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# Recent progress in understanding immune activation in the pathogenesis in HIV-TB co-infection

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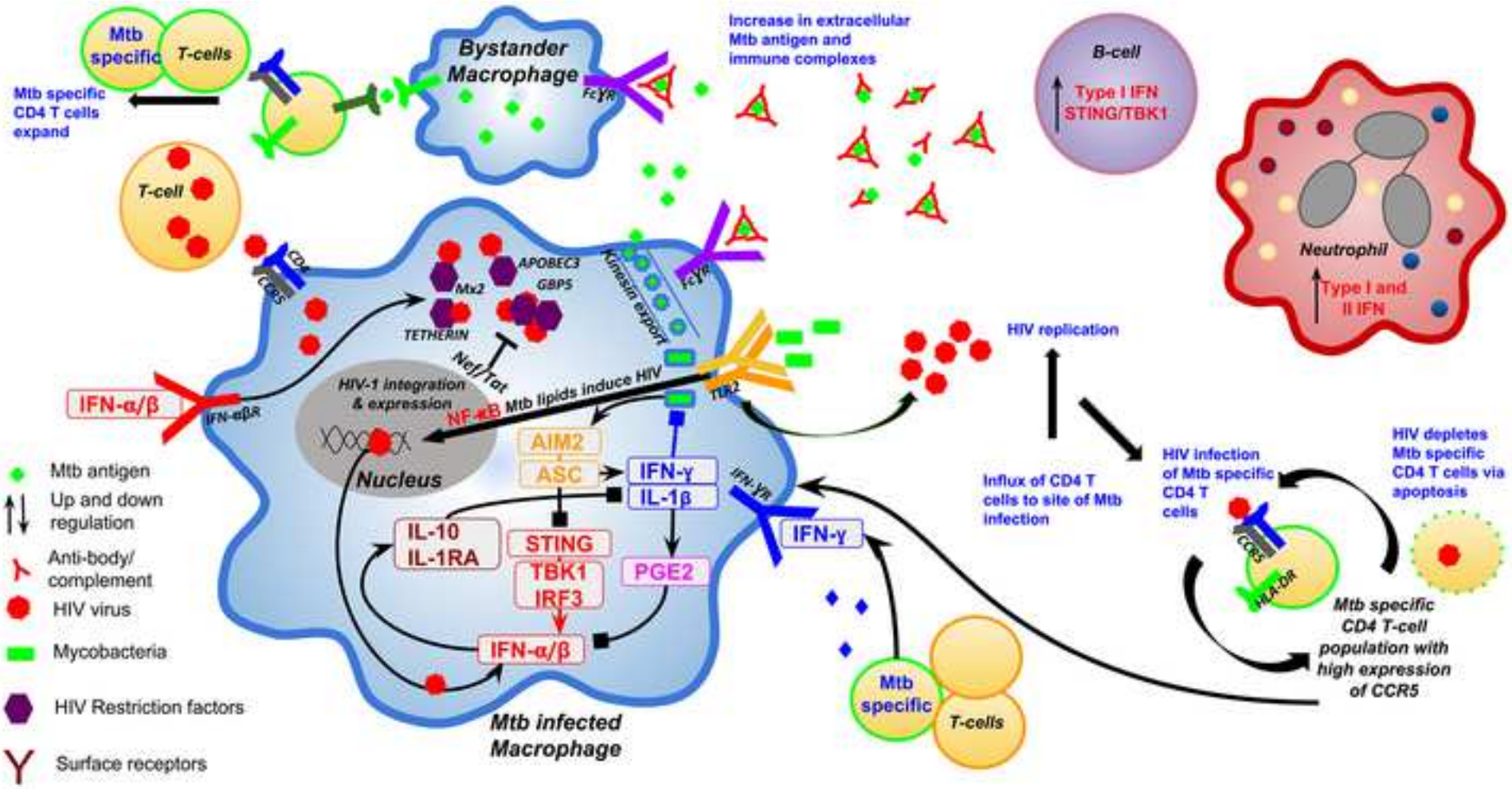
Figure


