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Pathogenesis of HIV-1 and *Mycobacterium tuberculosis* co-infection

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8 Glossary

Term	Definition
Acid-fast bacilli (AFB)	Bacteria which are resistant to decolourisation during laboratory staining procedures, which is a recognised property of *Mycobacteria*. This arises due to the high mycolic acid content of the bacterial cell wall. Several diagnostic tests for TB rely on this property, including Ziehl-Neelsen staining.
Bacillary load	The measurable quantity of bacteria within a host organism or sample.
Efferocytosis	The process by which dead or dying cells are cleared by phagocytosis.
Extra-pulmonary	Anatomical locations beyond the thoracic cavity or lung.
Granulomatous pathology	Chronic inflammatory foci within tissues, primarily made up of a core of activated macrophages surrounded by $CD4^+$ T cells.
Giant cells	Multinucleated cells derived from macrophages, typically found within granulomatous inflammation.
HIV-1 long terminal repeat	Repetitive non-coding sequences at each end of the HIV-1 proviral DNA, which are formed during reverse transcription and play important roles in integration and regulation of viral gene expression.
Immunoregulation	Mechanisms by which the immune system self-regulates via negative feedback loops, e.g. the production of immunosuppressive cytokines.
Independent risk factor	A variable that improves the prediction of outcome in a statistical model which already includes other variables.
Inflammasome	Multimeric molecular complexes formed during innate immune signalling which activate caspase enzymes, control maturation of the pro-inflammatory cytokines IL-1β and IL-18, and may lead to cell death via pyroptosis.
Immunosenesence	The observable decline in immune function associated with ageing.
Immunodominant	The antigenic epitopes most commonly targeted by the adaptive immune response.
Lung apices	The upper lobe of each lung.
Mycobacteraemia	Circulation of mycobacteria in the bloodstream, identified on culture of blood.
Necrotic granuloma	Granulomatous inflammation with a core of dead cells.
Phagosome	A cytoplasmic vesicle formed as a result of the cellular uptake of particles >0.75μm in diameter.
Pleural effusion	An accumulation of fluid in the pleural cavity, the anatomical compartment which surrounds the lungs. This can arise due to a range of causes, one of which is infections such as TB.

Pro-inflammatory cytokines	Extracellular signalling molecules secreted chiefly by immune cells, which specific cell-surface receptors to trigger inflammatory processes.
Pulmonary cavitation	Formation of large airspaces in the lung parenchyma due to tissue destruction.
Pyroptosis	A specific cell death pathway triggered activation of caspase 1.
Resting T cells	T cells which have not been activated by binding of their cognate antigen to the T cell receptor or stimulation by mitogens.
Quasispecies	A genetically heterogenous population arising from a process of mutation and selection.
Sentinel cell	Tissue resident cell that initiates a host immune response.
Serodiscordant couples	A sexual partnership in which one partner is HIV-1 infected and the other is not.
Sympatric speciation	The evolutionary process by which one species adapts to another with which it overlaps geographically.
Tuberculin skin test	Intradermal injection of a standardised preparation of purified protein derivative of killed and homogenised *M. tuberculosis*.
Th17 cells	CD4 positive T helper cell subset that produces interleukin (IL) 17 on stimulation, which in turn has a canonical role in augmenting neutrophil responses to infection.
Viral rebound	Development of a detectable plasma viral load in a HIV-1 positive individual following a period of virological suppression, typically associated with an interruption in ART or the development of drug resistance.

Abstract

Co-infection with *Mycobacterium tuberculosis* is the leading cause of death in HIV-1 infected individuals. It has long been known that HIV-1 infection alters the course of *M. tuberculosis* infection and substantially increases the risk of active tuberculosis (TB). It has also become clear that TB increases levels of HIV-1 replication, propagation and genetic diversity. Therefore, co-infection provides reciprocal advantages to both pathogens. In this Review, we describe the epidemiological associations between the two pathogens, selected interactions of each pathogen with the host and our current understanding of how they affect the pathogenesis of TB and HIV-1/AIDS in co-infected individuals. We evaluate the mechanisms and consequences of HIV-1 depletion of T cells on immune responses to *M. tuberculosis*. We also discuss the effect of HIV-1 infection on the control of *M. tuberculosis* by macrophages through phagocytosis, autophagy and cell death, and we propose models by which dysregulated inflammatory responses drive the pathogenesis of TB and HIV-1/AIDS.

Key points

- There were 1.14 million new cases of HIV-1/TB co-infection and 400,000 deaths that were attributed to co-infection in 2015.
- The risk of TB increases by 2-5 fold in early HIV-1 infection and by more than 20-fold in advanced HIV-1 disease. Approximately 4-fold increased risk of TB persists in HIV-1 infected patients treated with antiretroviral therapy.
- HIV-1 infects $CD4^+$ T cells and macrophages. *Mycobacterium tuberculosis* primarily infects macrophages, which require $CD4^+$ T cells to augment intracellular clearance of microbial pathogens. Hence the depletion of $CD4^+$ T cells that is associated with HIV-1 infection is thought to have a major role in the increased risk of TB in HIV-1 infected people.
- Co-infection of HIV-1 and *M. tuberculosis* at the level of individual macrophages may also occur, but has not been demonstrated in vivo. This is important because experimental models show that HIV-1 infection of macrophages can attenuate phagocytosis and intracellular killing by the autophagy pathway.
- Progressive HIV-1 disease and TB are both characterised by chronic inflammation driven by the failure to clear either pathogen. The chronic nature of these responses may undermine host protection by promoting an immunoregulatory phenotype that is characterised by attenuated T cell responses.
- Advanced HIV-1 infection is associated with reduced immunopathology of TB co-infection, but the introduction of antiretroviral therapy can exacerbate the immunopathology of TB, giving rise to immune reconstitution inflammatory syndrome (IRIS). This reflects recovery of innate immune inflammatory responses to *M. tuberculosis*, which may be exacerbated by the recirculation of *M. tuberculosis* reactive T cells and failure of the normal homeostatic control of inflammatory responses.
- The proinflammatory response to *M. tuberculosis* may exacerbate HIV-1/AIDS disease progression by increasing virus propagation through increased transcription and cell-cell transmission.

Introduction

The retrovirus HIV-1, which causes Acquired Immunodeficiency Syndrome (AIDS), transmitted to humans from primates in the 20th century[1]. The causative agent of tuberculosis (TB), *Mycobacterium tuberculosis*, is an unencapsulated acid-fast bacillus which has been a pathogen of humans for millennia[2]. HIV/AIDS and TB are each among the 10 leading causes of death world-wide. The interaction between these two pathogens substantially contributes to this high incidence of mortality. Estimates for the incidence of HIV-1 and *M. tuberculosis* infection in 2015 highlights the scale of these epidemics (**Table 1**)[3,4]. The geographical convergence of the HIV/TB 'syndemics' in Africa and in Eastern Europe, as well as the demographic convergence in particular at-risk groups, such as prisoners[5] and miners in Southern Africa[6], further exacerbate the burden of co-infection morbidity and mortality. The impact of TB is also increasing with the emergence of multi-drug resistant (MDR)-TB (**Table 1**), for which HIV-1 is an independent risk factor[7]. Moreover, the rates of undiagnosed TB in HIV-1 positive individuals as revealed by post-mortem studies[8], suggest that the disease burden that is associated with co-infection has been underestimated.

