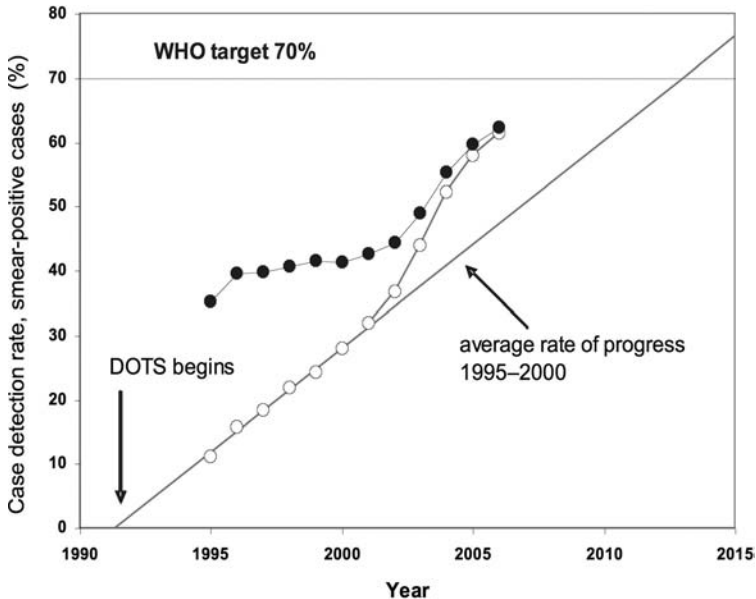


Figure 9.3 Global case detection of TB under DOTS programs. The open circles mark the number of new smear-positive cases notified under DOTS 1995–2006, expressed as a percentage of estimated new cases in each year. The solid line through these points indicates the average annual increment from 1995 to 2000 of about 134 000 new cases, compared to the average increment from 2000 to 2006 of about 242 000 cases. The closed circles show the total number of smear-positive cases notified (DOTS and non-DOTS) as a percentage of estimated cases. For the Africa region, however, case-detection remains under 50%. Adapted from WHO 2008 Global Tuberculosis Control Report.

9.2.1.1 Trends in the Global HIV/TB Epidemic Since 2000

9.2.1.1.1 The Example of Sub-Saharan Africa With 11% of the global population, Africa has the greatest regional burden of the global HIV/TB epidemic, with about 68% of the estimated burden of HIV in 2007 and 85% of all cases of HIV/TB [17]. Africa's weak health systems have resulted in very slow progress towards TB control even before HIV [5]. By 2000, an estimated 38% of African TB patients were HIV-positive, and 31% of TB cases and 39% of TB deaths were considered directly attributable to HIV, with much higher coinfection rates in Southern Africa. In Botswana, for example, 64% of TB cases were directly attributable to HIV in 2000 and, among HIV-positive Africans, TB was the cause of at least 12% of all deaths [19]. (This is likely to be a gross underestimate because of difficulties in quantifying deaths from undiagnosed TB.) Notably, it is estimated that only 37% of all TB cases in Africa were diagnosed and treated [4], and thus undiagnosed TB cases made up the bulk of estimated HIV-positive TB deaths.

Between 2004 and 2007, TB case-notification rates have decreased in a number of severely affected African countries, and the global trend in TB incidence may now have turned around into decline [17]. This may reflect different combinations of declining HIV prevalence and better TB control. However, revised methods for calculating HIV prevalence in countries with generalized epidemics, which reduced

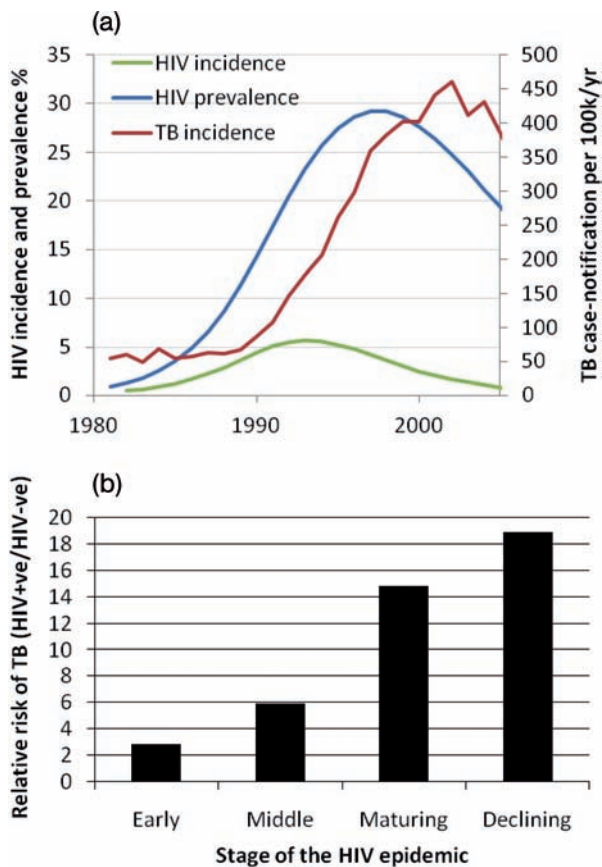


Figure 9.4 Evolving HIV/TB epidemics in Southern Africa. (a) Temporal relationship between HIV and TB epidemics in Zimbabwe. HIV incidence (green) peaked in 1993, with steep declines in HIV prevalence (blue) from a peak adult prevalence of 29% in 1997. TB case notifications have followed the HIV prevalence trend, but with a lag of four to five years; (b) Cohort estimates of the relative risk of TB from being HIV-infected, according to the stage of HIV epidemic. Four estimates of the relative risk of TB from being HIV-infected

are shown. The first three points are from a gold-mining cohort in South Africa, the last from a cohort of factory workers in Zimbabwe. These show a progressive trend towards an increasingly strong HIV effect, which may primarily reflect changes in the distribution of immunosuppression as the HIV epidemic evolves (e.g., in the first few years of an HIV epidemic most individuals will have had their infection for a short period, and so most will still be in the early stages of immunosuppression).

adult HIV prevalence estimates particularly in Africa, has almost certainly provided erroneously low estimates of HIV/TB for the last few years (Stop TB Dept., WHO, personal communication).

The underlying trend of the intertwined relationship between HIV and TB is illustrated in Figure 9.4a, which shows the temporal relationship between HIV and TB epidemics in Zimbabwe. The HIV incidence peaked in 1993, with steep declines in HIV prevalence from a peak in adults of 29% in 1997. Other recent cohort studies

have reported higher relative risks for TB from being HIV-infected (i.e., the risk of TB in HIV-infected individuals relative to the risk of TB in HIV-negative individuals is now higher) than were apparent during the early years of the HIV pandemic [20, 21]. Adjusted estimates of the global burden of HIV/TB are likely to be almost doubled from the 2007 estimate of 700 million cases, when this trend is taken into account (Stop TB Dept., WHO, personal communication.). This is due to the natural evolution of the HIV epidemic, with changes occurring in the distribution of early/moderate/advanced HIV-related immunosuppression at the population level as the HIV epidemic rises, matures, and goes into decline. As shown in a cohort from South Africa (Figure 9.4b), this will tend to cause such a trend. Another contributing factor is the increasing association of the HIV epidemic with poverty in resource-poor and highly TB-burdened countries where a lack of access to ART is common [22].

The other striking figure for which no current update is available is the very high rate of latent TB/HIV coinfection, which has reached 5% or more in the general adult population in eight African countries, with South Africa alone estimated to have over two million coinfecting individuals. There is a high risk of breakthrough of latency to active TB disease in HIV coinfecting persons, estimated to be a lifetime risk of approximately 30–40% if no intervention is received [4]. This pool of individuals is clearly driving increases in TB, and efforts to diagnose HIV early, to provide access to ART, and to diagnose and prevent HIV-related TB are urgent priorities.

9.2.1.1.2 HIV/TB Incidence in Other Global Regions Although no other global sub-region has seen HIV prevalence rise to the same high rates as Southern Africa, generalized HIV epidemics, which reached highs of 4% in Cambodia, 2.1% in Thailand, and 2.0% in Myanmar, have also fueled substantial epidemics of HIV-related TB [17, 21]. Cambodia, for example, which is one of the highest-TB-burdened countries, has a prevalence of all forms of TB of 665 per 100 000 [23]. The screening of new TB patients in 2007 in two rural provinces revealed that 1.4% were HIV-positive and 24% of AIDS patients presenting for care at rural HIV care sites in two provinces had evidence of TB (Cambodian Health Committee).

Eastern Europe, which has one of the most severe MDR-TB problems worldwide, also has a rapidly rising HIV epidemic, driven by an explosive epidemic of HIV among intravenous drug users (IVDUs) [24]. Notably, in other parts of the world, including Northern Thailand, Spain, and the United States, there is a strong epidemiological link between being an IVDU, clinical TB, and imprisonment. These may explain some of the epidemiological links between HIV status, imprisonment, and MDR-TB being reported from Eastern Europe [25, 26].

9.2.2

HIV as a Risk Factor for TB in the Pre- and Post-ART Era

HIV greatly increases the risk of progressing from recent or remote (latent) TB infection to TB disease, with relative risk estimates from individual cohort studies ranging from below 5% to over 20% [4]. The relative risk of TB increases with worsening immunosuppression, and so tends to increase during the course of an

HIV epidemic as the median duration of HIV infection rises [22, 27]. Thus, the high risk of TB in HIV-infected individuals can be reduced by the treatment of LTBI [28], especially if the patient is tuberculin skin test (TST) -positive, and by provision of ART [29, 30].

Studies from the United States, Italy, South Africa, and Brazil showed that ART reduced the incidence of TB in HIV-infected persons by 80% or more [31–34], with the greatest impact in patients with the lowest CD4 cell counts [31]. Importantly, even with treatment the incidence of TB does not return to HIV-negative rates. This situation was apparent in a series of studies from South Africa, which showed that:

- there is a very high risk of incident TB being diagnosed in the first month of ART treatment, which most likely reflects undiagnosed TB at the time of commencement of therapy [35]; and
- the risk is strongly related to the CD4 cell count thereafter, regardless of the duration of ART treatment [36].

TB incidence rates on ART were, respectively, 3.4, 1.7, and 2.0 per 100 person-years for CD4 cell counts $<200\mu\text{l}^{-1}$, $200\text{--}350\mu\text{l}^{-1}$, and $>350\mu\text{l}^{-1}$ [31]. These observations are consistent with the hypothesis that clinically important immune dysfunction is likely to persist even when the CD4 counts are restored to normal values [37]. This raises the possibility that, in the long term, ART programs may contribute to the HIV-associated TB epidemic by creating an expanding cohort of patients receiving ART who survive for prolonged periods but remain at increased risk of TB [37, 38].

The risk of recurrent TB is also increased by underlying HIV infection. This represents a combination of increased risk of recurrent TB disease if suboptimal TB regimens are used, and an increased susceptibility to progressive TB disease in the event of reinfection [39]. In high TB transmission settings, reinfection is the dominant cause of recurrent HIV-related TB. This is again of greater risk at lower CD4 counts [40], but suggests the need for secondary preventive therapy [41].

9.2.3

The Secondary Impact of HIV-Related TB on Global TB Transmission Rates and Population Genetics of *M. tuberculosis*

Given these major changes in global epidemiology, several groups of investigators have begun to address three critical questions regarding the secondary impact of HIV that may have profound implications for the next stage of the global HIV epidemic:

- To what extent is the epidemic of HIV-related TB fueling increases in TB transmission?
- Are we seeing the evolution of *M. tuberculosis* strains adapted to immunosuppressed hosts, with consequent shifts in population genetics?
- How are drug sensitivity patterns being affected by the HIV epidemic at national and regional levels, especially given the relative ease with which Beijing-W TB strains acquire drug resistance, and is this reflected in shifting TB population genetics?

TB transmission rates are difficult to measure accurately, and only a few countries measure them periodically. In Africa, typical rates are 0.5–2% risk of TB infection per annum. Rates in Asia have been much higher (rates of over 10% per annum were recorded in Hong Kong), but have progressively declined in many countries since TB treatment became available, with the exception of conflict and post-conflict areas, a notable example being Cambodia [42]. Although there are no conclusive answers, the available data indicate that in the majority of countries, the potential for large numbers of HIV-infected TB patients has had little effect on TB transmission rates at a *community* level, although there clearly has been a major impact at *health facility* level (deteriorating TB infection control and institutional transmission) [11], with extremely high transmission rates reported from African hospitals [43, 44] along with well-publicized outbreaks that have come to light because of drug resistance [10].

The population genetics of *M. tuberculosis* have only recently been addressed on a global scale [45], but already there are some indications of potential change resulting from the HIV epidemic in very high TB transmission areas of South Africa. These changes have coincided with, and could conceivably be secondary to, the HIV epidemic [46, 47], although a direct link has not yet been established.

9.2.3.1 HIV and TB Transmission Rates at the Community Level

The critical period with respect to infectiousness is prior to diagnosis (diagnosis of TB disease and/or diagnosis of drug resistance), because most patients become noninfectious soon after starting appropriate treatment, even if HIV-infected [48, 49]. A key feature of the epidemiology of HIV-negative TB is a very prolonged period of infectiousness that is usually estimated to last for one or more years on average before diagnosis in resource-poor settings, and which can be increased by inadequate TB programs that do not support treatment completion [50]. This prolonged period of infectiousness thus results in a high burden of persistently infectious undiagnosed TB in the community compared to the number of new cases diagnosed each year.

Both, the intensity and duration of infectiousness of TB disease are highly variable, with some individuals being very much more infectious than others [49, 51], and others remaining infectious for prolonged periods, sometimes with apparently minor symptoms [52–55]. HIV-positive TB patients tend to be less infectious as they are less, or relatively less, smear-positive. Population-level TB transmission data that are available show a much more stable picture of transmission than would be anticipated: Malawi and Tanzania appear to have contained TB transmission rates successfully during a period when smear-positive TB case notifications increased fourfold or more [5]. In Kenya, however, there has been a documented rise in the annual risk of *M. tuberculosis* infection, and an association between rising transmission rates and higher HIV prevalence at the district level [5]. However, the magnitude of increase has been only a fraction of that of the preceding rise in TB case-notifications. That TB transmission rates can be contained by standard TB control programs in HIV-burdened countries is further supported by studies from several African countries and from Thailand, that show stable or

declining HIV-negative TB incidence rates during the course of epidemics of HIV-related TB [5].

The likely explanation for this otherwise surprising lack of effect of HIV infection in many countries is likely due to a major difference in the duration of infectiousness between HIV-negative and -positive TB infections. It is thought that relatively little of the burden of undiagnosed infectious TB at the community level is attributable to HIV, because of a much briefer mean period of smear-positivity before diagnosis or death in HIV-related TB [5]. This probably reflects a basic difference in the natural history of TB in immunosuppressed individuals, resulting in a more rapid and aggressive clinical progression that does not usually support prolonged smear-positivity [5] (Figure 9.5). The implications are that, on average, each HIV-negative TB patient contributes far more to TB transmission than each HIV-positive TB patient (having a longer infectious period in which to transmit) and that the brief infectiousness of HIV-related TB to a large extent mitigates the impact of the HIV-related TB epidemic on overall community TB transmission rates [5]. This carries with it the clear message that if prolonged transmission from HIV-negative TB

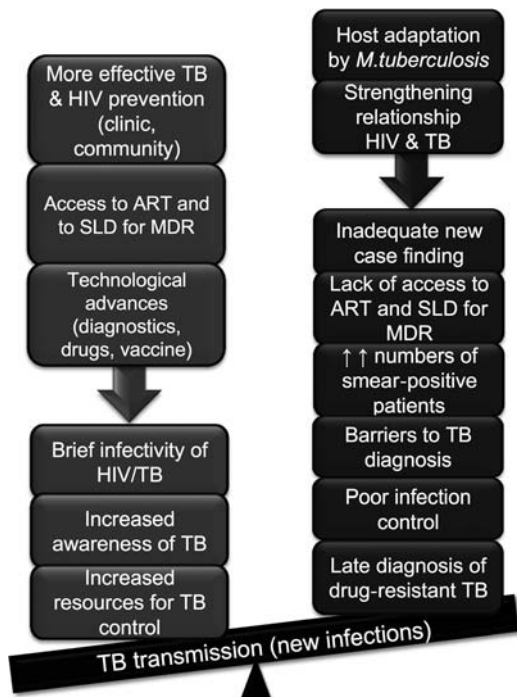


Figure 9.5 A hypothetical schematic illustration of the balance between current (lower part of figure) and emerging or future (upper part of figure) factors tending to either reduce or increase the impact of HIV on control of TB transmission. The overall balance will vary from

one setting to the next, with some countries (such as Malawi) successfully controlling transmission even in the face of a severe epidemic of HIV-related TB. Other countries, such as South Africa, may be in an upward spiral of rising TB transmission.

patients is a key driver of the epidemic of HIV-related TB even in high HIV prevalence settings, then interventions to improve TB control in general, including HIV-negative disease, is necessary in order to make an impact on TB/HIV.