Mtb specific
T-cells
Mtb specific CD4 T cells expand
Bystander Macrophage
FcγR
Increase in extracellular Mtb antigen and immune complexes
B-cell
Type I IFN STING/TBK1
T-cell
CD4
TCRS
APOBEC3
GBP5
Mx2
TETHERIN
Nef/Tat
Kinesin export
FcγR
Neutrophil
Type I and II IFN
HIV replication
IFN-α/β
IFN-αβR
HIV-1 integration & expression
NF-κB
Mtb lipids induce HIV
TLR2
Nucleus
AIM2
ASC
IFN-γ
IL-1β
IL-10
IL-1RA
STING
TBK1
IRF3
PGE2
IFN-α/β
IFN-γR
IFN-γ
Influx of CD4 T cells to site of Mtb infection
HIV infection of Mtb specific CD4 T cells
HIV depletes Mtb specific CD4 T cells via apoptosis
CCR5
HLA-DR
Mtb specific CD4 T-cell population with high expression of CCR5
Mtb specific
T-cells
Mtb infected Macrophage
Mtb antigen
Up and down regulation
Anti-body/ complement
HIV virus
Mycobacteria
HIV Restriction factors
Surface receptors

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**Conflicts of interests:**

**None**

**Abstract（200 words, including titles）:**

**Purpose of review**

Tuberculosis is the leading infectious cause of death worldwide, and HIV-1 the best recognized risk factor for active TB. This review focuses on immune complex formation; the interplay of type I and II interferon signaling; and T cell activation in HIV-TB pathogenesis.

**Recent findings**

Circulating immune complexes and complement, and Fcγ signaling in whole blood act as early markers of TB disease in HIV-1 infected persons. HIV-1 is associated with a type I interferon response in whole blood, reducing the specificity of TB biomarkers dependent on type I and II interferon genes. Type I and type II interferons are implicated in both protection and TB pathology, a protective outcome may depend on modulating these pathways. Whilst *Mtb*-specific CD4 T cells are preferentially depleted during HIV-1 infection, activation markers on *Mtb*-specific CD4 T cells, in particular HLA-DR, reflect immune activation and have promise as biomarkers of *Mtb* disease activity in individuals with HIV-1.

**Summary**

TB pathogenesis in HIV-1 involves a complex interaction of underlying activation of both the innate and adaptive immune systems. Further research is required to understand whether biomarkers of activation could be used to predict or quantify TB disease in the context of HIV-1 infection.

**Keywords:** tuberculosis, HIV-1, interferon, immune complex, systemic activation

**The pathogenesis of HIV-TB co-infection（2472 words)**

## Introduction

Tuberculosis (TB) recently replaced HIV-1 as the leading infectious cause of death worldwide, causing an estimated 1.7 million deaths in 2016, of whom 374,000 were HIV-1 infected persons(1). The true burden of TB in those with HIV-1 may be an underestimate, as post mortem studies have found TB in 40% of those who had died of HIV-1(2). HIV-1 significantly increases the risk of active tuberculosis (3). Equally, TB is thought to accelerate HIV-1 progression, creating an environment that promotes viral replication(4).

Understanding the pathogenesis of HIV-TB co-infection is essential to the development of much needed new biomarkers, diagnostics and vaccines, as previously reviewed(4). This review focuses on three recent and notable facets in our understanding of HIV-TB co-infection; firstly, the role of immune complex formation in early co-infection, secondly, the role of type I and II interferon signaling, and finally, the impact of markers of T cell activation during co-infection.