An increased risk of TB throughout the course of HIV-1 disease has been established through epidemiological studies[9,10] (**Figure 1a**). HIV-1 co-infection also influences the clinical phenotype of TB. HIV-1 positive patients with $CD4^+$ T cell counts in the normal range present with classic symptoms of pulmonary TB, but disease that is restricted to the lung apices is less frequent, whereas pleural effusions and lymph node disease are more likely[11]. In advanced AIDS, *M. tuberculosis* frequently causes disseminated extra-pulmonary disease and mycobacteraemia[12,13].

TB may also exacerbate HIV-1 disease and AIDS progression (**Figure 1b**). Active TB is associated with higher HIV-1 viral loads in the blood[14] and cerebrospinal fluid (CSF)[15], and an increased genetic heterogeneity of the viral quasispecies[16]. This is likely to be driven by increases in HIV-1 replication at the site of co-infection[17,18]. In addition, *M. tuberculosis* co-infection may contribute to higher levels of systemic immune activation that is associated with HIV-1 disease progression[19,20], even in the context of latent TB infection (LTBI)[21]. The importance of these phenotypes in driving HIV-1 disease progression is unknown, but based on our understanding of how high viral loads[22] and immune activation[23] can drive immunosuppression, co-infection with *M. tuberculosis* may accelerate AIDS progression. Accordingly, TB has been associated with an increased incidence of additional opportunistic infections[24], and may be an independent risk factor for progression to AIDS[25]. This hypothesis is supported by the observation that an episode of successfully treated TB is associated with a four-fold increase in all-cause mortality associated with HIV HIV[26].

Understanding the mechanisms that underpin HIV-1 and TB co-infection is paramount to identifying the best approaches to overcome the global burden of disease that is caused by these pathogens. In this Review, we describe the specific host-pathogen interactions which have emerged as common features of both organisms. In particular, their ability to infect macrophages, induce type 1 interferon (IFN) responses and the role of chronic or dysregulated inflammation in the pathogenesis of disease. We then describe our current understanding of the mechanisms by which each organisminfluences the pathogenesis of the co-infecting pathogen, and consider the future challenges for research in this field.

HIV-1 infection

Cellular targets of HIV-1

CD4+ T cells are considered the primary target cells for HIV-1. Macrophages are also infected by HIV-1 and may have an important role in HIV/AIDS pathogenesis. CD4+ T cells and macrophages are also thought to be crucial for host defence against *M. tuberculosis*[27] (Figure 2). Given that macrophages are the primary intracellular niche for *M. tuberculosis*, their permissivity to HIV-1 raises the possibility that the two pathogens can co-infect individual cells. HIV-1 infection of monocyte-derived macrophages *in vitro* is well described despite the fact that macrophages express SAMHD1, an endonuclease that is active in non-dividing cells. SAMHD1 has dNTPase activity and therefore depletes the cell of nucleotides that are required for DNA synthesis, thereby restricting HIV-1 reverse transcription[28]. The simian immunodeficiency virus (SIV) accessory protein Vpx (which is absent in HIV-1) degrades SAMHD1 and makes human myeloid cells more permissive to retroviral infection. Interestingly, Vpx deficient SIV still infects myeloid cellsin non-human primates[29], suggesting that Vpx is not necessary for infection of myeloid cells in vivo. Nonetheless, the observation that HIV-1 has not evolved to counteract SAMHD1 restriction had led to the hypothesis that HIV-1 infection of macrophages does not occur in vivo. However, sub-populations of human macrophages enter a G1-like state in which SAMHD1 activity is downregulated, making the cells substantially more permissive to HIV-1[30]. These data provide a mechanism by which a retrovirus that cannot counteract SAMHD1 restriction is able to infect non-dividing myeloid cells. A subset of human alveolar macrophages that are infected with HIV-1 in vivo, have been identified by RNA fluorescence in situ hybridisation, suggesting that the virus in these cells may be transcriptionally active[31]. Furthermore, sustained HIV-1 replication in the absence of T cells has been demonstrated in non-human primates and in humanised mouse models[32,33].

Long-lived memory CD4+ T cells are thought to be the dominant cell type in which HIV-1 can establish latency and evade clearance by antiretroviral therapy (ART). Proviral DNA can also be detected in alveolar macrophages in patients on ART[34]. Moreover, viral rebound following ART treatment interruption has been described in humanized myeloid-only mice, indicating that macrophages may also act as a long term viral reservoir[35]. Importantly, the viral reservoir during ART is not completely latent, but exhibits low levels of HIV-1 replication[36]. This may occur in ‘sanctuary sites’ where drugs do not reach sufficient concentrations. Lymphoid tissue has been proposed as one such site[37,38]. Macrophage populations within the central nervous system may be another[39]. Therefore, HIV-1 replication may still influence *M. tuberculosis* infection even in patients receiving effective ART, particularly if this occurs within macrophages.

Induction and evasion of type 1 IFN responses

Type 1 IFNs represent the canonical innate immune response to viral infections[40]. This response coincides with the peak in viraemia that follows primary HIV-1 infection[41]. In various models, HIV-1 has been shown to activate innate IFN responses through pattern recognition receptors (PRRs), including toll-like receptor (TLR)-7[42], RIG-I[43] and DDX3[44] RNA sensors, as well as cGAS[45], PQBP1[46] and IFI16[47] DNA sensors. This response is thought to contribute to suppression of primary HIV-1 viraemia[48], albeit without achieving complete suppression owing to the range of counter measures that are employed by viruses to overcome IFN-inducible antiviral host proteins[49]. Interestingly, HIV-1 infection and replication in macrophages, representing key tissue resident innate immune sentinel cells, fails to induce innate IFN responses as a result of interactions between the viral capsid and host proteins that shield the nascent DNA products of viral reverse transcription from host DNA sensors[50]. Macrophages successfully restrict HIV-1 in response to type 1 IFN[51]. Therefore, the ability of

the virus to evade innate immune detection in macrophages may have an important role in establishing persistent HIV-1 infection.

HIV-1 infection as a chronic inflammatory disease

HIV-1 infection is increasingly considered a chronic inflammatory disease that leads to immunodeficiency. Inflammatory markers are elevated throughout the asymptomatic phase of infection[52] and correlate with the rate of progression to AIDS[23]. The same phenotype is not evident in non-pathogenic SIV infection of non-human primates and in HIV-1 infected children who do not progress to immunodeficiency[53].