South Africa may be one place where community TB transmission has been tipped into an upwards spiral in the context of HIV infection. For example, reports from one gold mining workforce show a rising TB incidence among HIV-negative employees [22], in contrast to stable rates in another workforce [19]. In Cape Town, three recent community-based population surveys have shown very high rates of infectious undiagnosed TB (about 2% of adults were culture-positive in each case), with one also finding a higher prevalence of undiagnosed smear-positive TB in HIV-positive than HIV-negative patients, although the sample size was small [56, 57]. A hypothetical scheme of the impact of HIV upon TB transmission is shown in Figure 9.5.

9.2.3.2 HIV and Institutional TB Transmission

The world woke up to the resurgent epidemic of TB and the new threat of HIV-related disease during the early 1990s, after decades of neglect, when New York City and other parts of the USA were gripped by a TB epidemic that included multifocal outbreaks of MDR-TB centered around HIV care facilities, homeless shelters, and correctional institutions [58].

Since then, numerous other outbreaks of MDR-TB have forced high-income countries to adopt much more stringent approaches to institutional TB control. Each of the outbreaks has been characterized by extremely high mortality rates in HIV-infected in-patients who were infected by MDR-TB before the nature of the outbreak was recognized. These high case-fatality rates reflect the extreme severity of immunosuppression in HIV-infected in-patients, as well as a high mortality from inadequately treated MDR-TB [10, 11]. Outbreaks are likely to be centered around one or more highly infectious individual(s), consistent with data on individual infectivity that show a few individuals to be very much more successful at generating infectious aerosols than others, with the majority of even smear-positive TB patients showing little evidence of any infectivity to others [11, 49, 59].

Low- and middle-income countries have been very slow to implement basic TB infection control, even when the burdens of TB and HIV are very high. Furthermore, the recognition of outbreaks has been delayed due to limitations in surveillance and a lack of routine drug susceptibility testing. The outbreak of XDR-TB in KwaZulu Natal exemplifies these institutional failings, and has engendered a sense of urgency into TB infection control in the era of expanding HIV care, with the “three Is” policy of the WHO (intensified case finding; TB infection control; and isoniazid preventive therapy) being strongly promoted [10, 11]. Much of the risk of prolonged outbreaks of drug-resistant TB could be avoided by basic TB infection control measures (screening patients for TB symptoms and separating them from other patients while being investigated) and routine drug sensitivity testing of all smear-positive TB patients. The line-probe assays, which provide drug susceptibility testing without requiring TB culture, are likely to make a major contribution to MDR-TB management and infection control as they offer the first feasible and affordable means to promptly identify drug-resistant TB in resource-poor settings [13].

9.2.3.3 HIV and TB Population Genetics and the Coinfected Individual

From the perspective of the TB bacterium, HIV-infected hosts pose different challenges from immunocompetent hosts. Infection may be easier to establish, and progression to disease is certainly more easily achieved. However, reaching the “ideal” state of clinically stable or slowly progressive disease in a highly infectious individual will present a very different immunopathological challenge. Given these very different host attributes, and given that there is considerable phenotypic variability between the major TB strain lineages and subtypes, it is interesting to speculate that we will see changes in TB that reflect adaptation of the pathogen to the immunosuppressed host environment.

Data able to support such hypotheses are only recently emerging, but recent information from South Africa suggest HIV-driven shifts in the distribution of strain lineages and phenotypes. A compelling set of data in support of this concept is the rapid and progressive rise in the proportion of the W-Beijing lineage in disease-causing strains in children [46]. In this report, the strains are polyclonal, indicating that they are not attributable to a single successful transmitter. Beijing strains more readily acquire drug resistance than other lineages, and may also cause disease in a higher proportion of cases than some other lineages [60]. Mutations that lead to rifampicin resistance are accompanied by fitness costs to the pathogen, which limits their potential spread through immunocompetent populations [60]. For the reasons outlined above, however, it is conceivable that a lower pathogenicity could be of even less relevance, and even advantageous (through prolonging the disease course) in immunosuppressed hosts. In this context, the reports that show evidence of successful disease and transmission in South Africa by strains carrying mutations that are normally not found in clinical specimens may indicate adaptation to immunosuppressed hosts by drug-resistant organisms [47]. We may thus also see new TB control challenges emerging from an epidemic of HIV-related TB if the early indications from South Africa of successful pathogen adaptation to immunosuppressed hosts prove to be correct.

9.2.4

The Impact of Pathogen and Host Genetics on Disease Outcome in TB/HIV Coinfection

TB and AIDS coinfection can result in very diverse clinical outcomes in subjects who otherwise appear to have very similar baseline characteristics. Surprisingly, not all severely immune-suppressed AIDS patients latently infected with TB develop active TB disease, despite the importance of T cells in the containment of TB. Moreover, while some patients with very low CD4⁺ T-cell counts and extensive TB can be cured of their TB with a six-month regimen of TB drugs and show a marked improvement in immune suppression with the initiation of ART, others display a rapidly lethal course of infection and die within weeks of diagnosis. Although certainly influenced by environmental factors such as nutrition and by other coinfections, these distinct disease outcomes are undoubtedly in a large part also a result of specific host immune susceptibility and resistance factors to TB and HIV (Figure 9.6).

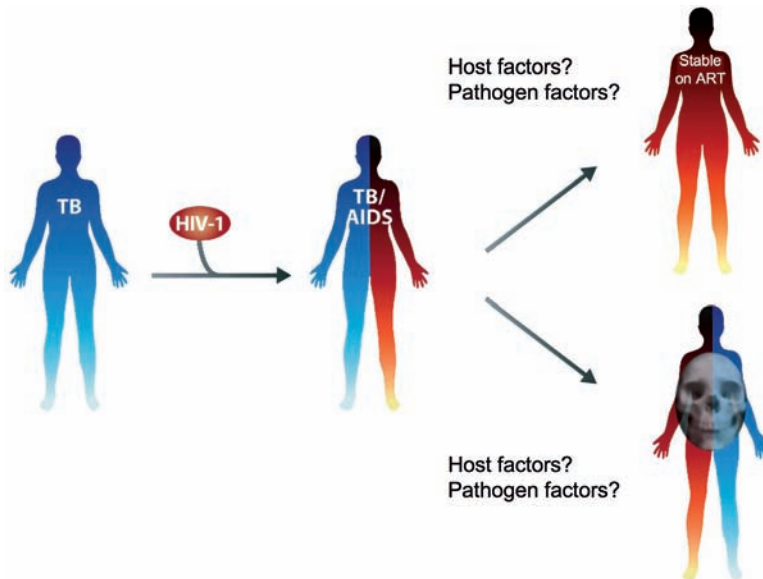


Figure 9.6 A schematic representation of potential factors impacting clinical outcome in TB/HIV coinfection.

9.2.4.1 The Impact of HIV Subtype Specificity on HIV Regulation and Disease Outcome in TB/HIV Coinfection

HIV-1 subtypes have spread unevenly, and while the HIV-1 B subtype is predominantly associated with HIV infection in North America and Europe, subtypes C and E have spread in Africa and Asia, respectively [61] (the regions of high TB prevalence), and are currently the most prevalent subtypes globally, spreading faster than other subtypes [61, 62]. The relationship between virus subtype and pathogenicity is not well defined in general, and the impact of HIV subtype on virus–host interactions and TB/HIV coinfection remains to be determined.

The activation of HIV-1 replication in coinfecting cells by TB, or by the immune activation that accompanies TB infection, has been well documented [8]. Thus, if an HIV subtype of a particular strain was particularly responsive to activation during TB infection or reactivation, it could in theory impact outcome during coinfection. In fact, several laboratories have shown that the long terminal repeats (LTRs) from different HIV subtypes have different activation profiles in response to a variety of stimuli. For example, E subtype LTRs appear relatively less responsive to activation by the cytokine tumor necrosis factor (TNF) than a B subtype LTR [63]. Furthermore, C subtype LTRs are relatively more responsive to NF- κ B p65 and to the Tat protein from a B subtype than E subtype LTR representatives [64]. E subtype LTRs were also less responsive to T-cell activation in the absence of Tat [65, 66], and infection of cells with HIV-1 subtype E isolates inhibits transcription of the TNF gene, which drives HIV replication [67]. Another transcription factor, NFAT5, has recently been shown to be

involved in the regulation of replication of all HIV clades [68]. Thus, it is interesting to speculate that TB infection may result in the differential regulation of distinct HIV subtypes at the molecular level, and that this could contribute to the different regional burdens of TB/HIV. In this regard, it is of interest to note that in *in vitro* laboratory studies, HIV subtype C – the clade associated with TB/HIV coinfection in Africa – appears to be generally more transcriptionally active than subtypes B and E [63]. Identification of the LTR sequences and activators involved in HIV transcription after TB infection or during TB activation thus provides an area of future research, which could potentially identify molecular targets for the inhibition of HIV replication during coinfection.

9.2.4.2 The Impact of TB Strain Variability on HIV Regulation and Disease Outcome in TB/HIV Coinfection

The impact of distinct TB strains and their variation on HIV replication and AIDS progression in coinfection is unknown, and is another area that warrants further study. Different clinical TB strains have been shown to have distinct immunomodulatory properties upon host cells [69, 70], including distinct effects on the regulation of cytokines that are produced in response to TB infection. This led to the hypothesis that different TB strains may influence HIV replication via the manipulation of cytokine networks, and thus influence disease progression in coinfection in a strain-specific manner. Data in support of this contention have been provided using an *in vitro* system of TB/HIV coinfection showing that a distinct activation of the host immune response by phenotypically distinct *M. tuberculosis* clinical strains directly regulates HIV-1 replication in a strain-specific manner. Specifically, the infection of human peripheral blood mononuclear cells (PBMCs) in an *ex vivo* coinfection model with the well-characterized immunogenic TB strain CDC1551, resulted in higher levels of replication of primary HIV subtypes B, C, and E as compared to the infection of PBMCs with the less immunogenic clinical TB strain HN878 [71–73]. Thus, it is intriguing to speculate that, certain TB strains differentially drive HIV-1 replication and disease progression in the human host. An investigation of the impact of TB strain on HIV replication could thus lend some basic insight into mechanisms resulting in high viral loads during coinfection. This could also lead to the identification of bacterial factors and potential drug targets to interrupt TB-driven HIV replication and increases in viral load.

9.2.4.3 The Impact of Host Variability and Disease Outcome on TB/HIV Coinfection

Genetic factors, including natural mutations in CCR5 (one of the receptors that HIV-1 uses for cellular entry) and HLA molecules, have been shown to influence the progression of AIDS and natural resistance to infection (for reviews, see Refs [74, 75]). Furthermore, there is a clear association between HLA-class II molecules and the progression of TB [76]. However, little is known about the genetic or epigenetic factors that determine outcome in TB/AIDS. Undoubtedly, disease outcome is

influenced by a combination of host resistance factors to each pathogen, and also to the host response to the TB/HIV disease complex.

Host variability in the immune response to TB has been demonstrated in a laboratory model to have a profound impact on HIV replication. In a study using PBMCs from former TB patients who were HIV-negative, the immune response to TB as measured by the protein-purified derivative (PPD) skin test reaction to TB antigens was shown to influence HIV-1 replication in an *in vitro* model system. When PBMCs from HIV-negative anergic versus TST-positive former Cambodian TB patients [77, 78] were infected *ex vivo* with HIV and secondarily stimulated by TB antigens, the level of HIV replication was greatly influenced by whether or not the patient was TST-positive or -negative. The infection of PBMCs with HIV and stimulation with from patients who had a vigorous immune response to TB, as measured by a positive TST, resulted in relatively higher levels of TNF and lower levels of interleukin (IL)-10, and higher levels of HIV replication than what was observed if the PBMCs were from a former patient with an undetectable TST [79]. Future experiments delineating the impact of host and pathogen factors on HIV and TB and on coinfection (see Figure 9.6) will thus be useful in developing a greater understanding of immune mechanisms to coinfection, and may in the future also serve as biomarkers of disease outcome.

9.3

Clinical Aspects of TB Disease in the HIV-Infected Patient

TB is often the first manifestation of HIV infection in resource-poor settings, and is the leading cause of death among HIV-infected individuals in these settings [1–3, 80–82]. In a hospital-based series of HIV-infected Africans with respiratory disease, between 40% and 65% had TB [80, 81]. For example, TB was diagnosed in 43% of adults presenting to primary health care clinics with cough for three weeks or longer (chronic cough) in Harare, Zimbabwe [82], and in 48% and 70%, respectively, of patients attending chest clinics with chronic cough in Kenya and Malawi [83–85]. Furthermore, TB was the leading cause of pneumonia in immune-suppressed AIDS patients in South-east Asia [86, 87].

HIV-related TB differs in a number of important ways from TB in the immunocompetent host. As a general principle, the more immunosuppressed the host, the more extreme these differences are [88]:

- A presentation of disseminated TB in an HIV-infected person is more commonly found than in an immunocompetent host, and if there is pulmonary TB, it more commonly presents with associated extrapulmonary or multifocal disease. This is also reflected by major differences in the immunohistopathology of diseased sites and the distribution and total body load of pathogens. Paradoxically, for pulmonary TB this leads to a reduced likelihood of patients with HIV-related TB presenting with a positive sputum smear. By contrast, positivity of the more sensitive test of culture, is similar in HIV-positive individuals and HIV-negative patients, regardless of the specimen taken (sputum, blood, pleural fluid, cerebrospinal fluid, etc.), and may even be higher.

- The rate of clinical progression is much more rapid for HIV-related TB, with a much a briefer interval of subclinical or minimally symptomatic infectious disease.
- Serious morbidity and mortality in HIV-related TB is common, and frequently relates to organ failure associated with overwhelming mycobacterial sepsis, as compared to the classical tuberculous complications in immunocompetent hosts of tissue destruction and fibrosis.
- HIV-positive individuals are at high risk of other opportunistic infections and malignancies that are rare in immunocompetent individuals. These tend to have a spectrum of clinical manifestations that overlap those of TB.

Thus, HIV coinfection affects the common clinical presentations, radiology, histology, prognosis, and differential diagnosis of TB.

9.3.1

Chronic Cough and other Common Clinical Presentations of HIV/TB

Chronic cough (usually defined as being present for at least 2–3 weeks) is a cardinal symptom of pulmonary TB. Respiratory symptoms and cough are, however, among the most common manifestations of HIV infection, with a wide spectrum of differential diagnoses including nontuberculous bacterial pneumonias and lower respiratory tract infections (LRTIs), bronchiectasis (often post-tuberculous) and, in Africa, Kaposi's sarcoma [86, 87, 89, 90]. In many parts of the world, HIV-positive people are also more likely to be smokers than are uninfected individuals, thus also putting them at risk of smoking-related diseases [91].

In the setting of HIV, cough also is a major indicator of TB infection. For example, the results from one study investigating the causes of chronic cough in a series of TB suspects recruited from a primary care clinic in Harare, Zimbabwe, showed that although the burden of TB was high in both HIV status groups in this resource-poor country, it was significantly higher in the HIV-infected TB suspects [91] (Figure 9.7). In this particular series, HIV prevalence was high (83%) and differed little between patients with TB (88%) and those with other causes of chronic cough (79%) [91]. Similarly, in a Kenyan series of 5457 TB suspects, HIV prevalence was 61% in patients with cough from other causes, compared to 63% in TB patients [84]. In two other recent smaller series from Malawi and Kenya, HIV prevalence was higher in patients with cough from other causes than it was among those with cough due to TB [92, 93]. It can be concluded from these data that it is critical for anyone who is HIV-positive and presents with chronic cough to be evaluated for TB; and conversely, that all those with chronic cough being evaluated for TB also be offered HIV-testing.