## Immune complex formation in the pathogenesis of early HIV-TB

The clinical presentation of TB in HIV-1 infected persons varies by CD4 count. Those with high CD4 counts (>350 cells/$mm^3$) present similarly to HIV-1 uninfected persons, albeit with increased incidence(4). The higher incidence of unique isolates of *Mycobacterium tuberculosis* (*Mtb*) by molecular typing in early HIV supports the notion that previously acquired *Mtb* infection is reactivated (5). By contrast presentation in those with low CD4 counts (<100/$mm^3$) is frequently disseminated reflecting progression of disease following recent infection or reinfection, supported by increased clustering of *Mtb* by molecular typing in persons with advanced HIV-1 infection(5).

The importance of the granuloma and the role CD4 T cells play in the initial control of TB infection is clear, however less is known about the pathogenesis of early disease that occurs following failure of granulomatous control. We have recently shown that 2-deoxy-2-[$^{18}$F]fluoro-D-glucose positron emission and computed tomography (FDG-PET/CT) can be used as a research tool to investigate early disease. In a study of 35 asymptomatic, HIV-1 infected persons with CD4 > 350 cells/mm$^3$, no previous history of TB treatment, positive IFN-γ release assay, and negative microbiological screening culture we demonstrated that 10 had abnormalities consistent with subclinical disease. Of these, 90% had evidence of infiltrates and/or fibrotic scarring in the upper lobes in a distribution typically seen in HIV uninfected adults. Those with evidence of subclinical disease were significantly more likely to experience symptomatic disease progression(6*).

HIV-1 infected adults with subclinical TB, identified by FDG-PET/CT, had an increased abundance of transcripts in whole blood relating to the classical complement pathway and Fcγ receptors, when compared to HIV-1 infected adults without active TB(7**). This increase has subsequently been observed in HIV-1 uninfected persons prior to TB disease presentation(8). Increasing complement transcript abundance correlates with increasing levels of circulating immune complexes (CIC), which bind complement and Fcγ receptors and C1q, that were also elevated in those with subclinical disease (Figure 1.) (7**). Elevated levels of CIC in active TB, even in those culture negative, in HIV-1 uninfected persons is recognised(9). The role that immune complexes may play in the pathogenesis of early tuberculosis disease in persons of differing levels of immunocompetence is yet to be fully determined(9), however recent evidence suggests this may be a critical stage in disease progression(10).

Mycobacterial antigen can be detected within *Mtb* uninfected cells, in early stages of disease even when overall numbers of visible acid-fast bacilli are low(11,12). Of note, it has previously been shown that mRNA levels of the secreted Ag85B increase 14.6-fold in comparison to 16S ribosomal rRNA in the first 24-hours of intracellular infection, and are

enhanced by exogenous and endogenous TNF-α(13). Recently, Srivastava *et al.* described intracellular *Mtb* as able to release free antigen extracellularly via kinesin-2 dependent antigen export vesicles, which facilitate cell-to-cell antigen transfer in part as an immune evasion strategy(14,15). B-cell clusters are frequently identified within TB lesions and free antigen released extracellularly could form immune complexes with locally produced antibody(12,16,17). Solubilization of immune complexes is critical to prevent local precipitation in tissue and *in situ* triggering of the pro-inflammatory complement cascade(18). This solubilisation is initially facilitated by binding of C1q to immune complexes, which prevent larger complexes forming, and may account for increases in C1q expression at the site of disease(19,20). Similarly, increased expression of Fcγ receptor would facilitate intracellular take up of antigen. Ridley and Ridley observed in a detailed histopathological study of 31 cases of TB, that when antigen was phagocytosed within mononuclear cells there was little noxious effect to surrounding tissue. However, where antigen was externalised, bound to the interstitial connective tissue and associated with complement this was associated with evidence of localised necrosis(12). Hunter established that antigen increase precedes tissue necrosis leading to a significant increase in bacillary numbers(11). Taken together this suggests that, in both HIV-1 infected (with high CD4 counts) and in HIV-1 uninfected adults, extracellular free antigen release may occur early in TB disease while bacillary numbers are low. Immune complex formation may simultaneously benefit the pathogen (facilitating cell-to-cell transfer of antigen and immune evasion) and be harmful to the host (resulting in complement mediated immunopathology).