Several mechanisms are thought to lead to the chronic immune activation that is associated with HIV-1 infection[52]. Chief among these is the translocation of microbial products from the gastrointestinal lumen into the bloodstream, following massive T cell depletion in gastrointestinal-associated lymphoid tissue (GALT) during primary HIV-1 infection. This is thought to be caused by lytic infection of GALT Th17 cells[54], which are particularly permissive to retroviral infection[55]. Non-productive HIV-1 infection of resting T cells by cell-cell spread in lymphoid tissue may also contribute to T cell depletion and immune activation. In this model, incomplete DNA products of reverse transcription are recognised by the cytosolic DNA sensor IFI16, leading toactivation of the inflammasome, leading to cell death by pyroptosis and the release of proinflammatory IL1β[56,57], thereby linking $CD4^+$ T cell depletion and chronic inflammation[58,59]. In contrast to this inflammatory mechanism of T cell death, HIV-1 proviral DNA integration may activate DNA damage pathways that can cause T cell apoptosis[60]. Chronic immune activation may also lead to premature immune senescence or compensatory immunoregulation[61–63]. Research efforts have focussed on the role of chronic type 1 IFN responses in persistent HIV-1 infection. In a non-human primate model of pathogenic HIV-1 infection, chronic IFN stimulation caused IFN desensitisation and consequently, reduced expression of IFN-dependent antiviral factors, leading to increased viral replication and further T cell depletion[48].

The strong link between HIV-1 infection and chronic inflammation raises the question of whether the virus gains an advantage by driving this phenotype. One which, co-infection with *M. tuberculosis* may be expected to compound as it also induces chronic inflammation. Inflammatory signalling may benefit HIV-1 by directly stimulating viral replication (discussed below). In addition, the recruitment of leukocytes could potentially provide HIV-1 with a source of target cells to infect. This may be particularly important as HIV-1 infects new cells most efficiently by direct cell-cell transmission via a 'virological synapse' actively orchestrated by the virus[64]. Cell-cell transmission also shields the virus from neutralising antibodies[65], leads to more rapid viral gene expression[66] and enables the virus to overcome cell tropism barriers, such as infection of macrophages by non-macrophage tropic viruses[67].

[Au: Please insert a brief summary paragraph (2-3 sentences) to conclude this section and to refocus the reader back to HIV/TB co-infection.]

M. tuberculosis infection

Infection of macrophages

M. tuberculosis is a facultative intracellular pathogen of macrophages. Once inside, *M. tuberculosis* quickly adapts to the environment within the phagosome through transcriptional reprogramming in order to upregulate iron scavenging mechanisms, switch to anaerobic respiratory pathways, and to use cholesterol as a carbon source and aspartate as a nitrogen source[68]. Within macrophages, *M. tuberculosis*-containing phagosomes

fail to undergo the normal process of maturation and acidification that is associated with phagolysosomal fusion and by which phagosomal cargo is usually degraded. Multiple *M. tuberculosis* virulence factors are thought to contribute to this phenotype[69].

Macrophage cell death is a key feature of granulomatous pathology in TB. Central to this process is the bacterial ESX-1 secretion system and the secreted effector molecule ESAT6. These are encoded by the RD1 locus which is deleted in the live attenuated *Mycobacterium bovis*, bacille Calmette–Guérin (BCG) vaccine. ESX-1 is required for *M. tuberculosis* to escape from phagosomes into the host cell cytoplasm, and for triggering cell death pathways[70]. ESAT6 can also trigger macrophage cell death through apoptosis[71]. In addition, ESAT6 has been reported to activate the inflammasome[72], suggesting that *M. tuberculosis* may cause cell death through inflammasome/caspase 1-mediated pyroptosis. The triggering of cell death promotes bacterial dissemination through efferocytosis[73] and by releasing bacteria into the extracellular space for onward transmission to new hosts.

Induction of innate immune type 1 IFN responses

Mycobacterial lipids, lipoproteins and nucleic acids trigger a range of innate immune responses when they are sensed by host PRRs within macrophages[74,75]. These responses are thought to be crucial for further immune cell recruitment and for the production of antimicrobial peptides[76]. Innate immune responses to *M. tuberculosis* include type 1 IFN responses that have conventionally been associated with antiviral responses[77]. The observation that type 1 IFNs are immunosuppressive in chronic viral infections has led to studies to determine whether type 1 IFNs counteract immunoprotective IFNγ-dependent or IL1β-dependent mechanisms of *M. tuberculosis* clearance[78]. Induction of type 1 IFN responses is principally mediated through the recognition of *M. tuberculosis* nucleic acids by the cytosolic DNA sensor cGAS[75]. This is dependent on ESX-1-mediated *M. tuberculosis* phagosomal escape, which also drives inflammasome maturation of IL1β [79]. Interestingly, the levels of effector molecules (ESAT6) that are secreted by the ESX-1 system determines the outcome of host cellular responses polarised towards either type 1 IFNs or IL1β). *M. tuberculosis* strains that are more virulent have been found to produce more ESAT6 and more type 1 IFNs[80].

Chronic inflammation to promote transmission

Similar to HIV-1 infection, chronic inflammation is the hallmark of TB pathology. Immunopathogenesis may even be more important in this case because, unlike HIV-1, *M. tuberculosis* is an obligate pathogen. Its ability to escape the intracellular niche, cause pulmonary cavitation and induce coughing through chronic inflammation within airways, are necessary for its dispersal between individuals. Matrix metalloproteinase (MMP)-1 has a crucial role in pulmonary cavitation that is associated with *M. tuberculosis* infection[81]. This protein belongs to a family of host proteinases that degrade the extracellular matrix. MMPs are produced by macrophages, epithelial cells and fibroblasts in response to pro-inflammatory cytokines. In this context, chronic pro-inflammatory T cells may contribute to the pathology in response to persistent *M. tuberculosis*. The observation that virulent *M. tuberculosis* strains have highly conserved immunodominant T cell epitopes suggests that *M. tuberculosis* does not rely on antigenic variation to evade protective immunity[82,83]. In addition, it raises the possibility that the conservation of these immunodominant responses may be beneficial to the pathogen. Thereby, *M. tuberculosis* may commandeer T cell responses to promote immunopathology and consequently, its transmission.

HIV/TB co-infection

HIV-1 depletion of *M. tuberculosis* reactive T cells

Notwithstanding the hypothesis presented above that T cell responses in TB may contribute to pathogenesis of disease, they have long been thought to have an important role in immunological protection against *M. tuberculosis* by promoting intracellular bacterial killing or restriction (Figure 2). Genetic deficiencies in IL12 signalling (which is required for Th1 cell differentiation), or IFNγ signalling (representing the canonical product of Th1 responses), give rise to Mendelian susceptibility to mycobacterial disease (MSMD)[84]. HIV-1 co-infection further highlights the importance of T cell mediated immunity. Substantially increased risk of TB and its extrapulmonary dissemination is strongly correlated with $CD4^+$ T cell depletion in HIV-1 infected individuals. T cell depletion is evident in peripheral blood, in the respiratory tract and at the site of tuberculin skin test (TST) challenge[85–87]. Assuming that $CD4^+$ T cell protection against *M. tuberculosis* is conferred by the pro-inflammatory cytokines that they produce, it is notable that the proportions of polyfunctional *M. tuberculosis* reactive T cells**,** which produce the pro-inflammatory cytokines IFNγ, tumour necrosis factor (TNF) and interleukin (IL)2 are also depleted in HIV-1 infected individuals[86]. Hence, HIV-1 depletes T cell populations that are likely to be functionally important for protection against TB. Transcriptional profiling of biopsies taken from the site of the TST challenge in humans confirmed that T cell recruitment and IFNγ activity were both substantially reduced in HIV-1/TB co-infected patients, with blood $CD4^+$ T cell counts of <200 per mL, indicative of advanced HIV-1 disease[85].