Although there is an increased incidence of classical presentations of extrapulmonary disease in HIV infection, pulmonary TB is also common in HIV-positive patients, although atypical presentations are more frequent. For example, *M. tuberculosis* was isolated from 9% of adults with acute pneumonia in Kenya [94], from 35% of chest clinic attendees with cough for less than three weeks in Malawi [95], and from 13% of HIV-infected patients with chronic diarrhea in

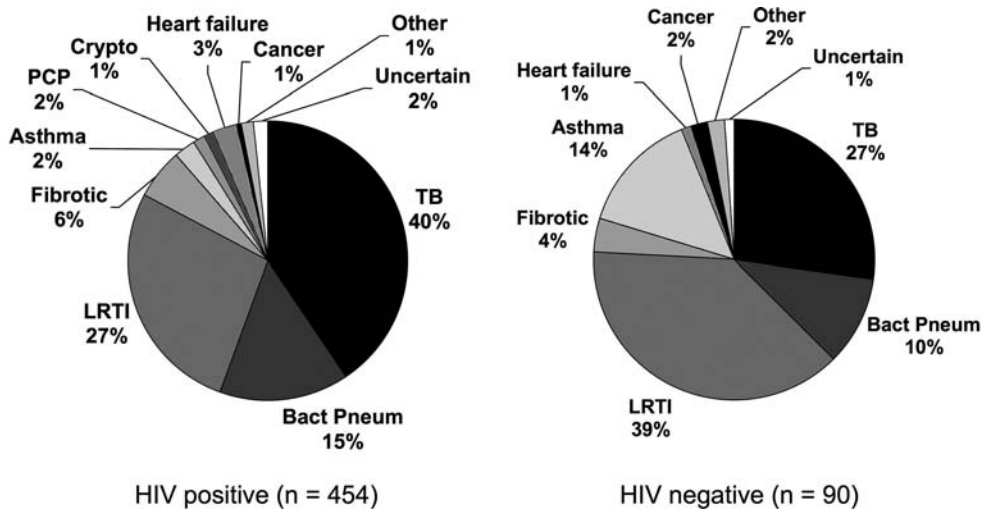


Figure 9.7 Cause of chronic cough in primary clinic attendees, Harare, Zimbabwe [91]. LRTI, lower-respiratory tract infection without radiological changes.

Kenya [96]. In a series from Cambodia, 17% of smear-negative HIV-positive patients with pneumonia with a median CD4 T-cell count of $25 \mu\text{l}^{-1}$ were proven to have TB by fiber optic bronchoscopy [87]. In another series from Asia and Africa, following ART initiation, 253 of 496 patients were diagnosed with TB, with 145 extrapulmonary cases [86]. Thus, TB must be very high in the differential diagnosis of HIV-infected individuals with respiratory symptoms, even when they present with smear-negative pneumonia.

The most common presentations of HIV infection at the hospital level are undifferentiated febrile illnesses (including respiratory illnesses) and diarrhea, each of which are commonly associated with a subacute history and accompanied by rapid weight loss, and meningitis. Notably, *M. tuberculosis* can be the etiological cause of each of these syndromes, and must be considered in the differential diagnosis of these presentations along with the other common pathogenic causes of these syndromes, such as *Streptococcus pneumoniae*, non-typhoidal *Salmonella* species (non-typhoidal enteric fever), and *Cryptococcus neoformans* [5, 81, 97, 98]. For example, case series investigating bloodstream infections in febrile admissions in hospitals in Malawi, Thailand, and Tanzania have consistently reported high rates of mycobacteremia among HIV-infected participants (from 8% to 23%), indicating that disseminated TB is a common and underdiagnosed clinical problem with an extremely high risk of death. A history of recent rapid weight loss has been suggested as an indication of underlying disseminated TB, a risk factor for mycobacteremia, and also for immune reconstitution syndrome associated with TB (TB-IRIS) in the Malawi National Programme [99].

As many cases of TB are unrecognized and are thus not treated, deaths and excessive early mortality during AIDS often occur because of the late diagnosis of

TB [100]. Death from undiagnosed TB disease is therefore likely to make, if anything, an even bigger contribution to TB deaths than death from diagnosed TB [4], with rapidly progressing disseminated TB in the very immunocompromised host undiagnosed. Consistent with this contention, in Cote d'Ivoire, the Democratic Republic of Congo, and Kenya, 38%, 41%, and 47%, respectively, of autopsies in HIV-positive adults indicated TB as the cause of death [1–3], while in Tanzania 23% of febrile HIV-positive patients had tuberculous mycobacteremia, of whom the majority had died by the time of diagnosis [101]. Furthermore, TB and cryptococcal disease emerged as the major cause of early death in five observational ART program cohorts [102]. Thus, there should be a low threshold for starting TB treatment in severely ill patients, as supported by recent guidelines from the WHO [100].

9.3.2

Diagnosis of HIV-Related TB Infection and Disease

Radiological features of HIV-related TB include a higher prevalence of hilar adenopathy, pleural effusions, and a lower prevalence of cavitation than is found in HIV-negative TB patients [103–105]. The high percentage of smear-negative sputum examinations [38], and the high percentage of extrapulmonary TB disease presentations [106] in HIV-associated TB, thus present a major diagnostic challenge.

Among the various options for rapid TB diagnosis, only sputum microscopy provides a diagnostic test with a sufficiently high specificity to allow treatment to be started without further investigation or delay; moreover, it is also currently affordable and accessible in resource-poor settings. The sensitivity, however, is on the order of 30% in routine programs, although this can be increased through concentration and the use of fluorescence microscopy [107, 108]. Even this test is difficult to maintain, requiring experienced, trained personnel and a supply chain of reagents and specialized equipment.

TB culture is considerably more sensitive, albeit slow (2 weeks to 2 months), and is currently accessible by only approximately 3% of the world's population. A number of promising new diagnostics are currently being evaluated. None has the potential to provide the highly sensitive and specific point-of-care test that could revolutionize TB control, but some have the potential to replace smear microscopy with a rapid, sensitive test based on nucleic acid amplification that could be run in peripheral laboratories with minimal training and supervision [13].

9.3.3

Excluding TB in the Context of HIV Care

A related problem to the diagnosis of active TB is how to effectively exclude TB in HIV-infected individuals, particularly in order to allow patients to start INH preventive therapy as part of their HIV care. This is of major importance as undiagnosed TB is present in a high percentage of HIV-infected Africans and Asians attending HIV-test services and entering HIV care programs, with typically between 5% and 10% of HIV-infected persons found to have active TB if screened when first seeking

knowledge of their HIV status [109]. Furthermore, subclinical TB (with no reported symptoms) is a consistently reported feature of all series where culture or chest radiography are used systematically to screen all (not just symptomatic) participants [53, 57, 110–112].

TST responses to PPD progressively decrease with the decline in CD4, making the test of little use for detecting LTBI in HIV patients with immune compromise. Several recent studies have emerged from Senegal, South Africa, and Zambia [113–117] that specifically address the effect of HIV infection on the use of the ESAT-6/CFP-10 (EC) ELISPOT test, which was designed to differentiate immune response to antigens specific to *M. tuberculosis* (ESAT-6 and CFP-10) versus Bacille Calmette-Guérin (BCG) and atypical mycobacteria. One major benefit of both the EC ELISPOT test and the EC interferon- γ (IFN- γ) whole-blood assay as diagnostic procedures is their superiority over PPD testing for latent or active TB. The whole-blood assay is the most sensitive of the three assays. It is a seven-day procedure that targets central memory T cells, which can proliferate in response to ESAT-6/CFP-10, unlike the end-stage effector memory CD4 T cells that the ELISPOT (which is performed overnight) stimulates and measures. A drawback to the whole-blood IFN- γ assay is that, like PPD testing, it also loses sensitivity as the CD4 counts decline. The ELISPOT, on the other hand, shows a small but insignificant decline in sensitivity as the CD4 counts decline. Thus, the ELISPOT appears to be the best test for use in advanced HIV patients.

Interestingly, HIV-positive patients who have undergone treatment for TB are much more likely to show no response in an ELISPOT test than are HIV-positive patients who have not undergone TB treatment. Treated patients remain sensitive to the whole-blood assay, however. It has been suggested that this phenomenon is due to the cells targeted by each assay – the central memory T cells have time to proliferate in response to CFP10/ESAT6 in the whole-blood assay, while there are few (if any) effector memory cells left in TB-treated HIV-positive patients because these patients have no circulating TB antigen to continually induce effector memory CD4 cell generation [114]. Thus, these tests are useful in the diagnosis of both latent and subclinical TB in HIV coinfection, although given their dependence on laboratory equipment, their application in their current form in resource-poor settings remains limited.

9.3.4

Treatment of Latent TB, and Preventive Therapy

Isoniazid preventive therapy, when continued for six to nine months, reduces the incidence of TB by about 60% in HIV-infected persons with a positive TST, and by about 40% when used irrespective of skin test results [28, 118]. Although recommended as an intervention to reduce the risk of TB in people living with HIV since 1999, this intervention has not been routinely implemented because of concerns about the promotion of drug resistance if INH monotherapy is inadvertently started in someone with active and unrecognized TB. In practice, however, the clinical and bacteriological consequences of the latter appear to be minimal [119]. The widespread national implementation of INH preventive therapy has only been attempted in

Botswana, although many other countries are aiming to scale-up services in the near future. Unresolved issues include determining ways of effectively promoting adherence to preventative therapy, and defining the optimal duration of preventive therapy, as protection wanes with time – at least in HIV-infected individuals not receiving ART [28, 118]. In this context, it is important also to consider the fact that the lack of a positive TST, even in HIV-negative documented pulmonary TB, is influenced by ethnicity [120], and thus lack of a positive TST is a poor indicator of latent infection even in HIV-negative individuals. As discussed above, the ELISPOT assay is more sensitive than TST, and thus could be useful in ruling out latent TB in HIV-infected individuals. Thus, the criteria that should be used to determine who to treat for latent TB in the context of HIV infection remains an unresolved issue.

It has also been suggested that INH preventive therapy may reduce the risk of recurrent TB when it is used as secondary prevention after a first episode of TB in the HIV-infected patient, and when given in combination with ART. However, data are still lacking regarding the status of immune reconstitution and other variables, including efficacy data of post-treatment prophylaxis in general. Alternative regimens based on rifamycins have been suggested to be more effective and to shorten the treatment of latent TB, but carry the problem of a higher risk of side effects and a greater propensity for drug–drug interactions [118].

9.4

Treatment of HIV-Infected TB Patients

The concurrent treatment of both TB and HIV is complicated by drug–drug interactions that can include overlapping and additive toxic side effects (Table 9.3). Additional problems may be caused by the need to adhere to two courses of prolonged multidrug therapy, especially if these are not provided through the same clinic or integrated health system. Paradoxical reactions (PRs) [121] associated with TB, or immune reconstitution syndrome [121], are common during ART, and occasionally can be life-threatening when affecting vital organs, although PRs can usually be well-managed by short-course steroids or nonsteroidal anti-inflammatory drugs. Another major issue is the “unmasking” of previously undiagnosed TB with the start of ART, and the reconstitution of the immune response, which can predispose the patients to PRs.

Most HIV-infected TB patients are cured by standard TB regimens, however. The outcomes can be further improved by starting cotrimoxazole prophylaxis immediately on diagnosis, and by the prompt introduction of ART in patients with a CD4 count less than $200 \mu\text{l}^{-1}$ [122, 123]. Even though MDR-TB/HIV coinfection, if untreated, carries a very dire prognosis, good treatment outcomes of MDR-TB can also be achieved in HIV-infected patients [124, 125], provided that there is rapid diagnosis of drug resistance. The timing of ART in MDR-TB coinfection, however, remains an unresolved question. Unfortunately, the issue of MDR-TB cotherapy for MDR-TB/HIV coinfection remains a moot point in many countries, as MDR-TB care is not currently widely available in most resource-poor settings, thus placing patients at a high risk of death from progressive MDR-TB/HIV coinfection. Furthermore, the

Table 9.3 Some specific drug-adverse effects reported from combined HIV/TB treatment, and the probable drug causing the effect.

Adverse effect	Most likely combined cause of specified adverse reactions	
	Used in HIV care	TB drug
Peripheral neuropathy	Stavudine	Isoniazid
Hepatitis	Nevirapine	Rifampin, isoniazid, pyrazinamide
Skin rash	Nevirapine, cotrimoxazole	(Uncommon) Rifampin, isoniazid, pyrazinamide
Central nervous system dysfunction (e.g., mood, personality, sleep disorder, psychosis)	Efavirenz	Isoniazid, cycloserine (a second-line drug)
Anemia	Zidovudine	Rifampin
Reproductive effect	Efavirenz (fetal toxicity)	Rifampin (increases likelihood of suboptimal contraceptive doses)
Paradoxical reaction/immune reconstitution syndrome	Early initiation of ART in patient with low CD4 count	Initiation of TB therapy prior to ART

negative consequences of a delayed diagnosis of drug resistance have been apparent during institutional outbreaks of drug-resistant TB strains, which are usually associated with extremely high mortality rates [12, 126].

9.4.1

Reducing Mortality in HIV-Infected TB Patients

Case fatality rates in TB/HIV coinfection can be dramatically reduced when a positive HIV status is known, and by the concurrent administration of cotrimoxazole prophylaxis and ART; this is particularly critical if the patient is immunocompromised. However, there remains a considerable excess mortality in patients, especially in resource-poor settings, where there is a high mortality rate due to TB, particularly within the first few weeks of starting TB therapy [102, 126]. Thus, evidence based research to optimize care for TB/HIV coinfection is urgently needed.

9.4.2

Antituberculosis Regimens

Both U.S. [123] and European [122] recommendations are consistent in the antituberculosis regimens used in HIV-infected adults, and follow the same principles that are in place for adults without HIV infection. This includes four drugs in the intensive phase of therapy during the first two months of therapy with INH, rifampin

or ribabutin, pyrazinamide, and ethambutol, followed by INH and rifampin for an additional four months (“standardized treatment”).

The U.S. and European guidelines also both recommend individualizing the TB treatment for HIV-coinfected patients by prolonging the continuation phase of therapy for patients with cavitary lung disease and certain types of extrapulmonary TB, including central nervous system (CNS) disease, and bone or joint TB. The exact duration is then left to the discretion of the treating clinician [123], although recommendations include extending rifampin/INH treatment in cavitary disease to three additional months for a total of nine months; for extrapulmonary TB a six- to nine-month regimen is recommended, with four to seven additional months of INH/rifampin after the intensive four-drug phase. For CNS TB (tuberculoma or meningitis) or bone and joint TB, it is recommended that there be a total of nine to 12 months of therapy [123].

In patients with MDR-TB, case-fatality rates are much higher in HIV-positive patients than in HIV-negative patients, especially if drug resistance is not diagnosed at an early stage [127]. In resource-poor settings, relatively little is known about the treatment outcomes of HIV-related MDR-TB, other than in the context of institutional outbreaks, where survival prospects are poor, particularly with XDR-TB [10, 12]. However, it appears that unusually severe immunosuppression contributed to the very high mortality of institutionally acquired MDR/XDR-TB, and that the prognosis is better for HIV-related MDR-TB presenting in any other context [10].

Despite the magnitude of the TB/HIV problem, major unresolved issues in cotherapy persist, including the question of the timing of when to start ART, the choice of ART regimens (especially in women of child-bearing age, as efavirenz is contraindicated in pregnancy), the potential role of ribabutin as a substitute for rifampin, and the role of fluoroquinolones. Furthermore, the optimal treatment duration of drug-resistant forms of TB, such as MDR/XDR-TB, in HIV-coinfection are currently unknown. The need to conduct clinical trials that will address these questions and provide rigorous answers cannot be overstated.

9.4.3

Choice of Antiretrovirals in the Context of Treating TB

The diagnosis and treatment of clinical TB in patients with HIV requiring antiretroviral (ARV) drugs will in turn influence the choice of ARV agent. Several guidelines have recommended that the first line of ARV treatment in an ARV-naive patient should include efavirenz (EFV) and two nucleoside reverse transcriptase inhibitors (NRTIs), since EFV metabolism is less affected by rifampin than other options. However, in low-income countries, the poor access to EFV, its teratogenicity, and the absence of a fixed-dose combination (FDC) formulation containing EFV, leads to management difficulties, especially for women of child-bearing age [15]. The results of a study from Thailand indicated that, in HIV/TB coinfecting patients administered rifampin, the efficacies of a standard dose (600 mg) EFV-based ART and a standard dose (400 mg) nevirapine (NVP)-based ART may be similar, although adverse events

tended to occur more frequently with the NVP-based ART [128], further supporting a role for EFV.

Given that rifampin results in a substantial reduction in NVP levels in patients taking both drugs [128], and is incompatible with most protease inhibitors (PIs), there are relatively few combined TB/ART regimens. Thus, for example, in Malawi the decision was made to routinely introduce NVP-based regimens into the National Programme, with patients starting at two months after TB therapy initiation (mostly without CD4 cell counts) [99].

An alternative rifamycin, *rifabutin*, should become available in generic form during the coming years, and provide an affordable alternative TB regimen that is compatible with commonly used first- and second-line ART regimens. Another new drug, *raltegravir* [129], an integrase inhibitor, represents an alternative to the use of a PI in TB/AIDS cotherapy. Because of its low interactions with rifampicin, raltegravir represents another future option in TB/HIV cotherapy.