**Interplay of interferon signalling in the pathogenesis of HIV-TB**

One of the most well studied groups of signalling proteins involved in HIV-1 and *Mtb* infections are the interferons (IFNs). Type 1 interferons (IFN-α/β) are classically induced by HIV-1 infection and type 2 IFN (IFN-γ) by Mtb infection(21). Blood transcriptional profiling of TB patients has identified a role for neutrophil-driven type I and II IFN in TB disease(7**,22), suggesting there may be an interaction between these signalling pathways in individuals infected with HIV-1 and *Mtb*.

Whole blood transcriptomics has provided insight into disease pathogenesis in tuberculosis and more recently in predicting disease development(7**,22,23). Zak *et al*. have shown that the whole blood abundance of 16 gene transcript pairs, recently reduced to 11 gene transcript pairs in peripheral blood mononuclear cells (PBMC) with equal sensitivity and specificity(24), can predict disease progression over a 12 month period in HIV-1 uninfected adolescents(8). This signature is dominated by IFN-inducible transcripts and has reduced diagnostic specificity for HIV-TB disease (7**,23,25*). Moreover, whilst IFN-based transcriptional signatures poorly discriminate HIV-1 from TB-HIV, we found IFN-γ and CXCL10 protein levels in serum contributed to a signature predicting TB risk in HIV-1 infected individuals (*unpublished data*), which was verified in a cohort of advanced HIV-TB co-infected patients with a CD4 count <100 cells/$mm^3$ (7**,25*). Interestingly, high circulating CXCL10 inhibits CXCR3+ NK cell function during HIV-1(26), suggesting that excessive interferon signalling induced by HIV-1 may inhibit host *Mtb* responses, due to diminished NK IFN-γ production. Therefore understanding the role of interferons in disease progression is key, as the higher baseline threshold of IFN signalling in individuals with HIV-1(27) may lead to quicker TB disease progression.

Transcriptomic modular approaches combined with whole blood deconvolution have indicated that IFN signalling in HIV-1 is not restricted to a single cell type, but occurs in multiple cell types, including neutrophils and B cells (Figure 1) (28). Interestingly, B cells from mice infected with *Mtb* induce type I IFN via a key protein, Stimulator of IFN Genes (STING) and, to a lesser extent, the C-type lectin Mincle signalling. This modulates macrophage polarization towards an M2-type anti-inflammatory phenotype(29) which would be predicted to lead to a poorer outcome following *Mtb* infection. Furthermore, B cells isolated from pericardial fluid from TB patients display higher type I IFN transcripts than in the blood, indicating B cells also contribute to type I IFN signalling at disease site(29).

IFNs play a crucial antiviral role during acute HIV-1 infection, preventing productive viral infection through induction of host restriction factors, including the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3) family of proteins, tetherin and recently identified guanylate binding protein 5 (GBP5),(30–32*) the latter also being a frequent component of whole blood signatures predicting TB risk(8). HIV-1 infection in turn inhibits type I IFN production by T cells through expression of HIV encoded proteins, Nef and Tat, inhibiting IFN-β promoter stimulator-1 (IPS-1), an innate immune viral RNA sensing adaptor protein. Blocking of antiviral IPS-1 signalling, restriction factors and therefore IFN induction, results in increased HIV-1 replication (reviewed in (33)).

Although type II IFN is generally thought to promote protection during initial *Mtb* infection, both type I and II IFN signalling have each been shown to promote protection as well as drive pathology, depending on the timing of infection and disease and the context of modulation (reviewed in (34–36)). Excessive type I IFN signalling is associated with eicosanoid imbalance resulting in inefficient bacterial containment and disease exacerbation, suggesting that type I IFN may counteract the immunoprotective effects of type II IFN on *Mtb* infection(37).