HIV-1 infected T cells may also contribute to the increased risk of TB in early HIV-1 disease before substantial depletion of peripheral blood CD4 counts[87]. HIV-1 DNA was detected more frequently in *M. tuberculosis* specific T cells. These T cells produced high levels of IL2 which made them more permissive to HIV-1 infection. In comparison to the total memory T cell population or memory T cells that specifically recognise human cytomegalovirus, *M. tuberculosis* specific T cells were preferentially depleted in early HIV-1 infection. Taken together these data suggest that the depletion of these cells may be a direct result of HIV-1 infection. Transcriptional profiling of the TST challenge site biopsies in HIV-1/TB co-infected patients with blood $CD4^+$ T cell counts >200 /mL also revealed less T cell recruitment at the site of the antigenic challenge, compared to HIV-1 negative patients with active TB[85]. However, the functional significance of the reduced T cell recruitment observed in this study is currently unknown, as comparable levels of IFNγ inducible gene expression were found in the HIV-1 positive and negative groups. Hence, IFNγ activity as a surrogate of robust CD4 T cell responses to mycobacterial antigens was preserved in early HIV-1 disease, despite previous reports of preferential depletion of *M. tuberculosis* specific T cells. These data suggest that increased risk of TB in HIV-1 infected patients is not solely mediated by T cell depletion.

Of the other T cell populations that may contribute to HIV-1-associated TB, Th17 and Th22 cells are the most plausible candidates. A functional role for these T cell populations in immunological protection against TB is primarily based on data obtained from experiments in mice and by their role in the recruitment of phagocytic cells including macrophages[88–90]. The depletion of these T cell subtypes during primary HIV-1 infection[54,91] may therefore contribute to differences in the immune response to *M. tuberculosis* in HIV-1 co-infected patients compared to HIV negative patients. Another T cell population that becomes depleted in HIV-1 infection are mucosal associated invariant T (MAIT) cells[92]. These are $CD8^+$ innate lymphoid cells which recognise bacterial metabolites of vitamin B that are presented by a non-polymorphic MHC-like molecule, MR1[92]. MAIT cells are activated by *M. tuberculosis* and are enriched at the site of TB disease[93]. Therefore, their depletion in HIV-1

infection may attenuate a component of host immune responses to *M. tuberculosis*. MAIT cells are not infected by HIV-1. Their depletion is thought to be caused indirectly by immune activation. Importantly, by comparison to the general population, the risk of active TB remains higher in HIV-1 infected patients even after becoming established on effective ART[94]. In this context, the failure of ART to restore the T cell repertoire, including MAIT cells[95,96], may also be a significant factor in the persistently elevated risk of TB.

HIV-1 inhibition of phagocytosis and autophagy in macrophages

M. tuberculosis has evolved to survive and grow within macrophages. Nonetheless, *M. tuberculosis* phagocytosis by macrophages is thought to restrict mycobacterial growth. The best evidence for this is comes from experiments in the zebrafish *Mycobacterium marinum* model in which bacillary uptake by macrophages is elegantly visualised. In this model, macrophage depletion, delayed macrophage recruitment or necrotic macrophage cell death are all associated with increased microbial burden[73,97,98]. Once the bacteria are phagocytosed, phagolysosomal fusion that would lead to bacterial killing is inhibited by *M. tuberculosis*. This may be overcome by the autophagy pathway[99,100], and by inducible production of bacteriocidal nitric oxide or a range of antimicrobial peptides, all of which are generally upregulated by the action of IFNγ.

HIV-1 infection has been reported to inhibit macrophage phagocytosis dependent on diverse cell surface receptors and mediated by HIV-1 infection of the affected cell[101]. In this study, the HIV-1 accessory protein Nef was found to be both necessary and sufficient to inhibit phagocytosis by directly interacting with adapter protein AP-1 to inhibit the recruitment of recycling endosomes that are required for phagosome biogenesis[101]. It is also possible that these effects may be mediated indirectly by the action of circulating virus-free accessory proteins on uninfected macrophages, which can be detected *in vivo*[102,103]. Moreover, impaired phagocytosis has also been observed in HIV-infected alveolar macrophages *ex vivo*[31]. Interestingly, HIV-1 Nef also inhibits the autophagy pathway by blocking the maturation of autophagosomes through a direct interaction with the autophagy regulator, Beclin-1[104]. This inhibition was found to protect nascent virion assembly from autophagic degradation. Consistent with these effects of HIV-1 infection on phagocytosis and autophagy (Figure 2), HIV-1 co-infection in macrophages that are infected with *M. tuberculosis* has been associated with increased mycobacterial growth[105]. Interestingly, however, vitamin D treatment of co-infected macrophages was reported to restrict both *M. tuberculosis* and HIV-1 replication by an autophagy-dependent mechanism, suggesting that the inhibition of autophagy by HIV-1 is easily overcome by the action of vitamin D[106]. Vitamin D deficiency is undoubtedly prevalent amongst populations at greatest risk of co-infection[107]. Therefore, if HIV-1 inhibition of autophagy is an important determinant for increased risk of TB, vitamin D supplementation may substantially reduce the incidence of TB disease in HIV-1 infected patients while at the same time supporting immune control of the virus. However, this hypothesis has yet to be tested in clinical trials.

Macrophage cell death and tissue necrosis in HIV/TB co-infection

HIV-1 does not cause macrophage cell death[51]. A number of reports suggest that HIV-1 proteins reduce *M. tuberculosis*-associated macrophage apoptosis; potentially by HIV-1 Nef inhibition of TNF responses to *M. tuberculosis*[108–110]. Ccellular apoptosis has been considered as a mechanism for limiting intracellular *M. tuberculosis* growth. Therefore, by inhibiting apoptosis, HIV-1 infection may compromise *M. tuberculosis* restriction. However, live cell imaging data has recently contradicted this hypothesis by demonstrating that cell death is associated with *M. tuberculosis* growth rather than restriction[111]. A key observation in HIV-1 infected patients with pulmonary TB is the presence of fewer necrotic granuloma and less pulmonary cavitation. Interestingly, in active TB MMP1 levels are significantly lower in respiratory tract samples from HIV-1 infected

patients with severe CD4+ T cell depletion, compared to HIV-1 negative patients[112]. These observations have largely supported the hypothesis that T cell responses to *M. tuberculosis* contribute substantially to cellular necrosis and tissue damage. In agreement with this cellular necrosis and tissue damage are reduced in patients with AIDS, but enhanced host cell viability in advanced HIV-1 disease does not achieve better *M. tuberculosis* control. Consistent with the reduction in pulmonary cavitation, HIV-1 co-infected patients may transmit less *M. tuberculosis*[113]. Nonetheless, at the population level, an increased incidence of active TB in HIV-1 infected patients ultimately promotes the onward transmission of *M. tuberculosis*.