9.4.4

When to Start ART?

Despite the clinical impact of coinfection, and the extraordinary mortality of TB/HIV, the clinical trial data to guide the timing of cotherapy are still not available. Although other guidelines exist, those of the WHO (see Table 9.4) are not only representative but also probably the most widely followed [130].

As discussed above, early mortality during the first two months of TB treatment is extremely high, and is inversely related to the CD4 cell counts. Active TB at HIV diagnosis is a risk factor for death, both before starting ART and during the first months after treatment is initiated [102, 126]. A treatment cohort of just under 1000 patients in Malawi reported high case-fatality rates (20%) that were little changed from historic rates when ART was routinely introduced at two months, with 60% of deaths occurring before the start of ART [131]. In an ART program in Cape Town, South Africa, 6.2% of patients enrolling for treatment died within the first three months, with 66% of deaths occurring before ART was started [132]. A significant survival benefit of starting ART earlier has, however, been reported from a small study in Thailand, where the initiation of HAART at two to four weeks after starting TB therapy led to a significant reduction in mortality, especially in patients with a CD4 cell count $<100 \mu\text{l}^{-1}$ [133].

Table 9.4 Current WHO guidelines on when to start ART in patients presenting with HIV-related TB.

CD4 cell count (μl^{-1})	ART recommendations	When to start ART
<200	Recommend ART	2 to 8 weeks into TB treatment
200–350	Recommend ART	After 8 weeks
>350	Defer	Re-evaluate at 8 weeks and at end of TB treatment
Not available	Recommend ART	2 to 8 weeks into TB treatment

The critiques of the early start of ART included concerns about adverse events, including drug interactions and immune reconstitution inflammatory syndrome (IRIS), when ART is started two to four weeks into the TB treatment [15, 126, 134]. For example, one study in South Africa reported a 36% risk of IRIS in patients starting ART treatment within two months [134] although, as IRIS is rarely fatal, current opinion is moving towards an earlier initiation. This decision will soon be supported by clinical trial data, which are anticipated and summarized in Table 9.5 from the report of Blanc *et al.* [14].

The CAMELIA (Cambodian Early versus Late Introduction of Antiretrovirals) study (see Table 9.5), for example, addresses the question of whether early (2 weeks after TB treatment is initiated) versus late (8 weeks after initiation) will result in a decreased mortality measured at one year after the last patient's entry into the trial (the trial scheme is shown in Figure 9.8). All patients must have a positive AFB culture sample, a CD4 cell count $<200 \mu\text{l}^{-1}$, and all must receive a standard six-month TB treatment regimen [unless MDR-TB is found on drug sensitivity testing (DST)] and receive standard ART according to Cambodian national guidelines, with stavudine + lamiduvine + efavirenz. All 660 patients have been recruited as of May 2009 and it is anticipated that the results will be available in mid-2010. In addition to the trial's major goal of determining the impact on mortality of early versus late ART, the trial has enhanced integrated TB/HIV care to its five clinical sites in collaboration with the Cambodian Health Committee and with local and international partners. Furthermore, a variety of other clinical and basic scientific questions, including the definition of basic immunological mechanisms of the paradoxical reaction and genetic correlates with disease outcome, will be addressed through the nesting of basic scientific discovery within the clinical trial.

The START study in South Africa (see Table 9.5) was also designed to address when to start ARVs at three different time points in standardized TB treatment (2 weeks, 2 months, and 6 months) in smear-positive patients presenting with CD4 cell counts of less than $500 \mu\text{l}^{-1}$. The follow-up in the arm that initiated ART at 6 months after TB treatment was completed was halted by the trial's Data Safety Monitoring Board in 2008 because of a substantial mortality reduction (55%) from an earlier ART commencement during TB therapy, with a significant benefit even in patients with CD4 cell counts of $200\text{--}500 \mu\text{l}^{-1}$, as well as in the more immunosuppressed arm. The follow-up for the 2-week versus 2-month arms is continuing, with results expected in 2010. Inclusion criteria included acid-fast bacillus (AFB) + sputum and a CD4 cell count $<500 \mu\text{l}^{-1}$. All patients receive standard antituberculosis therapy and cotrimoxazole prophylaxis and, when ART is begun, daily didanosine, lamivudine, and efavirenz [14].

9.4.5

Immune Reconstitution in TB/HIV Coinfection

Although the subject of IRIS is detailed elsewhere in this book (see Chapter 3), a brief discussion of this issue will also be provided here as the incidence of IRIS in TB/HIV treatment is approximately 20–30%, and it is a major consideration for TB/HIV

Table 9.5 Summary of strategy clinical trials in TB-HIV coinfectd patients. Adapted from Ref. [14].

Trial	Clinical Trials. gov Identifier	Sponsors	Countries	No. of patients	Culture-confirmed TB	CD4 cell count at entry (μl^{-1})	TB regimen	HAART regimen	Arms	Duration	Primary outcome (end of the trial)	First inclusion
CAMELIA (ANRS 1295, CIPRA KH 001): Early vs. late introduction of ART in naive HIV-infected patients with TB in Cambodia	NCT 00226434	ANRS (France) + NIAID (USA)	Cambodia	660	Yes	<200	Standard RHEZ2-RH4	D4T-3TC (generic) + EFV	Early: HAART 2 weeks after initiating TB treatment Late: HAART 8 weeks after initiating TB treatment	12 months	Survival	January 2006
AACTG A5221: A strategy study of immediate versus deferred initiation of ART for HIV-infected persons treated for TB with CD4 count <200 μl^{-1}	NCT 00108862	NIAID (USA)	Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, Zimbabwe	800	Not mandatory (AFB-positive smear or probable TB on clinical judgment)	<200	Rifampin or other rifamycin-based TB regimen according to WHO and national treatment guidelines	TDF-FTC (Truvada [®]) + EFV	Early: HAART within 2 weeks after initiating TB treatment Late: HAART 8 to 12 weeks after initiating TB treatment	12 months	Survival without AIDS progression	Not yet recruiting
START: Implementing ART in resource-constrained settings: a randomized controlled trial to assess the effect of integrated TB and HIV care on the incidents of AIDS-defining conditions or mortality in subjects coinfectd with TB and HIV	NCT 00091936	NIAID (USA)	South Africa	592	Not mandatory (AFB-positive smear)	>50	Standard RHEZ2-RH4	ddI-3TC+EFV	Integrated HAART concurrent with standard TB therapy through DOT Sequential: after completion of TB treatment, HAART without DOT	18 months	Diagnosis of an AIDS-defining illness; 18 month mortality	Not yet recruiting

TB-HAART	Trial registration number not yet provided	WHO	South Africa, Tanzania, Uganda, Zambia	1900	Yes	>200	Standard RHEZ2-RH4	ZDV-3TC-EFV or placebo	HAART initiated 2 weeks after commencement of TB treatment concomitant with TB treatment until 6 months, then continuation on ARV alone	HAART placebo initiated 2 weeks after commencement of TB treatment concomitant with TB treatment until 6 months, then HAART	24 months	Composite endpoint of TB treatment of failure or death at 6 months after initiation of TB treatment.	Planned for June 2006
PART(NIH 1 R01AI051219-01A2): punctuated ART I HIV-associated TB	NCT 00078247	NIAID (USA) + Makerere University (Uganda)	Uganda	350	Not mandatory (AFB-positive smear)	≥350	Standard RHEZ2-RH4	ZDV-3TC-ABV (Trizivir)	Initial HAART	Delay HAART until CD4 cell counts fall below 200 μl^{-1}	24 months	-CD4+ decline (slope) - time to (AIDS)	October 2004
BKVIR (ANRS 129): Efficacy of a once-daily HAART regimen associating tenofovir-emtricitabine and efavirenz in HIV-1-infected patients with active TB: a pilot study	NCT 00115609	ANRS (France)	France	100	Yes	No entry criteria	Standard RHEZ2-RH4	TDF-FTC (Truvada) + EFV	NA		12 months	Treatment success rate - plasma HIV-1 RNA <50 copies ml^{-1} - TB cured	January 2006

ANRS: Agence Nationale de Recherches sur le Sida et les Hépatites Virales; CIPRA: Comprehensive International Program of Research on AIDS; NIH: National Institutes of Health; NIAID: National Institute of Allergy and Infectious Diseases; WHO: World Health Organization.

3TC: lamivudine; ABV: abacavir; AFB: acid-fast bacilli; ART: antiretroviral therapy; D4T: stavudine; ddI: didanosine; DOT: directly observed therapy; E: ethambutol; EFV: efavirenz; FTC: emtricitabine; H: isoniazid; HAART: highly active antiretroviral therapy; R: rifampin; TB: tuberculosis; TDF: tenofovir; Z: pyrazinamide; ZDV: zidovudine.

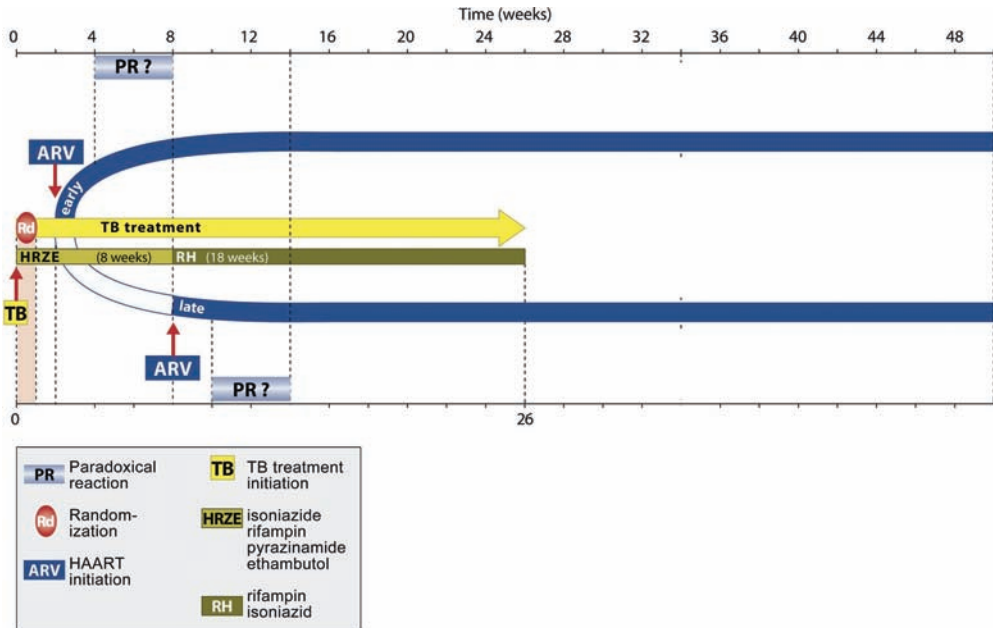


Figure 9.8 The design of the CAMELIA (Cambodian Early versus Late Introduction of Antiretrovirals): Determining the optimal time of starting ARVs in immune-suppressed AIDS patients with TB. A total of 660 patients randomized into early ART (or HAART) (at 2

weeks) versus late ART (at 2 months) after initiation of TB therapy. ART is according to Cambodian national guidelines: stavudine, lamiduvine, and efavirenz. A major outcome is mortality at 50 weeks after initiation of TB therapy.

cotherapy. This syndrome, which is due to a reconstitution of the immune response during ARV treatment during TB therapy, typically manifests as a transient worsening or appearance of symptoms or radiographic manifestations of TB, especially of increased mediastinal lymphadenopathy [135, 136]. This usually occurs within two months after the initiation of HAART, and after the patient has shown an initial improvement in TB disease [137, 138]. Clinically, PRs can also occur in immunosuppressed AIDS patients being treated for TB in the absence of HAART, although much less frequently. For example, in one study, non-HAART-related PRs occurred in 7% of 28 TB/HIV coinfecting patients who were not receiving HAART, compared to 36% of 33 TB/HIV coinfecting patients who were [139]. The mechanism of PR remains undefined, but it appears to be an immune-mediated phenomenon as it occurs in the setting of immune reconstitution and decreasing TB burden and HIV viral load, and appears to involve T cells [8]. This is supported by the return of delayed-type hypersensitivity (DTH) to PPD (or TST) in PR patients who had been anergic (no DTH) to PPD before HAART, despite persistently low CD4 cell counts ($<100 \mu\text{l}^{-1}$) at the time of the PR [139].

In general, the treatment of PRs with corticosteroids or nonsteroidal anti-inflammatories is effective, and the interruption of ARV treatment is not necessary [135, 140]. A poor clinical outcome of PRs is associated with severe immune compromise

(CD4 count $<50 \mu\text{l}^{-1}$) and a high viral load prior to therapy [138]. In an intriguing case report, infliximab was used successfully to control a steroid-resistant TB PR involving the CNS in an HIV-negative patient [141]. This clinical observation was consistent with basic laboratory data indicating that PRs are associated with an “explosion” of Th1 cytokines [142], and also consistent with the hypothesis that PRs are due to an imbalance between effector cytokines and immune suppressive cytokines. Ongoing basic science studies, such as those nested in the CAMELIA trial, are investigating immunological and genetic markers and the role of natural killer (NK) and T-cell responses in the predisposition to, and in the evolution of, PRs. Studies to determine diagnostic and predictive markers are urgently required, and should elucidate the basic mechanisms of the immune response to TB/HIV coinfection in general [8].

9.5

Critical Issues in the Delivery of Coordinated TB and HIV Prevention and Care

The meaningful integration of HIV and TB services has proved difficult to achieve in many countries during the initial phase of scaling-up HIV care services, largely because of the vertical nature of TB and AIDS programs historically, to the lack of acknowledgement of the overlapping nature of the two epidemics, and to a lack of leadership on the issue.

Key lessons from experience gained so far are that the high uptake of HIV testing among TB patients can only be achieved through routine provider-initiated testing and counseling. Ideally, this should occur at the same facility or via the same program through which the patient is accessing TB care. Similarly, success rates for starting ART are much better if such therapy can be initiated by an integrated service. Notably, a low uptake of HIV testing and ART (approximately 20% or less) has been reported from countries requiring patients to access TB and ART from separate facilities or clinics [143]. The main constraints appear to be the eight-week delay between starting TB treatment and becoming eligible for nevirapine-containing ART, as well as the logistical difficulties in accessing ART services from an independent health structure [143]. This illustrates a major limitation of nonintegrated TB and ART programs that are linked only by cross-referrals, and highlights the need for ART and TB management to be integrated. Integrated programs not only have major advantages for the patients but are also cost-saving through increased efficiency [144], *de novo* case finding, and by using trained TB personnel who are well versed in the issues of adherence and medication side effects [149, 150]. When well integrated, TB services can identify a substantial group of individuals in need of HIV care [145, 146].

9.5.1

An Example of Linking TB and HIV/AIDS Care at the Community Level

One example of integrated TB/AIDS care in a rural setting is provided by The Cambodian Health Committee's TB/AIDS Program in rural Svay Rieng and Kompot

provinces, Cambodia [147]. This HIV program was built upon an extensive provincial community TB program, including a home care component (Home DOTS) where mobile teams deliver TB medications, together with a patient supporter to supervise and assist patients to complete their therapy, and also a village health worker network [148]. In January 2004, 36 patients who were identified in the context of the Cambodian Health Committee's TB outreach program to be HIV-positive and to have a CD4 cell count $<200 \mu\text{l}^{-1}$ were begun on stavudine, lamiduvine, and nevirapine, using the Home DOTS approach of delivering medicines to them at their homes with supervision by the patient supporter. Of these first 36 patients, 85% had a CD4 count $<50 \mu\text{l}^{-1}$ at the time of initiation of ART. In July 2004, in conjunction with the national AIDS program, an ART program was initiated in the TB ward of the Svay Rieng Provincial Hospital. New patients were recruited and care of the first 36 patients initiated at home was integrated into the clinic. Of the original 36 patients, 33 were alive and stable on ART at four years after ART initiation (T. Sok, D. Laureillard and A.E. Goldfeld, unpublished results).

Program elements at the Svay Rieng ART clinic were adapted from the Cambodian Health Committee's community-based TB treatment program [148]. HIV-positive patients received counseling, education, and a CD4+ cell count. Cotrimoxizole was offered, with ART being offered to patients with a CD4+ cell count $<200 \mu\text{l}^{-1}$ after additional education, and after the patient and supporters had signed a treatment contract. These strategies, along with home visit follow-up, were previously demonstrated to enhance adherence with TB drugs and have been found to be extremely effective in AIDS care [148]. Furthermore, the Svay Rieng Clinic is one of the clinical sites of the CAMELIA study, which has further enhanced care. Another rural ART clinic was established in a neighboring province, Kompot, in May 2005, also in conjunction with the Cambodian Health Committee's TB program.