This intricate balance was further dissected by Yan and colleagues who showed that mice deficient in Absence in Melanoma 2 (AIM2) expressed higher levels of IFN-β and suppressed IFN-γ signalling, resulting in higher infection burdens and more severe pathology (38**) . They showed that AIM2 induction of apoptosis-associated speck-like protein (ASC) results in interaction with STING, inhibiting the interaction between STING and downstream TANK-binding kinase 1, (TBK1) which interacts with interferon regulatory factor 3 (IRF3), consequently reducing the release of type I IFN, in bone marrow–derived macrophages and bone marrow–derived dendritic cells (Figure 1).

A recent study has shown that the product of one of the most highly induced IFN stimulated genes (ISGs), ISG15, can dually promote and protect against *Mtb* infection depending on the

stage of disease. They demonstrated that ISG15 along with type I IFN promote bacterial replication during early infection. However, as the infection progresses, ISG15 switches to a more protective role, demonstrated by an increased susceptibility to infection observed in mice deficient of ISG15(39). The authors hypothesise that differential regulation of ISGs between people may lead to different disease outcomes.

**Depletion of *Mtb*-specific CD4 T cells and the impact of T cell activation during HIV-TB co-infection**

Although TB risk is associated with lower peripheral CD4 T cell count(40) it is also observed early after HIV-1 seroconversion in persons with relatively well-preserved peripheral CD4 T cell counts(5,41), as well as in persons on antiretroviral therapy (ART) (42,43). This has prompted further investigation into the role of selective depletion and qualitative differences in function of *Mtb*-specific CD4 T cells during HIV-1 infection.

There is evidence that *Mtb*-specific CD4 T cells are preferentially depleted during HIV-TB co-infection, a phenomenon not observed during co-infection with other pathogens such as cytomegalovirus(44). This is thought to be driven by increased surface expression of CCR5 (a co-receptor used by R5 strains of HIV-1 to gain cellular entry) and low expression of macrophage inflammatory protein-1β (CCL4, a natural ligand of CCR5) on *Mtb*-specific CD4 T cells, with this being a prominent feature of lung resident *Mtb*-specific CD4 T cells(45–47). A study using Mtb-specific major histocompatibility complex (MHC) class II tetramers reported a 52% lower absolute number of Mtb-specific tetramer+ CD4 T cells in HIV-1 infected versus uninfected participants with LTBI(48**). Interestingly, despite low CD4 T cell counts (median 105 cells/mm$^3$), HIV-1 infected participants with active TB had comparable absolute numbers of *Mtb*-specific tetramer$^+$ CD4 T cells to those of HIV-1 uninfected participants with active TB. This demonstrates that co-infection with HIV-1 does not impair *Mtb*-specific CD4 T cells' ability to expand in response to replicating *Mtb.*

HIV-1 infection also mediates depletion of *Mtb*-specific CD4 T cells at the site of disease (Figure 1). Four studies have reported decreased frequency of *Mtb*-specific CD4 T cells in

broncho-alveolar lavage (BAL) samples of HIV-1 infected compared to HIV-1 uninfected healthy persons(47,49–52). The most recent of these studies by Bunjun *et al*. included HIV-1 infected, IFN-γ release assay (IGRA) positive participants who were ART-naive with relatively preserved CD4 T cell counts (median of 619 cells/mm$^3$) as well as HIV-1 uninfected controls. In contrast with previous studies, a significantly higher number of CD3$^+$ lymphocytes were observed in the BAL of HIV-1 infected participants compared to HIV-1 uninfected participants(53*). This comprised a 26-fold higher number of CD8 T cells and 7-fold higher number of CD4 T cells(53*), with both CD4 and CD8 T cell numbers showing significant correlation to BAL HIV-1 viral load, as seen in HIV-1 associated lymphocytic alveolitis(54). There was no significant difference in absolute number of *Mtb*-specific CD4 T cells in BAL of HIV-1 infected versus HIV-1 uninfected participants once adjusted for the higher number of CD4 T cells found in the HIV-1 infected group. The decreased frequency of *Mtb*-specific CD4 T cells was thus counteracted by HIV-1 mediated CD4 T cell influx, resulting in comparable absolute numbers of *Mtb*-specific CD4 T cells to that of HIV-1 uninfected participants. Although evidence from longitudinal studies are lacking it could be postulated that the findings of Bunjun *et al*. are representative of early HIV-1 infection, with the three prior studies being representative more advanced HIV-1 infection as evidenced by lower median CD4 count in their HIV-1 infected participant groups.