Immunopathology of *M. tuberculosis* in HIV-1 infected patients

The most direct evidence to support the hypothesis that there is a reduction of *M. tuberculosis* immunopathology in patients with AIDS, is the phenomenon of TB immune reconstitution inflammatory response syndrome (TB-IRIS). TB-IRIS is the development of increased inflammatory pathology in patients following the commencement of ART, and may manifest by either a worsening of known TB disease, or 'unmasking' of previously asymptomatic *M. tuberculosis* infection[114]. TB-IRIS occurs in ~15% of HIV-1 infected patients starting ART[115] and is most commonly found in patients with very low peripheral blood CD4+ T cell counts and evidence of a high *M. tuberculosis* bacillary load before starting ART. The pathological features of TB-IRIS include systemic responses such as fever and increased acute neutrophilic inflammation at the site of *M. tuberculosis* infection. Comparisons of peripheral blood transcriptional profiles in cohorts of HIV/TB co-infected patients with and without TB-IRIS revealed that in cases of TB-IRIS, there were increases in the expression of IFN, MyD88 and inflammasome-dependent innate immune responses[116]. These data suggest that TB-IRIS may be caused by the recovery of innate immune responses to *M. tuberculosis*, and presumably failure of immunoregulation that would ordinarily control pathogenic innate inflammatory responses. By inference these data suggest that HIV-1 may downregulate innate immune and immunoregulatory responses to *M. tuberculosis*, as well as classical Th1 responses. With the notable exception of type 1 IFNs, the wide repertoire of pro-inflammatory transcriptional innate immune responses at the site of TST challenge, were found to be lower in patients with active TB and advanced HIV-1 co-infection compared to.HIV-1 negative controls[85]. By contrast, patients presenting with unmasking TB-IRIS had substantially higher pro-inflammatory transcriptional responses to the TST compared to HIV-1 negative patients with active TB, consistent with exaggerated inflammatory responses. In this study, unmasking TB-IRIS was associated with features that are associated with Th2 responses and increased granulocyte colony stimulating factor (CSF3) expression that is known to augment neutrophil responses[85].

How HIV-1 might inhibit innate immune responses to *M. tuberculosis* remains unclear. Both pathogens can infect macrophages, which are widely recognised to generate pro-inflammatory innate immune responses. Attenuated innate immune responses to prototypic stimuli such as lipopolysaccharide (LPS) by alveolar macrophages from HIV-infected individuals has been reported[117,118]. The HIV-1 accessory proteins Nef, Vpu and Vpr have each been reported to inhibit innate immune intracellular signalling pathways[119]. Consistent with these reports, HIV-1 infection of macrophages attenuated activation of the canonical NFkB pathway in response to LPS[120]. However, genome-wide transcriptional responses to LPS were largely preserved. In the same experimental model of macrophages that were infected with HIV-1, pro-inflammatory innate immune responses to *M. tuberculosis* co-infection were also preserved[121–123]. Instead, HIV-1 infection was associated with attenuated immunoregulatory IL10 expression, leading to exaggerated pro-inflammatory responses at subsequent time points[121]. These data suggest that any HIV-1 associated inhibition of innate immune

pro-inflammatory responses to *M. tuberculosis* by HIV-1 does not arise because of co-infection at the cellular level.

Indirect effects on macrophage innate immune responses may also result from HIV-1 modulation of T cells, for example, by alterations in the cytokine milieu that acts on uninfected macrophages. Therefore, an alternative hypothesis may be that pathogenic innate immune responses to *M. tuberculosis* are amplified by T helper cells. In such a model, the immunopathogenesis of TB-IRIS may be driven by the recirculation of *M. tuberculosis* reactive T cells, leading to the recovery of innate immune inflammatory responses and compounded by high bacterial loads which have accumulated in the immunosuppressed patient providing a higher dose for stimulation of innate immune responses[114]. In this context, HIV-1 attenuation of IL10 responses in macrophages may represent foci of deficient immunoregulation that leads to pathological inflammation. In this regard, we found that deficient IL-10 responses persist in infected macrophages in the presence of antiretrovirals in vitro[121], and others have demonstrated the persistence of dysregulated phagocyte phenotypes after antiretroviral treatment, such as increased TLR-2 expression[124] and a dysregulation of complement pathways[125]. Hence, TB-IRIS may be caused by a combination of high bacterial burden, T cell recovery and failure of immune regulation in HIV-1 infected macrophages (Figure 3). Interestingly, TST challenge experiments revealed attenuated IL10 responses in HIV-1 infected patients with $CD4^+$ T cells >200 /mL [85]. Therefore, an increased risk of active TB in early HIV-1 disease may also partly reflect a propensity for immunopathology as a result of inadequate IL10 regulation.

Effects of TB on HIV-1 replication

Increased HIV-1 viral loads in the lungs of co-infected patients with pulmonary TB is well established[17,18,126]. This is commonly associated with increased viral load in peripheral blood[14]. Whether the increase in circulating virus arises from replication in the lung alone or is also due to increased systemic virus replication is not known. As most HIV-1 replication occurs in activated T cells, their recruitment to sites of granulomatous inflammation in TB may facilitate rapid virus propagation through the accumulation of tightly packed permissive cells and hence cell-cell transmission. However, the host immune response to *M. tuberculosis* also increases HIV-1 transcription. The HIV-1 long terminal repeat (LTR) includes binding sites for several host transcription factors which are activated by innate immune and cytokine signalling pathways. These include the NFκB, AP1, CCAAT/enhancer binding protein (C/EBP), CREB/ATF and NFAT families of transcription factors[127]. Innate immune activation by *M. tuberculosis* or mycobacterial products increased HIV-1 transcription and replication in myeloid cell lines, through the action of C/EBP, NFκB and NFAT5[128–130].

Experimental data on the effects of *M. tuberculosis* infection on HIV-1 transcription in macrophages are inconclusive. In macrophages that were infected with HIV-1, co-infection with BCG caused a dose dependent suppression of virus production. This was attributed to the C/EBP binding motif in the viral LTR and associated with production of a type 1 IFN inducible inhibitory isoform of C/EBPβ, leading to a model in which mycobacterial induction of type 1 IFNs may restrict HIV-1 transcription[131]. These data are somewhat inconsistent with the current view of ESX-1-dependent induction of type 1 IFN responses by *M. tuberculosis*[79] given that BCG lacks ESX-1[132]. Interestingly, alveolar macrophages from healthy lung tissue express high levels of inhibitory C/EBPβ, but this is strongly downregulated in cells that are isolated from the site of pulmonary TB[131], suggesting that the pro-inflammatory milieu that are present in active TB granuloma overcomes type 1 IFN-mediated inhibition of virus transcription. Direct evidence for this hypothesis was shown in HIV-1 infected macrophages where co-infection with *M. tuberculosis* led to an initial decrease in HIV-1

transcription followed by a substantial increase[121]. The increase in virus transcription was co-incident with sustained pro-inflammatory responses as a result of HIV-1 attenuation of early IL10 regulatory responses, and complementation of deficient IL10 responses reversed the increase in viral transcription in *M. tuberculosis* co-infected macrophages[121]. IL10 has been reported to inhibit HIV-1 transcription by STAT3-dependent induction of inhibitory C/EBPβ and by inhibition of cyclin-T1 that is required for HIV-1 Tat-dependent transactivation of viral transcription[133–135]. Conversely, it has long been established that some canonical pro-inflammatory cytokines, for example, TNF, IL6 and IL1β, individually or synergistically upregulate HIV-1 transcription[136–138]. Notably, IFNγ (which is enriched within TB granuloma) shows substantial overlap with the antiviral effects of type 1 IFNs[51]. Despite this, IFNγ may still act synergistically with TNF to promote virus transcription[139].