An interim evaluation of both program sites in September 2007 based on clinical follow-up and laboratory tests, including a CD4+ cell count, revealed that 1246 patients were receiving ART, with 947 in active follow-up and 140 with a documented transfer to another ART clinic elsewhere in the country. There were only 18 defaulters and 140 deaths. The mean increase in CD4 count was $131 \mu\text{l}^{-1}$ after six months, with continuing steady increases as shown in Figure 9.9. These results demonstrate, therefore, that providing HAART in rural resource-poor settings by leveraging successful TB treatment strategies is extremely effective [147] (also T. Sok, D. Laureillard and A.E. Goldfeld, unpublished results).

Integration of the TB and AIDS programs also facilitated active TB case finding in HIV-infected patients. For example, between 2004 and 2008, a total of 343 of 1404 HIV-infected patients in Svay Rieng, and 222 of 1206 HIV-infected patients in Kompot, were also diagnosed and treated for TB (T. Sok, T. Chanthé and A.E. Goldfeld, unpublished results). This should be compared to the national statistic of 10% of HIV-infected patients attending ART clinics being codiagnosed with TB (Cambodian National TB Program 2007 statistics, personal communication). The collateral benefits of using a TB infrastructure, and staff trained not only in the general issues of drug interactions, adherence and long-term therapies but also in the specifics of diagnosis and management of TB, were keys to the success of the program and to the enhanced

	Svay Rieng Jan 04.–Sept. 07	Kompot May05–Sept. 07	Total
Total patients (adults)	1212	1036	2248
Total on ARV	673	573	1246
ARV alive	490	457	947
transferred	78	62	140
died	92	49	141
default	13	5	18
female (%)	50	51	
CD4 <50 at start (%)	47	36	

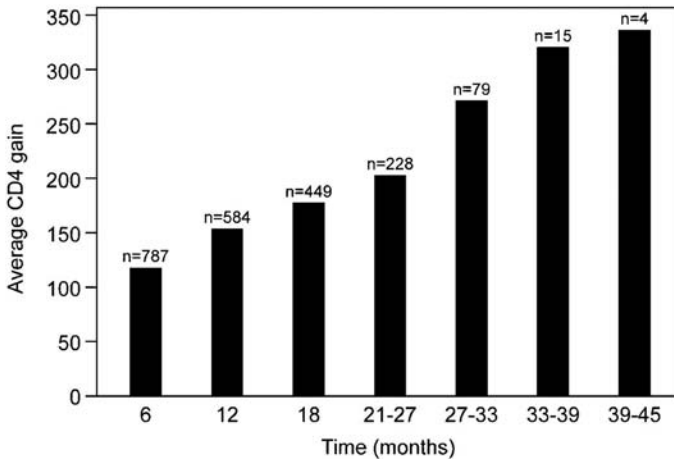


Figure 9.9 Linking TB and HIV/AIDS care using a community-based grass roots approach in Cambodia. A rural ART program was established by a local Cambodian NGO, the Cambodian Health Committee [151] beginning in January 2004 within its TB Home DOTS program network in Svay Rieng, Cambodia [148]. In July

2004, in collaboration with the National AIDS Program, an ART clinic was established in the TB ward of the provincial hospital and in May 2005 another provincial clinic was established in the CHC TB network in another rural province, in Kompong Trach, Kompot. The average gain in CD4 cell count is shown.

TB case detection. Results from an urban cohort that offered integrated TB/AIDS care by an international NGO, Médecins Sans Frontières-France, in Cambodia shows equally outstanding outcomes [149].

9.6 Conclusions

HIV has fundamentally changed TB and its global epidemiology, and the proportion of TB cases that are due to HIV-related disease will most likely continue grow as their epidemiologic association increases in strength at both country and regional levels. Of greater alarm is the number of untreated MDR-TB cases that occur globally, many in countries with a high HIV prevalence. The epidemic of XDR-TB/HIV in South Africa

has clearly demonstrated the consequences of the intersection of HIV and TB infections. The lack of access to second-line drugs to treat MDR-TB in general, and of the technical support to build MDR TB programs, are urgent problems that require immediate solutions. Clearly, new diagnostics and more effective first- and second-line TB drugs with fewer side effects are also urgently required. A knowledge of the optimal use of those drugs already in hand to treat coinfection is still lacking. Research into biomarkers and the mechanisms of host and pathogen factors involved in determining HIV/TB disease outcome is also urgently needed so as to maximize the cure of TB and treatment of HIV. More effective ways of bringing early diagnosis to the community level are also needed if these epidemics are to be brought under control. The current focus on early diagnosis and treatment of HIV as a potentially highly effective prevention tool [150] could open new options for combined HIV/TB prevention in the coming years, although access to ART and MDR drugs remains an impediment in this respect. Basic research and discovery represents one way of maximizing care and developing infrastructure and human capacity; the corollary of this is that delivery of care creates networks that facilitate basic research. Support for both approaches in the arena of TB and HIV is a global priority.

TB in HIV-infected persons is both preventable and curable in most instances with existing drugs and approaches. Timely and appropriate TB therapy, combined with ART and HIV care, can be accomplished in even the most resource-constrained settings. With the HIV/TB epidemic targeting those living in the poorest sectors of the world, access to integrated TB/HIV care, including ART and second-line MDR drugs, represents a humanitarian challenge. It is imperative that such a challenge is met.

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10

Extensively Drug-Resistant Tuberculosis and HIV/AIDS

Megan Murray and Ted Cohen

10.1

Introduction

In 2006, clinicians serving a rural Zulu community in the eastern KwaZulu-Natal province of South Africa reported the emergence of a strain of tuberculosis (TB) that was resistant to almost all of the drugs known to be effective in treating this disease. Of the first 53 patients reported at the Church of Scotland Hospital in Tugela Ferry with this highly drug-resistant form of TB, 52 died with a median survival of only 16 days after diagnosis [1]. All those who had been tested were also found to be coinfecting with HIV. In the months that followed, researchers at this hospital identified another 176 cases of extensively drug-resistant or “XDR” TB – that is, TB that was resistant not only to the two most potent first-line drugs, isoniazid (INH) and rifampin, but also to at least one fluoroquinolone and one of the injectable second-line drugs [2]. Although the Tugela Ferry cases catapulted XDR TB to global attention, this was not the first report on the emergence of XDR TB. The US Centers for Disease Control and Prevention and the World Health Organization (WHO) had surveyed *Mycobacterium tuberculosis* isolates that were identified at 27 supranational TB reference laboratories during the period 2000–2004; among the 17 690 strains collected from 48 different countries, 3520 (~20%) were multidrug-resistant (MDR), resistant to at least INH and rifampin, and 397 (~10%) of those that were MDR also met the case definition for XDR TB [3].

Although this set of isolates did not constitute a systematic sample of new cases of TB, the proportion of XDR among MDR cases found in this sample suggests a potential global incidence of over 30 000 cases of XDR TB in 2004 – around 0.4% of the total estimated TB burden worldwide [4]. Nor was the occurrence of drug-resistant TB among HIV patients a new phenomenon. Indeed, the first reports of the Tugela Ferry outbreak recalled the earlier experience of MDR TB among HIV-positive patients in New York during the late 1980s and early 1990s [5–10]. An excellent review of TB in New York City described the dramatic rise in the incidence of TB between 1978 and 1992 – a rise which ultimately led to an estimated 52 000 excess cases [11]. As TB rates increased, the proportion of cases due to drug-resistant strains

also rose – from 10% in 1983 to 23% in 1991. Much of this increase occurred in the context of the “social disarray” that afflicted New York City during the 1970s and 1980s. The authors cited increases in poverty, homelessness, substance abuse and especially HIV, as the central contributors to high rates of treatment failure that allowed resistance to emerge and the subsequent spread of drug-resistant TB. Failure to diagnose and effectively treat MDR TB among the HIV-infected persons in this setting led to high mortality rates and created a pool of untreated institutionalized patients who went on to spread disease to other vulnerable hosts.

These much-publicized reports of highly lethal drug-resistant TB among HIV patients suggest that HIV may be linked to MDR or XDR TB, either directly through a causal pathway, or by modifying the course of drug-resistant TB in coinfecting patients. In this chapter, we consider the evidence for a casual link between HIV and MDR/XDR TB, the possible mechanisms through which HIV could lead to the emergence of resistance, and the potential impact of HIV on the diagnosis and treatment outcomes of MDR/XDR TB.

10.2 The Burden of XDR TB and HIV

To date, there have been no systematic attempts to assess the population burden of XDR TB, or to determine either the prevalence of HIV among XDR TB patients or the prevalence of XDR TB among those infected with HIV [12]. Figure 10.1 displays the

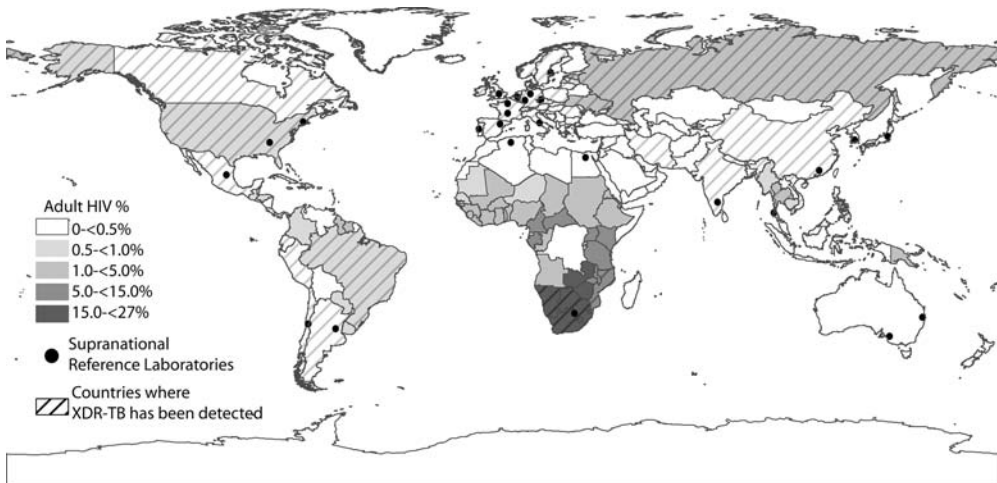


Figure 10.1 Global distribution of XDR TB and HIV. The map shows the estimated proportion of adults in each country that are infected with HIV (in 2007), the countries in which XDR TB had been detected (by June 2008), and the locations of the 26 supranational TB reference laboratories. Data obtained from Refs [12, 13].

estimated country-specific proportion of adults with HIV, indicates those countries in which XDR TB has been detected, and maps the location of supranational TB reference laboratories that can reliably test for the resistance to second-line antibiotics. In sub-Saharan Africa, where the prevalence of HIV is the highest, there have been relatively few countries where XDR TB has been detected. While it is plausible that highly drug-resistant forms of disease are less prevalent in these settings, this may also reflect the paucity of data available rather than the absence of XDR. In a recent ecological analysis of MDR TB rates for 39 out of 46 African countries, investigators found no correlation between the reported prevalence of MDR TB and HIV incidence or HIV/TB coinfection [13]. The authors of this study emphasized the urgent need for more TB drug resistance surveys to accurately estimate the burden of drug-resistant TB, and to elucidate the factors that contribute to the emergence of drug-resistant TB in this setting.

10.3

Evidence of a Causal Association Between HIV and Drug-Resistant TB

Several epidemiologic studies have demonstrated a higher proportion of HIV coinfection among patients with drug-resistant TB compared to patients with drug-sensitive TB [14–18], while others have found no correlation between individuals' HIV status and TB drug resistance [19–26]. Among studies which contributed data to the recent fourth WHO report on Anti-tuberculosis Drug Resistance in the World [27], only two locations – Latvia and the Donetsk Oblast in the Ukraine – had sufficient power to examine the association between TB drug resistance and HIV status. Both studies found that an individual's HIV status was associated with multidrug resistance and resistance to any drug. In the Donetsk Oblast of the Ukraine, the analysts found that this association persisted even after adjusting for confounding by previous TB treatment and a history of imprisonment. In Latvia, however, patients with unknown HIV status were not distinguished from those who were known to be HIV-negative; thus, the strength of the association between HIV infection and MDR TB was difficult to determine. To date, only one study has examined the association between HIV and XDR tuberculosis. Among 132 patients diagnosed with MDR TB at a Portuguese hospital, 69 (52.3%) also met the criteria for XDR TB. Both HIV (odds ratio (OR) 2.5; confidence interval (CI) 1.24,5.05) and prolonged previous TB treatment (OR 1.2; CI 1.11,2.3) were associated with a diagnosis of XDR among MDR patients in univariate analysis; no multivariate analysis was reported [28].

10.4

Mechanisms by which HIV may Lead to Drug Resistance in TB

Resistance to antituberculosis drugs can occur in one of two ways: (i) through inadequate therapy; or (ii) through an initial infection with a drug-resistant strain of

M. tuberculosis. Patients with active TB can receive inadequate treatment either because their treatment is interrupted or because the effective doses of the antituberculosis drugs are too low to eliminate the bacteria. In that case, rare drug-resistant mutants will survive under the selection pressure of antituberculosis drugs, and initially sensitive strains will acquire “secondary” resistance during a course of failed therapy. Secondary resistance can be prevented by improving compliance or raising drug levels either in the sera or at the site of infection. In contrast, primary resistance occurs when a susceptible patient is infected with a TB strain that is already drug-resistant. Primary resistance can affect both those who have never been previously infected or treated for TB as well as in people who are reinfected with a new strain of resistant TB after a previous infection [29]. Figure 10.2 illustrates these two routes to drug-resistant TB.

In a recent review of strategies to beat MDR TB, Dye and colleagues enumerated mechanisms by which antituberculosis drug resistance might be more common in HIV-infected patients than in those who were HIV-negative [30]. Here, we review and expand on these pathways, classifying them into those that might result in acquired or secondary resistance, and those that could lead to primary resistance.

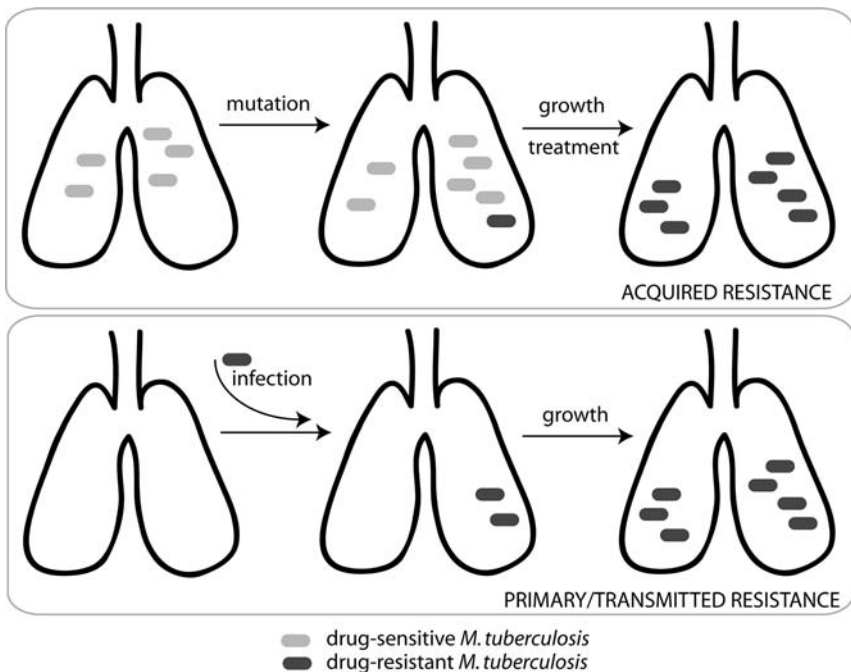


Figure 10.2 Routes to drug-resistant TB. An individual may acquire resistance through inadequate therapy of an initially drug-sensitive infection (top panel) or through direct infection with *M. tuberculosis* that has acquired resistance in another host (bottom panel).

10.4.1

Acquired Resistance

Most acquired drug resistance in *M. tuberculosis* occurs when patients do not receive adequate and sustained doses of antituberculosis drugs. HIV might affect patient compliance in several ways:

- The likelihood of noncompliance may increase as the complexity of a patient's medical regimen increases. Many coinfecting patients receive antiretroviral therapy (ART) and/or treatment for other opportunistic infections at the same time as they receive multidrug TB therapy; this may make it more difficult for them to adhere to the prescribed regimens.
- Interactions between drugs used for HIV and TB and overlapping toxicities may also compromise the patients' ability to adhere to regimens for both diseases.
- Risk factors for HIV/TB coinfection may also put patients at risk for noncompliance. For example, in some settings, coinfecting patients may be more likely to have an alcohol use disorder or to be injection drug users, and therefore less likely to complete a rigorous and prolonged course of therapy.