It is recognised that co-infection with *Mtb* contributes to immune activation observed in HIV-1 infection and that activation is associated with a higher risk of opportunistic infection and death(55–57). Several markers have been associated with immune activation e.g. CD38, programmed death receptor 1 (PD-1), Ki-67 and HLA-DR(60–63**). Riou *et al*. showed that there is higher HLA-DR expression on *Mtb*-specific cells from HIV-1 infected compared to HIV-1 uninfected persons both in LTBI and active TB(61**). In the HIV-1 infected LTBI group HLA-DR expression was similar to that observed in the bulk CD4 T cell compartment and thus indicative of HIV-1 mediated systemic immune activation. Activated CD4 T cells expressing CD26 and HLA-DR have also been implicated as sources of HIV replication during HIV-TB co-infection, thus accelerating HIV-1 disease progression(62). There is evidence that systemic immune activation also imparts higher TB risk. In a cohort of HIV-1 unexposed, BCG vaccinated infants the frequency of activated HLA-DR$^+$ CD4 T cells correlated with TB risk, with the highest risk being observed in those with the highest levels of response(63). In turn

these findings were confirmed in a cohort of HIV-1 uninfected adolescents(63). HLA-DR expression on *Mtb*-specific CD4 T cells holds promise as biomarker of disease activity during HIV-TB co-infection and could be explored by future longitudinal studies as predictive biomarker of TB risk in HIV-1 infection.

## Conclusions

Recent evidence emphasizes the diversity and complexity of the immune response to TB in persons infected with HIV. Using novel FDG-PET/CT imaging to identify HIV infected individuals with early TB disease, circulating immune complexes are a hallmark of TB risk and may contribute to disease progression. Type I and type II interferons have both been shown to promote protection as well as pathology, depending on timing and context of modulation. *Mtb*-specific CD4 T cells are preferentially depleted by HIV-1, but still retain their ability to expand in response to *Mtb*. CD4 T cell expression of HLA-DR may be a useful marker of systemic immune activation and disease activity in HIV-TB co-infection.

**Figure 1.**

**Interplay of immune activation driving *HIV-Mtb pathogenesis*.**

Elevated levels of circulating immune complexes found in early stages of HIV-TB co-infection are associated with localised tissue necrosis and may lead to increased bacillary numbers. The immunoprotective effects of type II IFN on *Mtb* infection in macrophages co-infected with HIV-1 are suppressed by the excessive type I IFN signalling, which leads to inhibition of IFN$\gamma$ and IL-1 signalling and inefficient containment of *Mtb* infection and disease exacerbation. AIM2 induction of ASC, which blocks STING interacting with TBK1 can inhibit IFN$\alpha/\beta$, potentially improving outcomes. Type I interferon can also induce expression of HIV restriction factors; a mechanism that is further inhibited by HIV proteins Nef and Tat. An increase in HIV replication leads to infection of *Mtb*-specific CD4 T cells mediated through their increased expression of CCR5 and decreased expression of CCL4. They are however able to expand numerically in response to replicating *Mtb*. The influx of CD4 T cells to the

lung during early HIV-1 infection of persons with latent TB infection (LTBI) present new targets for HIV-1 infection.

**Key points:**

- **Circulating immune complexes are markers of early disease progression in HIV-TB**
- **Type I and II interferon signalling are potential targets to reduce HIV-TB driven pathology**
- **Systemic immune activation precedes CD4 T cell depletion as a factor of HIV-TB risk but is reflected in HLA-DR expression on CD4 T cells that may be a useful marker of disease activity during HIV-TB co-infection**

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