Increased HIV-1 replication in macrophages as a result of pro-inflammatory responses to *M. tuberculosis* may also be accompanied by increased macrophage permissivity to nascent HIV-1 infection. Macrophages had previously been considered as terminally differentiated cells that are unable to replicate. Mouse experiments have demonstrated macrophage replication in response to inflammatory stimuli, for example modelled by helminth infection[140] and for maintenance of embryologically derived resident populations[141–143]. Recent findings show that polyploid giant cells within granuloma arise from macrophages that enter cell cycle but do not complete cytokinesis[144]. In addition, macrophages that enter the cell cycle but arrest in a G1-like state, downregulate SAMHD1 activity to allow DNA synthesis, and consequently become more permissive to HIV-1 by facilitating reverse transcription[30]. Taken together, the pro-inflammatory cytokine response to *M. tuberculosis* (facilitated by HIV-1 attenuation of immune regulation), recruitment and activation of T cells, and modulation of macrophage cell cycle phenotype act in concert to enhance virus replication and propagation (Figure 4). Hence these co-infecting pathogens successfully cooperate to usurp host defences to their own advantage.

Future perspectives

In resource-poor settings where the highest incidence of HIV-1 and *M. tuberculosis* co-infection is found, ART has substantially reduced the incidence of co-infection and improved clinical outcomes[145]. In the pre-ART era, the majority of co-infection was evident in people with advanced HIV-1 disease. Assuming that ART programmes continue to grow in these settings, the majority of morbidity and mortality that is associated with HIV/TB co-infection may be caused by the increased risk of TB in patients with early HIV-1 infection prior to ART initiation; TB-IRIS arising in patients starting ART; and the residual increased risk of active TB in patients on ART. Therefore, ongoing research that focusses on the mechanisms by which the two pathogens interact in these circumstances, and identification of possible therapeutic targets, is necessary. Specific priorities include being able to (1) stratify the risks of active TB in order to inform optimal use of systematic screening for active TB or strategies for preventative therapy; (2) to identify opportunities for host directed therapies that treat or reduce the risk of TB-IRIS, or population level interventions to reduce the risk of active TB, such as vitamin D supplementation; and (3) to evaluate the effects of active TB or LTBI on subclinical viral replication and diversification that may promote HIV-1 drug resistance and persistence.

The application of whole-genome sequencing and single genome amplification[146] will offer greater depths of resolution to explore the effect of *M. tuberculosis* co-infection on HIV-1 diversity. Likewise, whole-genome sequencing of *M. tuberculosis* is expected to offer more insight into transmission chains[147] in order to assess

the impact of HIV-1 on the spread of *M. tuberculosis*, and whether HIV-1 influences sympatric speciation of *M. tuberculosis*[148]. Interestingly, a recent whole-genome sequencing study of *M. tuberculosis* strains in HIV-1 infected and uninfected individuals suggested virus-induced changes to bacterial evolution that disrupt the unusually high degree of epitope conservation in *M. tuberculosis*[149].

HIV-1, and for the most part *M. tuberculosis,* are exclusively human pathogens, often rendering conventional small models of disease misrepresentative. The recent developments in humanised mouse models for HIV-1 may also represent a significant opportunity to study co-infection. Likewise, the use of non-human primates in which the combination of blood sampling and functional imaging offers the opportunity for detailed and well-controlled longitudinal experiments to study co-infection. The prospect of human TB challenge models are also emerging[150]. In addition to the primary goal of developing tools to evaluate *M. tuberculosis* vaccine efficacy, these would offer unprecedented opportunities to identify correlates of protection and to study pathogenesis in co-infected individuals, particularly in the context of ART. These models could enable the assessment of variables other than T cell depletion during co-infection, and explore the effects of chronic inflammation, ageing, smoking, obesity and diabetes. Ultimately, the study of HIV/TB co-infection will also be crucial in the development of effective vaccines for these important pathogens, based on our understanding of how they undermine host defences through their interactions.

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Competing interests statement

The authors declare no competing interests.

Publisher's note

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Text

Co-infection with *Mycobacterium tuberculosis* is the leading cause of death in HIV-1 infected individuals. In this Review, Bell and Noursadeghi describe the epidemiological associations between the two pathogens, selected interactions of each pathogen with the host and our current understanding of how they affect the pathogenesis of tuberculosis and HIV-1/AIDS in co-infected individuals.

Subject categories

Biological sciences / Immunology / Infectious diseases / HIV infections

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Biological sciences / Immunology / Infectious diseases / Tuberculosis

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Biological sciences / Microbiology / Virology / Viral pathogenesis

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Biological sciences / Immunology / Immunological disorders / Immunological deficiency syndromes / HIV infections

[URI /631/250/249/1570/1901]

Health sciences / Pathogenesis / Immunopathogenesis

[URI /692/420/2780]

Tables

Table 1: HIV-1, active tuberculosis (TB) and HIV-1/TB co-infection in 2015[3,4].

Global burden of disease	
New HIV-1 infections	2.1 million
Individuals living with HIV-1	36.7 million
New cases of active TB	10.4 million
New cases of MDR-TB	480,000
Individuals with latent TB infection (LTBI)	1 in 4 individuals globally
Mortality	
Deaths attributed to HIV-1	1.1 million
Deaths attributed to TB	1.8 million
TB case fatality rate	17%
Burden of co-infection	
Cases of active TB among individuals with HIV-1	1.14 million
Deaths attributed to HIV-1/TB co-infection	400,000

Figure legends

Figure 1. HIV-1/TB co-infection increases the risk of active TB and HIV-1 disease progression.

(A) The risk of active tuberculosis (TB) increases to 2-5 fold above baseline soon after an individual is infected with HIV-1 during the early and chronic phases of infection. As HIV-1 progresses and causes severe immunodeficiency, the risk of TB is further increased to at least 20-fold greater than the general population. TB risk is accrued with longer times spent at low blood $CD4^+$ T cell counts. Moreover, antiretroviral therapy (ART) for HIV-1 does not fully restore the risk to baseline. There remains >4 fold increased rates of active TB even once $CD4^+$ T cell counts have reconstituted. **(B)** Incident *Mycobacterium tuberculosis* co-infection in HIV-1 infected people increases HIV-1 replication and consequently viral diversity. It may also potentiate chronic immune activation, accelerating the progression of HIV-1 disease.