Despite the plausibility of these scenarios, there is limited evidence to support the hypothesis that HIV-infected TB patients are less compliant than TB patients who are not HIV-infected. Most studies of risk factors for nonadherence to TB treatment have not included HIV among the covariates assessed [30–33]. Although several of the studies that have assessed HIV found it to be a modest risk factor for nonadherence in univariate analyses [32, 34], this effect has been abrogated in those analyses which have controlled for confounding by covariates such as injection drug use and homelessness. This suggests that much of the effect of HIV on the acquisition of drug resistance is mediated through these shared risk factors [34, 35]. Even fewer studies have documented the acquisition of drug resistance among patients who have not complied with TB therapy. A number of studies do show that adverse drug reactions contribute to nonadherence, but these reports do not distinguish between adverse reactions that occur in HIV-coinfecting patients and those that occur in others receiving TB treatment [36–39].

HIV-infected patients who comply with TB therapy may nevertheless receive inadequate doses of drugs to suppress bacterial replication if HIV interferes with a patient's capacity to absorb the drugs. Although one early study found no associations between HIV, CD4 cell count or diarrhea and variability in the pharmacokinetics of antituberculosis drugs [40], multiple subsequent studies have reported reduced serum and urinary levels of antituberculosis drugs as well as D-xylose in HIV-infected patients [41–45]. These data suggest that HIV-induced malabsorption can result in subtherapeutic concentrations of these drugs, that in turn may lead to treatment failures and the emergence of drug resistance. Although these findings support the recommendations that HIV-infected TB patients receive therapeutic monitoring of drug levels [46], such monitoring is not available at many sites where coinfection is common.

Another reason that may explain higher rates of antituberculosis drug resistance among HIV-infected TB patients is that the burden of TB bacilli present in an immunosuppressed host may be greater than in a normal host [30]. Drug resistance in *M. tuberculosis* stems from chromosomal mutations rather than through plasmid-encoded proteins that confer resistance to antibiotics. Since the probability of the sporadic appearance of a resistance conferring mutation increases with the size of the bacterial population, resistance is more likely to arise when there is a large pool of replicating bacilli. Here again, despite the plausible association between HIV and higher bacterial loads of *M. tuberculosis* as a result of impaired cellular immunity, it is not yet clear whether HIV-infected patients harbor more TB bacilli than do HIV-seronegative patients. Studies of chest radiology have shown that HIV-infected TB patients are either as likely or less likely [47–52] to have significant pulmonary disease and/or cavitory lesions than HIV-noninfected patients. Similarly, HIV patients are less likely to be sputum smear-positive [53–55]. As smear positivity correlates well with cavitory disease [56], these data suggest that the pulmonary bacillary burden may actually be higher in HIV-noninfected patients. However, even if HIV-infected patients with pulmonary TB are less likely to form granulomas and progress to cavitory disease, this does not mean that they have fewer bacteria in their lung tissue. Furthermore, even if the pulmonary bacillary burden of HIV-infected TB patients is lower than that of noncoinfected patients, the fact that HIV-infected patients are more likely to have extrapulmonary spread of TB indicates that they may nonetheless have higher overall bacterial loads.

While it is not entirely clear how HIV infection impacts mycobacterial burden at the individual level – even if coinfecting patients have lower overall bacillary burdens than their HIV-negative counterparts – the actual number of *M. tuberculosis* bacilli harbored by the *HIV-infected population* in any specific community may be higher than that in the *non-HIV-infected population*, especially in those areas with high HIV burdens. Since the risk of progression of TB in the HIV-infected is vastly higher than in the uninfected [57], it follows that HIV ultimately serves to increase the overall number of *M. tuberculosis* bacilli present within the entire community, thus increasing the probability of occurrence of drug resistance-conferring mutations within the community, even if the risk is lower among each individual with HIV coinfection. Therefore, a high HIV prevalence may be a community level risk factor for the acquisition of resistance, even when it is protective at the individual level. Few studies have examined HIV prevalence as an ecological risk factor for rates of either MDR/XDR TB, or compared the estimated burden of *M. tuberculosis* bacilli found within populations of HIV-positive and -negative hosts [58].

HIV-infected patients may also be more likely to acquire secondary resistance to TB drugs because they have increased exposure to antibiotics with some antituberculosis activity prior to a diagnosis of active TB. For example, HIV-infected patients are often coinfecting with a variety of other sexually transmitted or respiratory pathogens, and may receive drugs such as quinolones, aminoglycosides, or even rifampin, that are also active against *M. tuberculosis*. This may exert some selection pressure for resistant organisms even before TB treatment has been initiated [59, 60]. A recent report from the United States found that the proportion of TB patients

exposed to fluoroquinolone within the year prior to TB diagnosis increased from 9% in 2000 to 41% in 2004 [61]. In the same study, HIV status emerged as the strongest predictor of fluoroquinolone exposure prior to TB diagnosis, along with older age and calendar year.

The current HIV management guidelines recommend a six- to nine-month course of INH preventive therapy, not only for those with a positive tuberculin skin test (TST) but also for all persons living with HIV in whom active TB has been excluded [62]. While there is little evidence that resistance arises in latent TB bacilli exposed to preventive monotherapeutic regimens [63], those who have undiagnosed active TB and who receive single drug therapy are relatively likely to develop resistance to that drug. In a pilot study on the efficacy of TB screening in Botswana, Mosimaneotsile *et al.* found that only 0.2% of those who presented without signs or symptoms of TB were diagnosed with active TB by chest X-radiography (CXR) [64]. Other studies in high-burden African countries, however, have identified much higher rates of active TB diagnosed by CXR among patients without apparent disease [65, 66]. These findings raise the concern that HIV-infected persons who have not been screened by CXR and who are started on INH may develop monoresistance to that drug [17].

Lastly, HIV might contribute to the emergence of acquired drug resistance if drug-resistance mutations resulted in a loss of virulence that would be lethal to the organism if it were present in an immunocompetent host, but which would be tolerated by the bacteria if it occurred in an immunosuppressed host [30]. While some early studies have shown INH-resistant strains to be less virulent in animal models than sensitive strains [67], subsequent investigations have shown that these effects are heterogeneous, with some resistance mutations exerting more fitness costs than others [68–70]. Not surprisingly, those mutations most often found in clinical MDR and XDR strains are also those found to suffer the least fitness costs in laboratory experiments [71]. Nonetheless, with XDR strains harboring as many as ten drug-resistance mutations in the housekeeping (often essential) genes that are the targets of antituberculosis drugs, it is tempting to speculate that such strains must suffer accumulated fitness costs. As such, these highly resistant but potentially relatively unfit strains may be more viable among hosts in whom immunosurveillance is impaired.

10.4.2

Primary Resistance

In principle, HIV could contribute to primary MDR or XDR TB in one of two ways. Those infected with HIV might be more likely to be infected or to develop disease with an MDR or XDR strain, while those who have MDR/XDR HIV coinfection might be more likely to transmit to others. In this section, we consider evidence for both of these mechanisms.

The transmission of drug-resistant strains of TB can be assessed in two ways. Among patients infected with MDR and XDR TB, those who are newly diagnosed cases who have not previously received antituberculosis drugs are considered to

have primary resistance – that is, to have been infected with a drug-resistant strain of *M. tuberculosis* [72]. In its global surveillance surveys of TB drug resistance, the WHO reports the proportion of resistant cases in new and re-treatment cases separately in order to monitor the contribution of primary resistance to the overall burden of drug-resistant TB in a specific setting. For example, the most recent surveillance report shows that the prevalence of MDR TB among new case ranges from 0% in eight different countries to 22% in Baku, Azerbaijan [28]. While there are as of yet no systematic studies of the prevalence of XDR TB, several reports have been made of the occurrence of XDR TB in newly diagnosed patients who have not received antituberculosis drugs [1, 73]. Primary resistance can also be detected using molecular epidemiologic tools. Strains that share a genetic fingerprint are considered “clustered,” and clustered cases are assumed to be part of a transmission chain [74]. Some molecular epidemiology studies have found that MDR TB strains are less likely to be clustered (i.e., transmitted) than drug-sensitive strains [75, 76], while others have found the opposite – that MDR strains are more likely to be clustered than their drug-resistant counterparts [41, 77–79].

Among the first 53 cases of XDR TB reported in Tugela Ferry, 55% had never been previously treated for *M. tuberculosis* [1]; such evidence supports the contention that XDR strains were circulating in the community and infecting HIV patients. The fact that all of the XDR TB patients tested in this study were HIV-positive suggests that those who were not HIV-infected were at lower risk either for infection with an XDR strain or, more likely, for rapid progression to disease once infected. Molecular fingerprinting of the strains from the Tugela Ferry outbreak showed that 85% of the XDR strains were genetically similar, which also suggested that they were clustered and therefore recently transmitted [1].

Why might XDR strains preferentially infect or cause disease in HIV-positive patients? As discussed above, highly resistant strains that suffer fitness costs may be more likely to remain viable in patients who are unable to mount an effective immune response to infection. Another possibility is that while both HIV-positive and -negative patients may be equally likely to be infected with a currently circulating XDR strain, those with HIV are much more likely to develop disease soon after infection, whereas those who are HIV-negative may harbor latent strains of the infection that could reactivate in the future. As such, HIV patients may represent the vanguard of an epidemic of drug-resistant TB [30].

Another reason why HIV-infected patients in Tugela Ferry may have been infected with drug-resistant strains of *M. tuberculosis* is that they were more likely to have been hospitalized and to have been exposed to other hospitalized patients with XDR TB. Some 67% of the XDR TB patients had been admitted to the district hospital within the two years preceding their diagnosis; none of these patients had any known contact with each other, apart from receiving care at the same district hospital, and none had a known contact with a family member afflicted with active TB [1]. Basu *et al.* fitted a mathematical model to the epidemic trajectories of drug-susceptible and drug-resistant TB in Tugela Ferry, and used this model to estimate the future burden of XDR TB in that community [80]. The study results predicted the occurrence of 1300 new cases in the area by the end of 2012, over half of which were projected to be

nosocomially transmitted. The finding that HIV-infected patients may be at more risk of drug-resistant TB because they are more likely to contact those with resistant disease echoes the earlier experience of New York City, in which a sustained epidemic of drug-resistant TB occurred among HIV-infected patients who were living in congregate settings.

That XDR TB can result from primary transmission to HIV-infected patients was confirmed by a recent study demonstrating the reinfection of patients who had previously been treated for TB in Tugela Ferry [73]. Of the 17 patients who were found to have MDR and/or XDR TB after having had an episode of drug-sensitive TB, all harbored a new strain and were thus classified as having primary rather than acquired resistance. In an editorial accompanying this report, Horsburgh argued that the high rates of reinfection provided evidence that primary transmission must be the principal route through which MDR and/or XDR TB is acquired in this community [81]. While this was the first study to be published that examined reinfection by XDR TB, previous studies of recurrent MDR TB in China also supported the contention that much resistant TB is primary, even among patients who are not HIV-infected [82].

10.5

Role of HIV on the Infectiousness of XDR TB

HIV may also increase the likelihood that a person infected with resistant TB transmits the infection to others. Although it is clear that HIV-infected TB patients are less likely to be sputum smear-positive, and that smear positivity correlates with infectiousness, a meta-analysis on the impact of HIV on the infectiousness of TB found no difference in the proportion of healthcare workers or household contacts who converted their TST after exposure to TB cases who were HIV-positive or -negative [83]. Other recent research addressed the impact of HIV on the infectiousness of drug-resistant TB. Enscombe and colleagues measured the infectiousness of patients with TB by exhausting the air from an HIV-TB ward over a chamber containing guinea pigs [84]. The animals were subjected to monthly TSTs, and tissue was obtained from those that tested positive. The investigators inferred the identify of the patients who had infected the guinea pigs by comparing the drug-susceptibility patterns and DNA fingerprints of isolates obtained from patients and guinea pigs. The results of this study demonstrated that only 10 of 97 HIV-positive patients admitted to the ward had transmitted TB to the guinea pigs and of these, six were MDR TB patients who had received suboptimal therapy. It was concluded that, although the transmissibility of HIV-infected patients with drug-resistant disease is highly variable, some suboptimally treated HIV-positive patients with MDR TB are quite capable of transmitting disease.

HIV-infected patients with drug-resistant TB may also be more likely to transmit disease for operational rather than biological reasons. HIV-coinfected patients are those most likely to require hospitalization and to undergo procedures such as sputum induction that could lead to the nosocomial transmission.

10.6

Community Level Impact of HIV on Population Increases in Drug-Resistant TB

In the preceding sections, attention has been focused on the impact of HIV on drug-resistant TB in coinfecting individuals. Some public health authorities have suggested, however, that countries with rising burdens of HIV may experience an increase in drug-resistant TB due to the failure of health systems overtaxed by the HIV epidemic, rather than through mechanisms that link HIV and drug-resistant TB in individuals. This hypothesis was not supported by the previously cited study on MDR TB rates in 39 African countries, which found no correlation between national estimates of HIV incidence or rates of coinfection and rates of MDR TB [13]. On a more local level, relatively few data are available on trends in the availability of TB care and control services over the course of HIV epidemics in specific settings. Gilks *et al.* examined the patterns of general admissions and outcomes to Kenyatta hospital in Nairobi during the rise of HIV prevalence in Kenya [85]. While outcomes for HIV-related admissions did not change as the HIV prevalence rose, outcomes for patients who were not infected with HIV deteriorated over the first years of the HIV epidemic. This led Gilks to conclude that the HIV epidemic had affected patient care and the severity of disease among admitted non-HIV-infected patients. When this analysis was repeated several years later, however, the investigators found that while mortality among HIV-negative patients had risen from 14% to 23% between 1988/89 and 1992, it returned to baseline levels by 1997, despite a steady increase in HIV incidence and prevalence [86]. While the results of these studies did not distinguish admissions or mortality due to TB, they did suggest that a rise in the burden of HIV would not lead to an inevitable collapse of health systems and a consequent deterioration of TB services.

In summary, HIV may be associated with MDR TB and, by extension, to XDR TB by increasing the likelihood that patients with TB acquire resistance once infected with a drug-sensitive strain, by increasing the likelihood that patients are infected with drug-resistant strains of TB, or by increasing the likelihood that patients with MDR TB transmit their disease to others. While limited data exist to support the hypothesis that HIV is associated with MDR TB through each of these possible mechanisms, there are few data available as yet to draw specific conclusions about the role that HIV plays in facilitating the emergence or transmission of XDR TB.

10.7

Effect of HIV on the Diagnosis and Treatment of MDR and XDR TB

10.7.1

Diagnosis

The routine diagnosis of TB in most resource-constrained settings relies on the demonstration of acid-fast bacilli by sputum smear microscopy. Until recently, the diagnosis of drug resistance in TB required culture and phenotypic drug sensitivity

testing of the organism [87]. Since the laboratory capacity for such testing is limited in these settings, culture has been reserved for those with suspected drug resistance, those with apparent treatment failure and/or relapse, and in patients with pulmonary TB who have repeatedly negative smears. *M. tuberculosis* is a slow-growing mycobacterium that requires five to eight weeks to grow in culture on solid media; therefore, the diagnosis of drug-resistant TB is frequently associated with significant delays, which may in turn lead to disease progression and increase the risk of ongoing transmission. Those patients who are diagnosed on the basis of smear microscopy or clinical suspicion, usually receive standard short-course chemotherapy while awaiting the results of culture and drug sensitivity testing (DST); those who are resistant to two or more of these four drugs therefore receive functional monotherapy and/or dual therapy, and are more likely to go on to develop further drug resistance – a process referred to as resistance amplification [88].

Today, a vast array of new diagnostics is available to detect active TB and, in some cases, to measure drug susceptibility. Some alternative approaches to the diagnosis of TB, including fluorescent microscopy, rapid culture techniques [89], mycobacteriophage-based assays, nucleic acid amplification tests, immunodiagnostic tests, antigen-capture tests and expanded clinical case definitions that bypass laboratory diagnoses, are listed in Table 10.1. Of these, only rapid culture methods, mycobacteriophage-based assays, nucleic acid amplification tests and line probe assays can be used to determine antibiotic susceptibilities, and most of these currently only detect resistance to INH and/or rifampin, although efforts to expand the number of antituberculosis agents to which resistance can be detected are under way. Although, rifampin resistance has been shown to be an excellent marker for multidrug resistance, it provides no information on second-line drug susceptibilities, and does not aid in the diagnosis of XDR TB. Given the need for diagnostics that can help providers select treatment regimens when patients first present with symptoms, there is much enthusiasm for the expanded use of diagnostics such as line probe assays for the detection of resistance to other first- and second line antituberculosis drugs.

HIV has further complicated the diagnosis of both drug-sensitive and drug-resistant TB in these settings. A recent systematic review found that the frequency of smear-negative TB among HIV-infected patients ranged from 24% to 61%; hence, the early diagnosis and treatment of these highly vulnerable patients is often delayed until the results of culture are available. Culture of the sputum from HIV-infected patients has also been found to take longer than that from patients without HIV infection [90], resulting in further delays in the diagnosis of drug-resistant disease. Some novel tools for the diagnosis of TB in HIV-infected patients and the detection of TB drug resistance are reviewed in Table 10.1.

10.7.2

Treatment

The Tugela Ferry outbreak focused the world's attention on XDR TB, largely because of the extraordinarily high and rapid mortality reported among the initial cohort of

Table 10.1 Selected TB diagnostics.

Method	Principle	Advantages	Utility for diagnosing drug resistance	Reference(s)
Fluorescence microscopy	Uses acid-fast fluorescent dye and high-intensity light source, enabling use of lower power objective lens than conventional microscopy	Improves sensitivity by 10% overall with higher comparative benefits reported in HIV-infected patients with pulmonary TB	Does not detect drug resistance	[92–94]
Automated liquid culture systems	Uses broth for rapid culture. Mycobacterial growth detected by changes in gas pressure, CO ₂ production or oxygen consumption	Reduces time to culture positivity to 14–21 days. In principle, should detect TB in HIV-infected patients	Can be used to detect drug resistance by addition of antimycobacterial drugs to culture media	[90, 95]
Microscopic-observation drug-susceptibility (MODS)	Uses light microscopy to detect cording of <i>M. tuberculosis</i> in liquid culture	Reduces time to diagnosis of TB and drug resistance to 7 days. Preliminary data suggest efficacy in HIV-positive patients	Can be used to detect drug resistance by addition of antimycobacterial drugs to culture media	[91]
Solid culture systems	Detects early metabolic activity of dividing mycobacteria	In principle, should detect TB in HIV-infected patients	Can be used to detect drug resistance by addition of antimycobacterial drugs to culture media	[90]
Mycobacteriophage-based assays	Uses mycobacterial phage to infect <i>M. tuberculosis</i> in clinical isolates. Lysis of phage allows subsequent infection of <i>M. smegmatis</i> , leading to visible plaque formation	Reduces time to diagnosis to 1–2 days. Low specificity. May not detect paucibacillary <i>M. tuberculosis</i> , suggesting limited utility in HIV-positive, smear-negative patients	Detects resistance to rifampin in 48 h.	[96]

Nucleic acid amplification tests	Amplifies and detects genetic targets specific to <i>M. tuberculosis</i>	Reduces time to diagnosis. Variable sensitivity, especially in smear-negative patients	Detects drug resistance if specific mutation conferring resistance is included in test	[89]
Line probe assay	Amplifies genetic targets known to confer drug resistance	High sensitivity and specificity in smear-positive TB	Detects resistance to rifampin and INH, but not yet XDR	[97]
Immunodiagnosics	Detects TB infection based on either cell-mediated or antibody-based host immune response to pathogen	TST and some antibody tests detect infection, but do not reliably distinguish TB infection and disease	Does not detect drug resistance	[89]
Antigen capture	Detects TB antigens in urine or other host secretions	Moderate sensitivity and high specificity unaffected by HIV status	Does not detect drug resistance	[98]
Expanded case definition	Uses clinical prediction models to diagnose smear-negative TB in HIV patients	Moderate sensitivity and specificity	Does not detect drug resistance	[99]

TST: tuberculin skin test.

53 patients, most of whom were known to be coinfecting with HIV [1]. Subsequent reports have shown that aggressively treated XDR TB can be cured in some circumstances, and have provided early guidance in case management strategies. Only four of these studies, however, have included HIV-coinfecting patients with XDR TB, and these have reported much lower treatment success rates among those with HIV.

Among those studies that reported treatment outcomes for patients diagnosed with XDR TB, the proportion of patients with poor outcomes (i.e., those who died, failed to convert a positive sputum smear, or defaulted from therapy) ranged from a low of 0.35 to a high of 0.81 [3, 28, 101–106]. An editorial accompanying one of these reports cited specific approaches that were associated with relatively high rates of success; these included the use of at least five effective antituberculosis drugs selected on the basis of drug susceptibility testing, and the addition of a fluoroquinolone and the injectable drug, capreomycin, even in the presence of apparent resistance to those drugs [107]. Among the studies that included HIV patients, two reported mortality that approached 100%, although the sample sizes of these studies were small [1, 108]. A recent review of XDR TB cases in the United States found that 21 of the 30 (70%) XDR TB/HIV died within two years of treatment initiation [3].

These data suggest an urgent need for early detection and aggressive therapy of both HIV and TB among those coinfecting with XDR TB and HIV. To date, optimal treatment regimes for XDR TB have not been defined on the basis of clinical trials or systematic observational data [109]. Pending such data, the treatment of XDR TB should be guided by drug sensitivity profiles and should adhere to the principles of treatment for MDR TB [110]. These include early detection and initiation of appropriate regimens, the use of four to six drugs chosen on the basis of drug sensitivity data, maximum dosing of drugs for body weight, careful adherence monitoring, rigorous management of side effects, and prolonged therapy of at least 18 months' duration following sputum conversion. Regimens should be chosen from among the drugs to which patients remain susceptible, beginning with those with highest efficacy – that is, the remaining first-line drugs, pyrazinimide, ethambutol, and streptomycin, proceeding to the injectable second-line agents (kanamycin, amikacin, capreomycin), followed by fluoroquinolones (moxifloxacin and gatifloxacin which may retain some activity even in the presence of resistance to the less-potent agents such as levofloxacin, ofloxacin and ciprofloxacin), and finally including other second-line agents (thioamides, *para*-aminosalicylic acid, cycloserine, linezolid, and imipenem). In addition, the US Department of Health and Human Services' Panel on Antiretroviral Guidelines for Adults and Adolescents recommends that those patients coinfecting with HIV and XDR TB continue ART along with antituberculosis therapy. Those patients who had not previously started antiretrovirals should be started on therapy once a diagnosis of XDR TB has been made.

The incidence of specific toxicities and drug–drug interactions among those being treated for both diseases is, as yet, unknown. While rifampin is known to interfere with the metabolism of protease inhibitors (PIs) and to cause reduced levels of these drugs, such considerations do not pertain to XDR TB since this condition is, by definition, resistant to rifampin. As there is limited experience of treating HIV-infected patients with second- and third-line antituberculosis agents, there is an

urgent need for descriptive studies documenting clinical practice as increasing numbers of patients are started on therapy.

10.8

Future Directions

The emergence of XDR TB has occurred in the context of a global HIV epidemic, and has raised the possibility that the two diseases are associated through a causal link. Even in the absence of any direct biological association, there are many ways in which drug-resistant TB and HIV interact, and it is clear that the intersection of these epidemics creates new problems for the diagnosis and management of patients, as well as for the availability of resources to combat these threats. In light of the growing problem of MDR and XDR TB, the WHO has advanced an eight-step plan that emphasizes the need for a coordinated response to address both HIV and TB. Among the objectives identified, several explicitly call for the measures that focus on the co-occurrence of these diseases. Based on this plan, priorities for future research and action on XDR TB and HIV should include the following:

1. The strengthening of basic control programs for TB and HIV.
2. A scale-up of programmatic management of MDR/TB and operational research to understand optimal management in the context of coinfection.
3. The strengthening of laboratory services for the adequate and timely diagnosis of MDR and XDR TB, especially for HIV-infected patients who require early diagnosis and treatment to improve the current abysmal survival rates.
4. An intensified surveillance for MDR and XDR TB, to gauge the magnitude and trends of the epidemic and to clarify the link with HIV.
5. The implementation of sound infection control measures to prevent the transmission of MDR and XDR TB to patients, healthcare workers and others in congregate settings, especially in areas of high HIV prevalence.
6. To strengthen political commitment to addressing MDR/XDR TB and HIV.
7. The mobilization of resources to ensure an adequate global response.
8. The promotion of research aimed at improving diagnosis, treatment regimens and preventing XDR TB and coinfection with HIV.

There is a clear need for urgent action and a coordinated response that addresses both TB and HIV. With adequate resources and political will, efforts to reduce the emergence and transmission of XDR TB will not only serve to protect us from this threat but will also benefit the routine care of TB and HIV and help lead to their ultimate elimination.

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11

Clinical Issues (Including Diagnosis): Immune Reconstitution Inflammatory Syndrome (IRIS)

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11.1

The Problem of IRIS: An Overview

11.1.1

Introduction

When the function of the immune system improves rapidly following the start of highly active antiretroviral therapy (HAART), systemic or local inflammatory reactions may occur. This reaction is considered as frequently self-limiting, especially if the pre-existing infection is effectively treated. However, it may develop into a life-threatening condition in a few individuals [1, 2].

Antiretroviral therapy (ART) initiation may be associated with the onset of new symptoms related to the recovery of a host's immune system to resident opportunistic infectious agents such as *Mycobacterium tuberculosis* [3], atypical mycobacteria [4], *Mycobacterium leprae* [5], *Cryptococcus* spp. [6], cytomegalovirus (CMV) [7], *Toxoplasma gondii* [8], and many others; or noninfectious, immune-mediated pathologies such as AIDS-related lymphoma [9], Grave's disease [10], and systemic lupus erythematosus [11], to name a few.

At the end of October 2008, a PubMed Query literature search yielded 255 hits alone for this recrudescence of immune function now most commonly referred to as "immune reconstitution inflammatory syndrome" (IRIS), although several similar terms such as "immune recovery disease," "immune reconstitution disease," and others have been suggested before [2, 3]. By now, it has become clear that there is no limit to any HIV-related condition being at least considered, in the absence of any clear-cut diagnostic criteria, to be involved in IRIS following the initiation of HAART in HIV-infected individuals.

Although IRIS in AIDS patients who are receiving HAART is currently studied extensively, to date the details only of case reports and almost exclusively of retrospective case series have been reported. So far, to the best of our knowledge, one prospective study on the incidence and risk factors for IRIS has been

published [12]; high-quality data regarding putting possible diagnostic criteria to the test, and on the management of IRIS, are lacking so far.

In two large case series, up to 30% of HAART responders developed one or more inflammatory syndromes consistent with IRIS [13, 14].

In a case series and a literature review of 182 episodes of IRIS, the most frequently reported associated infections were localized herpes zoster (22%), *M. tuberculosis* (20%), *M. avium* complex (17%), CMV (12%), and *Cryptococcus* spp. (6%) [15].

A retrospective study of HIV patients who were coinfecting with *M. tuberculosis*, *M. avium* complex, or *C. neoformans*, found that one-third of these patients developed IRIS, with similar rates of IRIS across all three coinfections [16]. The overall incidence rate was about 15 cases per 100 patient-years. IRIS was more likely when HAART was initiated close to the time of diagnosis of the coinfection [2].

In general, the treatment of IRIS depends on using the optimal agent for the underlying infection and its clinical symptoms, as well as measures to dampen the immune response. However, concerns about stopping HAART, such as the development of viral resistance or AIDS progression, remain an issue.

The most beneficial outcome of an IRIS episode is the cessation of clinical symptoms, and the recovery of immune function with viral suppression, as evidenced by an increase in CD4+ cell counts which is observed in almost all individuals upon complete or even only partial suppression of viral replication [17].

Although not totally relevant here, it should be mentioned briefly that IRIS and related terms have been occasionally used for similar conditions in patients who experienced similar phenomena following the withdrawal of iatrogenic immunosuppression. Examples include the cessation of corticosteroid therapy, the recovery of neutropenia after cytotoxic chemotherapy, the withdrawal of immunosuppression in transplant recipients infected with *C. neoformans*, and the engraftment of stem cell transplantation [18–20].

11.1.2

Pathogenesis

IRIS appears to be a consequence of the interplay between the degree of an HIV-infected individual's immune restoration under HAART, the antigenic burden of a relevant pathogen, and host factors defining susceptibility to a pathogen (Figure 11.1).

Infection with the human immunodeficiency virus (HIV) produces a quantitative and qualitative time-dependent deleterious effect on the immune system. The likelihood and severity of IRIS correlates with two interrelated factors, namely the extent of CD4+ T-cell immune suppression prior to the initiation of HAART, and the degree of viral suppression and immune recovery following the initiation of HAART [2].

The half-life of HIV is generally between one and four days, and HAART may produce a greater than 90% reduction in the overall viral burden within one to two weeks of initiating therapy. A decline in the viral load usually persists during the next eight to 12 weeks, but this then stabilizes. An increase in immune effector cells is inversely proportional to the fall in HIV viral load in most treated individuals [2].

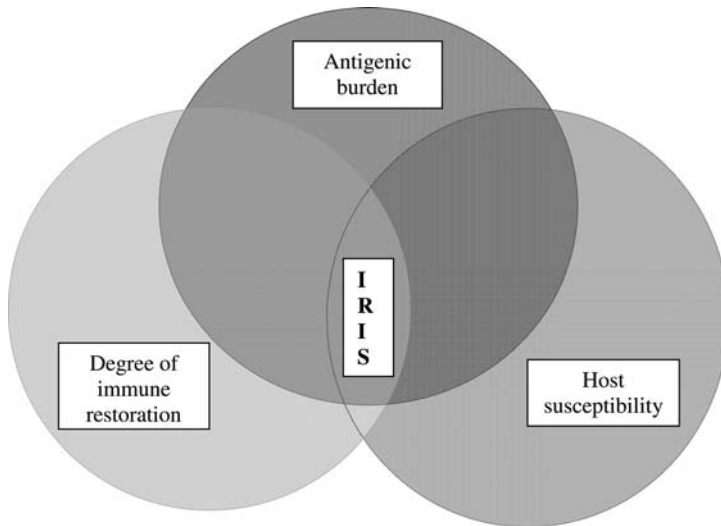


Figure 11.1 The pathogenesis of immune reconstitution inflammatory syndrome (IRIS).

Following therapy, the recovery of CD4 + T lymphocytes is biphasic [21–23]. There is a rapid increase in CD4 + cells during the first three to six months of HAART, due to an increase in CD45RO + memory T cells. This is presumed to be due to a combination of decreased apoptosis and a simultaneous redistribution of lymphocytes from the peripheral lymphoid tissues into the circulation. A slower increase of predominantly naive CD4 + T cells (CD45RA + , CD62L +) occurs after this; there is then a secondary increase in CD4 + T cells, which is thought to be due to the expansion of T-cell clones produced by the thymus prior to its age-related functional decline and/or secondary to thymopoiesis [24–26]. The CD8 + T lymphocytes also increase rapidly in number after starting therapy; however, their total numbers generally stabilize as the memory CD8 + cells decline and are replaced by naive CD8 + T lymphocytes [23].

An increase in T lymphocytes after starting HAART is also accompanied by increased *in vitro* lymphocyte proliferation responses and a pathogen-specific delayed hypersensitivity response [21, 23, 27]. This *in vitro* lymphocyte proliferation against *Candida* antigens, as well as an increase in *in vitro* stimulation indices with CMV antigens and tuberculin typically occur after initiating HAART in HIV-infected patients [21, 27]. In one study, it was documented that 90% of HIV-infected patients had cutaneous anergy prior to starting to HAART; 12 weeks later, 30% of these patients had recovered their ability to respond to skin test antigens [27].

A prospective study on the incidence and risk factors for IRIS in South Africa arrived at the conclusion that IRIS may affect 10% of patients initiating HAART in Africa, predominantly in those with advanced immune deficiency. However, the overall risk of developing severe life-threatening IRIS was considered low [12].

Various risk factors have been associated with the development of IRIS [1, 12–14, 16]. Amongst other possible factors, it has been suggested that IRIS in general—or at least IRIS in tuberculosis (TB IRIS)—may be a consequence of the swift restoration of antigen-specific immune responses under HAART [3, 28].

11.1.3

Defining IRIS

Although no agreed definition for IRIS has been accepted to date, most (or all) of the following should be present in order to make the diagnosis [2]: (i) the presence of AIDS with a low pre-therapy CD4 + count (often <100 cells μl^{-1}) [14], with an important exception to this rule being that TB IRIS secondary to pre-existing *M. tuberculosis* infection may occur in individuals with CD4 + cell counts >200 cells μl^{-1} ; (ii) a positive virological and (iii) immunological response to ART (which is defined as a greater than 1–2 log reduction in HIV load, along with a two- to fourfold or greater rise in CD4 + cell count within eight weeks).