Figure 2. HIV-1 Nef may compromise host control of *Mycobacterium tuberculosis* by inhibition of bacterial phagocytosis and autophagic clearance of phagosomal cargo.

Macrophage control of *M. tuberculosis* is thought to be mediated by bacterial phagocytosis and the clearance of *M. tuberculosis* containing phagosomes that fail to undergo phagolysosomal fusion via the autophagy pathway. HIV-1 co-infection can undermine this mechanism of host defence at multiple levels. *M. tuberculosis* clearance by this pathway is also dependent on vitamin D and augmented by Th1 responses through the action of IFNγ. Deficiency of IFNγ responses owing to depletion of *M. tuberculosis* reactive Th1 cells in progressive HIV-1 disease is thought to be the canonical mechanism by which HIV-1 compromises host defence against *M. tuberculosis*. In addition, the HIV-1 Nef accessory protein reduces macrophage phagocytic capacity by inhibiting AP-1-mediated endosomal recycling that is needed to form nascent phagosomes. Autophagosome assembly is increased in HIV-1 infected macrophages, but their maturation and clearance function by fusion with lysosomes is attenuated by the interaction of HIV-1 Nef with the autophagy related gene, Beclin-1. Interestingly, vitamin D supplementation may overcome this inhibition of autophagosome maturation to improve both *M. tuberculosis* clearance, and HIV-1 restriction by autophagy.

Figure 3. High bacillary burden, T cell recovery and HIV-1-induced failure of immunoregulation drive TB immune reconstitution inflammatory syndrome (TB-IRIS).

Exaggerated pro-inflammatory responses that are normally derived from innate immune activation of myeloid cells are the dominant feature of TB-IRIS, and T cell derived IFNγ responses are known to augment innate immune responses by macrophages (A). Therefore the most likely model for exaggerated macrophage derived inflammation in TB-IRIS is the combined effects of high bacillary burden in immunocompromised patients before the onset of antiretroviral therapy (ART) **(B)** and recirculation of *Mycobacterium tuberculosis* reactive T cells after ART initiation **(C)**. Although ART effectively blocks cell-cell propagation of the virus, the treatment does not clear integrated HIV-1 provirus in macrophages which continue to express HIV-1 proteins. **(D)** In this context HIV-1 inhibition of macrophage IL10 responses to *M. tuberculosis* may also contribute to exaggerated inflammatory responses by a failure of immunoregulation **(D)**. CC, chemokines.

Figure 4. Tuberculosis increases HIV-1 replication and propagation through innate immune signalling pathways, proinflammatory cytokines and failure of immunoregulation.

(A) Innate immune signalling pathways in macrophages can increase HIV-1 transcription through activation of NFkB, C/EBP, CREB/ATF and NFAT transcription factors. The host cell response to innate immune activation by *Mycobacterium tuberculosis* leads to the production of a range of proinflammatory cytokines and chemokines. These drive the local recruitment of T cells as part of a prototypic cell mediated immune response **(B)**. The accumulation of activated T cells provides a population of cells permissive to HIV-1 and allows for

rapid virus propagation by direct cell-cell spread **(C)**. The pro-inflammatory cytokines also serve to promote transctivation of virus replication through the action of NFkB and NFAT transcription factors **(D)**. HIV-1 attenuation of IL10 responses to *M. tuberculosis* favours the virus by reducing IL10 mediated inhibition of HIV-1 transcription via C/EBPβ and by promoting pro-inflammatory responses through a failure of immunoregulation **(E)**. Although *M. tuberculosis* induces type1 IFN responses in macrophages, which would be expected to promote an antiviral state, any autocrine or paracrine inhibition of HIV-1 replication is transient **(F)**. Recent data has emerged to show that *M. tuberculosis* causes macrophage polyploidy through activation of the cell cycle coupled to cytokinesis failure **(G)**. G1-like macrophages are more permissive to HIV-1. Whether, *M. tuberculosis* induction of multinucleated giant cells further increases the HIV-1 permissive host cellular niche, merits further investigation. CC, chemokines.

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Figure 1

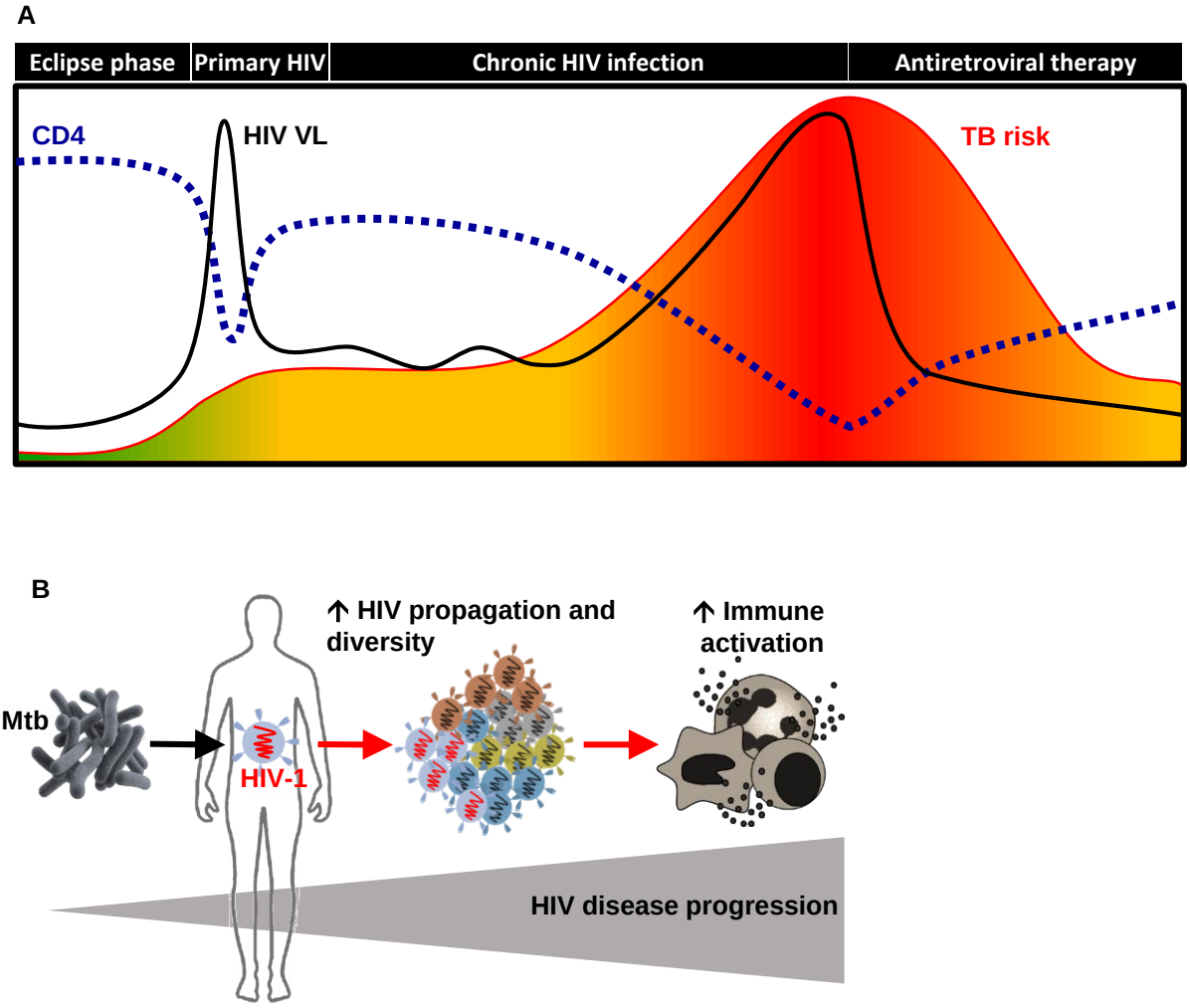
A
Eclipse phase
Primary HIV
Chronic HIV infection
Antiretroviral therapy
CD4
HIV VL
TB risk
B
Mtb
HIV-1
↑ HIV propagation and diversity
↑ Immune activation
HIV disease progression