One group has empirically defined the criterion for a significant response to HAART in patients with an IRIS to be a CD4 + cell count increase of 50 cells mm^{-3} or greater, and an absolute CD4 + cell count of 100 cells mm^{-3} or greater [29]; however, others found that there seemed to be no association of IRIS risk and the magnitude of increase in CD4 + cell count [14].

Although the CD4 + cell recovery may lag behind virological suppression, a retrospective multivariate analysis of 37 patients coinfecting with TB found a greater increase in CD4 + cell percentage (median 11%) after one month on HAART to be independently associated with IRIS occurrence [30].

There is also a temporal association between the starting of HAART and the symptoms of IRIS. However, the timing of onset of these symptoms is variable. An IRIS developed within the first two to three months of initiation of HAART in two-thirds of patients in three retrospective case series [13, 14, 31]. IRIS may occur within even a few days or weeks of starting HAART [18]. There have been reports of IRIS beginning as long as one to three years after the initiation of HAART, and hence it is difficult to know whether this is the same syndrome, or not. Rarely, reports have been made of IRIS beginning as long as four years after the initiation of HAART [32].

Hypersensitivity reactions that are seen with abacavir may be difficult to distinguish from an IRIS, such as that seen with *M. avium* complex (MAC). Symptoms of abacavir hypersensitivity become worse after each dose of treatment but then improve prior to the next dose, albeit with a trend towards aggravation over time. The relationship between doses and symptoms can help to distinguish abacavir hypersensitivity from an IRIS [2].

11.2

Tuberculosis-Related IRIS

11.2.1

Case Definitions and Diagnostic Criteria

To date, no unanimously accepted case definition exists. Case definitions proposed earlier, and as reviewed by Meintjes *et al.* (2008) [3] for IRIS in general [33, 34], do not appear to be widely acceptable in regions with limited laboratory and diagnostic

resources. Rather, these overlap widely with those parts of the world with the highest HIV and TB coinfection rates [3], and are too broad or unspecific for certain conditions such as TB IRIS. For TB IRIS, a first attempt towards a case definition considered to be of particular use in developing countries was proposed by Colebunders *et al.* (2006) [35]. Recently, Meintjes *et al.* (2008) [3] reported in detail proposed case definitions for TB IRIS, that had been consensually reached by recognized experts in the field during a meeting held in late 2006.

It appears to be commonly accepted that there are two broad categories (or main syndromes) of TB-associated IRIS:

- A reaction following HAART initiation in patients on TB treatment, commonly referred to as “paradoxical TB-associated IRIS.”
- An onset (“unmasking TB-associated IRIS”) of TB following HAART initiation, often with an exaggerated inflammatory clinical presentation [3].

Meintjes *et al.* (2008) [3] have provided detailed information on how those suggested case definitions evolved, in addition to a detailed list of suggested elements relevant to correctly diagnose those conditions. Whereas the establishment of “paradoxical” TB IRIS appears to offer fewer challenges in the view of most experts, “unmasking” TB IRIS is frequently more difficult to diagnose. In any case, these expert groups proposed that ART-associated TB be broadly defined as all cases of TB that are diagnosed during ART: (i) in patients not receiving TB treatment on HAART initiation; (ii) in patients diagnosed with active TB after HAART initiation; and (iii) whilst fulfilling WHO diagnostic criteria for smear-positive or -negative pulmonary TB, or extrapulmonary TB, respectively. The case study described in Figure 11.2 demonstrates that the establishment of a diagnosis of TB IRIS is not always clear-cut from the beginning.

Paradoxical inflammatory syndromes, as seen in HIV-infected patients, have also been described in non-HIV-infected patients following therapy for TB [36–39] or leprosy [40].

11.2.2

Epidemiology

Mycobacterium tuberculosis is the infectious agent most commonly associated with IRIS [12, 41], and may occur in any age group [42]. It is to be expected that in areas of extremely high HIV/TB coinfection rates, such as in Southern Africa [43], TB IRIS is the predominant IRIS to be encountered, and is aggravated in an environment where the majority of patients commencing HAART are in the advanced stages of CD4 + cell lymphocyte depletion.

The first prospective study on IRIS was to evaluate the incidence of paradoxical responses in patients on TB therapy and who were subsequently initiated on HAART. It was noted that, of 33 HIV/TB coinfecting patients undergoing dual therapy, 12 (36%) developed paradoxical symptoms [44]. The frequency of symptoms in this group was greater than was observed in HIV-infected controls receiving TB therapy alone, thus confirming the role of an exaggerated immune system response in the

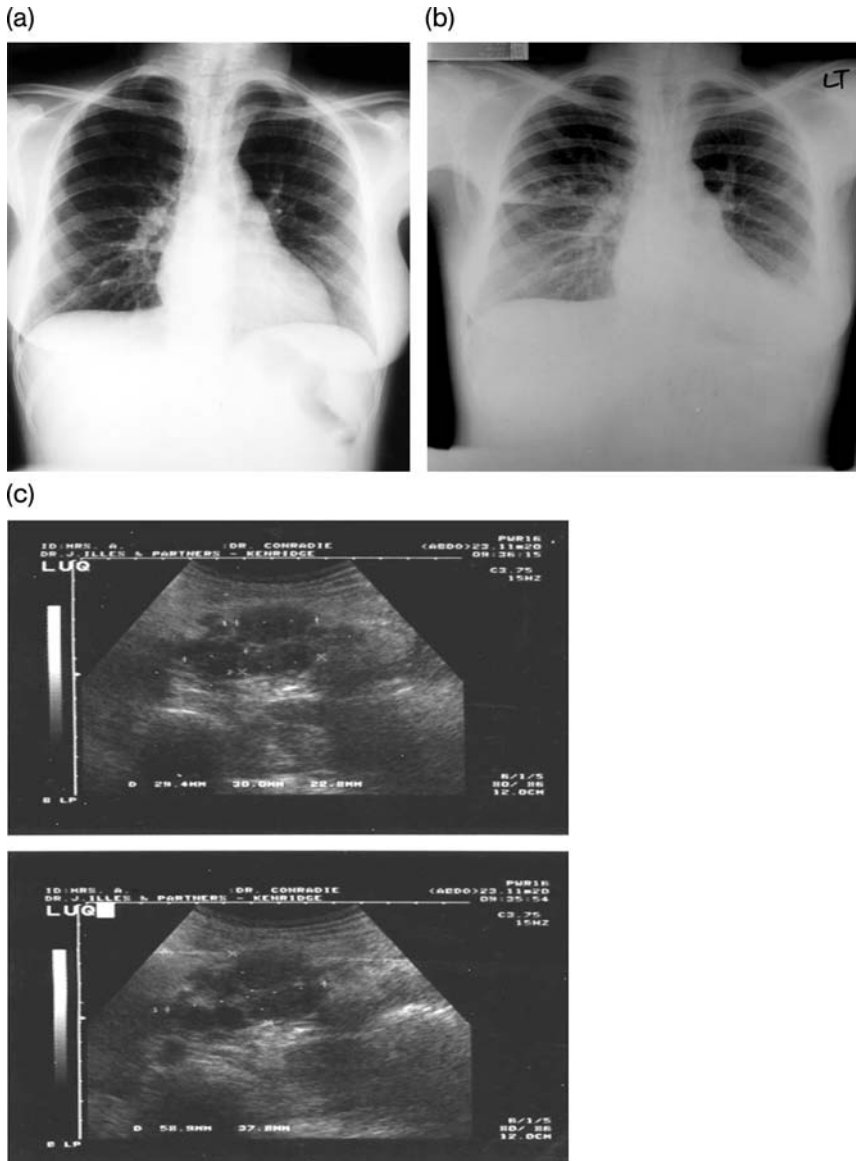


Figure 11.2 Case vignette: TB IRIS. (a) Chest radiography at two weeks after commencing HAART; (b) Chest radiography at four weeks after commencing HAART. Note the increased pulmonary infiltrates; (c) Ultrasonography of the abdomen, showing lymphadenopathy. This 25-year-old female patient was diagnosed with HIV infection in March 2000. In October 2000, her CD4⁺ cell count was 160 mm⁻³, with a viral load of >750 000 copies ml⁻¹. HAART with stavudine, lamivudine, and efavirenz was commenced. Two weeks later, the patient presented with cough, fever, and weight loss.

The CD4⁺ cell count at one month after commencing HAART was 674 mm⁻³, and the viral load had declined to 10 400 copies ml⁻¹. Four sputum specimens were negative for acid-fast bacilli, blood cultures for mycobacteria were negative, and a bronchoalveolar lavage was also negative for TB culture. Ultrasonography showed hepatomegaly with large para-aortic nodes (panel c). Combination TB treatment was commenced empirically, after which the patient's symptoms improved and pulmonary infiltrates resolved within two months (panels a and b).

pathogenesis of the syndrome. The results of several other retrospective studies also concurred with the finding that a significant proportion of HIV/TB coinfecting patients undergoing HAART have symptoms consistent with IRIS, with estimates ranging from 7% to 45% [30, 45–49]. In the first prospective study examining TB IRIS in South Africa, the majority of a series of 44 TB IRIS cases were new, or “unmasking” presentations of the syndrome, with only 20% manifesting the “paradoxical” form. While some investigators have found no difference in time from the initiation of TB therapy to the commencement of HAART between IRIS and non-IRIS subjects [30], others have reported significant differences between these groups [16, 47]. This association of a shorter lapse between TB therapy and HAART initiation remains an area of debate.

It was commonly noted that IRIS occurred in individuals who were started on HAART within two months of TB therapy initiation [47]. A study in Thai patients with active TB with CD4 + counts less than $100 \mu\text{l}^{-1}$ who were started on ART showed that a shorter time between TB treatment and ART onset was statistically significantly associated with the occurrence of IRIS [50]. A decision analysis exercise in hypothetical cohorts on ART initiation timing in TB patients also found the highest rates of IRIS occurrence estimates to occur in patients initiated on HAART within two months of starting TB treatment [51]. However, withholding or deferring ART until two to six months of TB therapy was associated with a higher mortality. It appears that many clinicians in developing countries agree that TB IRIS is controllable in most cases, and that the mortality is lower than the mortality from opportunistic diseases whilst delaying HAART initiation.

11.2.3

Clinical Manifestations

With regards to the timing of TB IRIS, among a retrospective cohort of 144 HIV patients with TB coinfection, 8% had a paradoxical reaction after a median time of 47 days following HAART initiation. The incidence was 15 cases per 100 patient-years [48]. Generally, the time interval from the initiation of HAART to the onset of IRIS associated with *M. tuberculosis* infection varies (according to the literature) from about 10 to 180 days, but tends to occur within the first 60 days of starting HAART [34, 52].

The symptoms of IRIS are related to the type and location of pre-existing infection, with symptoms being either localized or systemic. Pre-existing infections may or may not be clinically apparent prior to the initiation of HAART [2].

With regards to IRIS associated with mycobacterial infections, prior to the widespread emergence of HIV infection, an IRIS-like paradoxical inflammatory response was known to occur in some patients treated for TB [18, 36–38]. The paradoxical reaction that now occurs with the initiation of TB therapy for pulmonary TB is usually characterized by fever, malaise, weight loss, and worsening respiratory symptoms [2].

Signs and symptoms in line with TB-IRIS can, however, also be observed in individuals on HAART who had not been diagnosed and treated for TB prior to an

episode of acute illness suggestive of, yet microbiologically negative for, TB but responding well to TB treatment (see the case study in Figure 11.2). The radiographic features comprise transient worsening of abnormalities, including new parenchymal opacities, new pleural effusions, and progressive intrathoracic lymph node enlargement. Rarely pulmonary involvement may progress to severe respiratory compromise and adult respiratory distress syndrome (ARDS) [2].

TB-IRIS affecting the central nervous system (CNS) is a serious problem. As the availability of HAART increases in endemic countries, the incidence of CNS TB-IRIS may also increase [53, 54]. This may present as an expansion of pre-existing intracranial tuberculomas after TB therapy is begun [37, 52, 55, 56]. Cutaneous lesions [44], peritonitis [57], epididymitis [57], bowel perforation [58], or granulomatous nephritis [59] have also been documented.

Lymphadenopathy may occur, and the aspiration of fluctuant nodes often reveals purulent material; however, mycobacterial cultures are sterile and acid-fast stains yield few or no organisms. When bacilli are recovered from patients with paradoxical reactions, these organisms generally have not developed new antituberculous drug resistance [18].

The incidence of a paradoxical worsening of symptoms associated with pulmonary TB is higher in HIV-infected individuals following HAART initiation compared to those who are not coinfecting with HIV [60, 61]. In a prospective study, whereas paradoxical inflammatory reactions were documented in 36% of patients coinfecting with HIV and treated with HAART, similar reactions occurred in only 2% of patients without HIV [44].

Patients coinfecting with the MAC may also develop inflammatory reactions following the commencement of HAART [41, 62–64]. In the majority of cases, MAC IRIS manifests as fever and painful lymphadenitis that occurs one to eight weeks after HAART is initiated [41, 65]. Individual case reports or small cases series also exist, in which IRIS is described as presenting with necrotic subcutaneous nodules, osteomyelitis, bursitis [66], granulomatous hepatitis, paravertebral abscesses [67], brain abscess [68], worsening lung infiltrates, or diffuse intestinal involvement presenting with abdominal pain [69]. In two reviews, onset occurred within one month of starting ART in the majority of patients who developed MAC-related IRIS [64, 67]. The majority of patients with IRIS due to treated MAC infection have negative blood and bone marrow cultures. In contrast to findings in patients with AIDS and untreated MAC infection, biopsies of the lymph nodes of patients with IRIS secondary to MAC usually reveal well-formed granulomas, with relatively few visible organisms [70].

11.2.4

Management

To the best of our knowledge, no controlled trials for the management of IRIS have been published to date. Patients should be treated for the underlying infection in the best possible way, depending on the availability of drugs and the highest achievable quality of care, both of which may be an issue in resource-poor settings [2].

Treatment options for managing IRIS include the continuation of HAART. It has been suggested in various studies that it is reasonable to continue HAART in the majority of cases, if not life-threatening. Mild or moderate symptoms may be tolerable and patients can be reassured that the symptoms will resolve over time, and that they can be treated symptomatically [2].

Corticosteroids or nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the inflammatory response. Although such therapy is not consistently effective, it at least seems to control the symptoms in some patients [2].

Whilst the paradoxical worsening of symptoms of non-HIV-infected patients with pulmonary TB following the start of antituberculous therapy is assumed to be self-limiting in most cases, the results of several controlled studies have suggested that adjunctive corticosteroid therapy, usually given for four to eight weeks following the initiation of antituberculous therapy, results in a faster short-term symptomatic improvement and radiographic resolution [71].

Corticosteroid therapy may be harmful, and decisions relating to individual patients must be made before initiating therapy. It is important to ensure that an appropriate antituberculous drug regimen is in place, taking into account the possible occurrence of multidrug resistance [2].

In the absence of any clinical trials, it has been advocated that therapy with prednisone at a dose of 1 mg kg^{-1} per day (maximal daily dose 60–80 mg) may be used, followed by a tapering off of steroids whilst monitoring for any recurrence of clinical symptoms over the ensuing weeks to months. Dexamethasone (8–16 mg per day, divided into twice daily dosing) may also be used [2].

Occasionally, the interruption or discontinuation of ART in some patients may be necessary, especially in those who experience life-threatening IRIS or in whom localized IRIS threatens permanent neurological or other sequelae. Patients can then be treated for a period of time for their underlying infection before resuming HAART [2].

11.2.5

Prevention

Opportunistic infections (OIs) should be treated adequately with the appropriate antimicrobial therapy, as it may be possible to avoid IRIS in this way for at least one to two months prior to starting HAART [2].

If a patient is coinfecting with hepatitis B virus (HBV), then lamivudine and/or tenofovir should be included into the HAART regimen for HBV suppression [2, 72].

While awaiting additional evidence, and with the goal of minimizing both the risk of IRIS and the risk of death and other opportunistic infections, it is recommended that ART should be delayed for about two weeks in patients with TB and a CD4+ cell count below $100 \mu\text{l}^{-1}$ [52]. HAART may be delayed for two months in patients being treated for TB who have a CD4+ cell count above $100 \mu\text{l}^{-1}$, so as to decrease the risk of IRIS [73]. However, opinion on this subject may soon change as evidence is accumulating of a swift HAART initiation being favored with regards to a positive outcome.

It is of utmost importance, therefore, that before starting HAART in a patient with a low CD4+ cell count, a detailed history be acquired and a thorough physical examination carried out. Careful screening for any signs of infection, and appropriate laboratory tests to establish the correct diagnosis, should be performed if the situation is at all suggestive of an opportunistic infection.

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