Figure 2

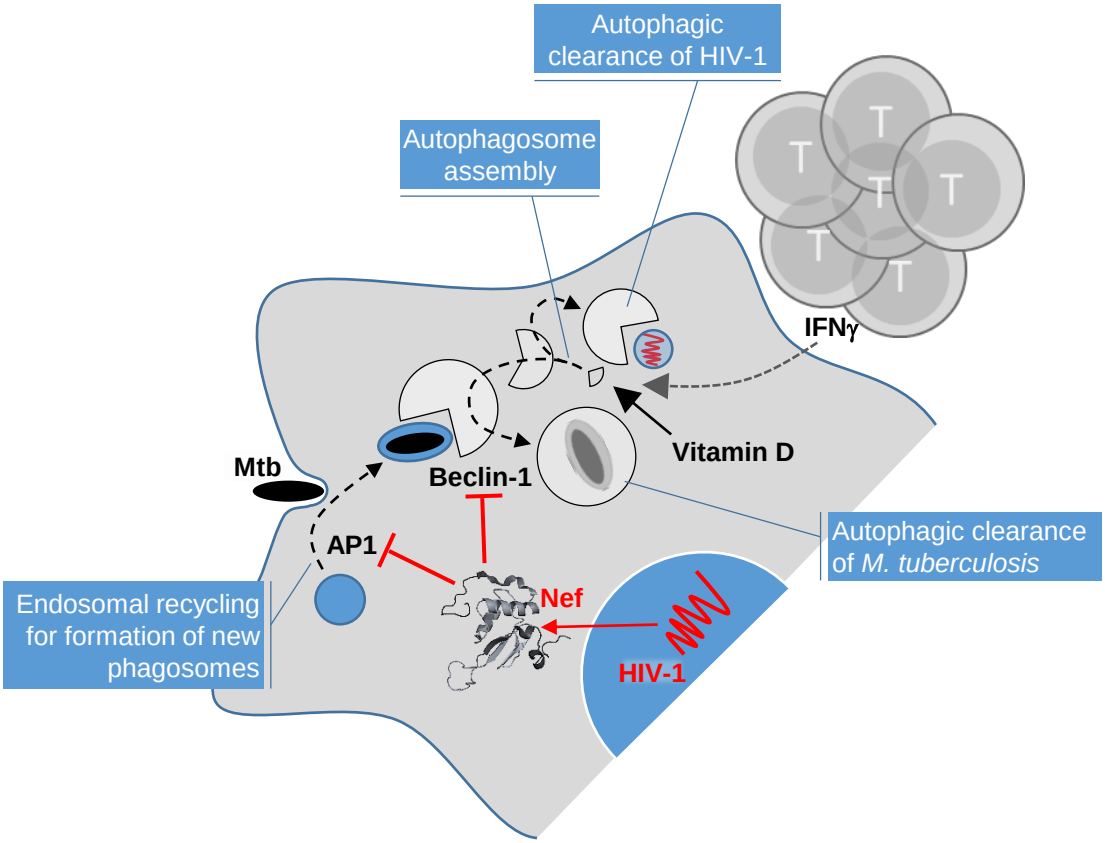
Autophagic clearance of HIV-1
Autophagosome assembly
T
IFNγ
Vitamin D
Mtb
Beclin-1
AP1
Nef
HIV-1
Autophagic clearance of M. tuberculosis
Endosomal recycling for formation of new phagosomes

Figure 3

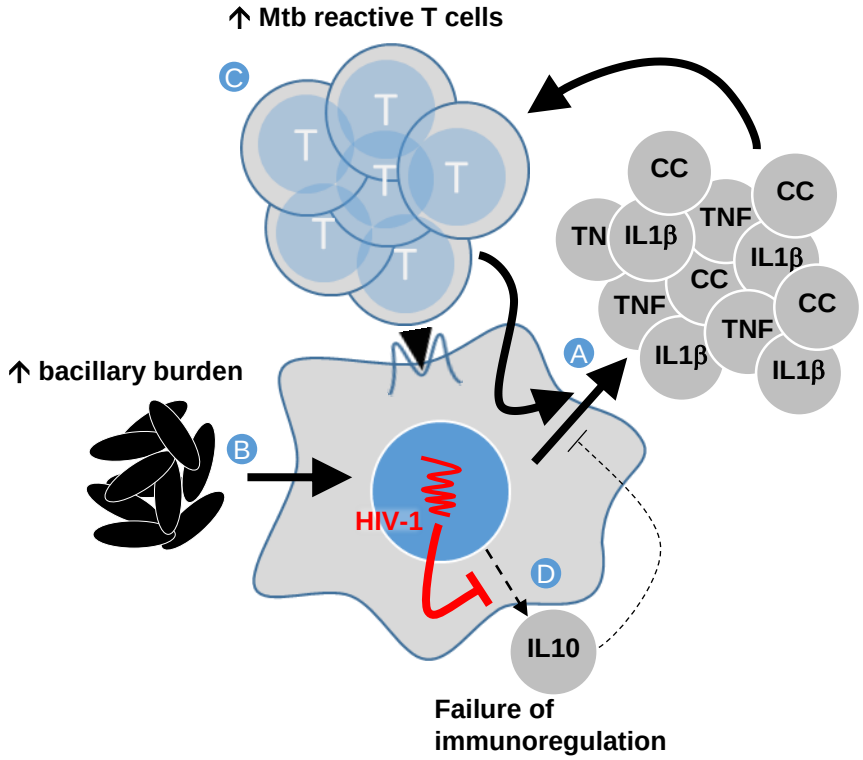
↑ Mtb reactive T cells
C
T
T
T
T
T
T
CC
CC
TN
IL1β
TNF
IL1β
CC
TNF
CC
TNF
IL1β
IL1β
A
↑ bacillary burden
B
HIV-1
D
IL10
Failure of
immunoregulation

Figure 4