M.Tuberculosis and HIV-Prolific Killers in Developing Countries are Syndemic Pathogens

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Mycobacterium tuberculosis(Mtb) infection accounts for the highest number of human deaths in low- and middle-income countries of the world. Mtb is also the most common opportunistic infection accompanying HIV infection. The death rate for combined Mtb/HIV infection is almost three-fold higher when compared to Mtb infection of HIV-seronegative patients, and the increase is not due simply to depletion of CD4 T cells due to HIV. The increased risk of mortality is due to a syndemic interaction between the two pathogens that leads to advanced immunodeficiency, chronic immune activation, and increased disease dissemination. Because of the synergy, eradication of MTB is dependent on the eradication of HIV. Though HIV/AIDS is often described as incurable, the recent "shock-and-kill" strategy to eradicate HIV holds promise for the eradication of MTB. Coinfection also amplifies transmission of multidrug-resistant tuberculosis (MDRTB), which besides requiring longer treatment has a very low success rate. Pending effective vaccine development, there is a need to identify newer targets for the development of drugs to treat MDRTB & XDR-TB.

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Introduction

According to a World Health Organisation (WHO) report, non-communicable diseases accounted for 74% of deaths globally in 2019 [1]. Major contributors to this class are neurological disorders, cancer and ischaemic heart diseases. However, when only low- and middle-income countries (LMICs) are considered, communicable diseases which include tuberculosis, HIV/AIDS and malaria, are the highest killers. Though tuberculosis (TB) is one of the oldest (>4000 years?) known human diseases, it is not yet eradicated, and still is one of the major causes of mortality. This is essentially because of the lifestyle of Mtb inside the infected host cell. We still do not completely understand how Mtb manages to evade the human immune system to survive and spread within eukaryotic cells. We also do not understand how the bacterium develops resistance against drugs. The problem is further compounded by the fact that Mtb infection is facilitated in HIV-positive individuals with a 20-fold increase in the risk of infection compared with HIV-seronegative individuals. Analysis of samples from coinfected patients was found to contain more drug-resistant Mtb. Besides, millions of people are developing cancers as a direct result of preventable infections by bacteria and viruses.Hence, infectious diseases will remain a major threat to humankind, especially in LMICs. The United Nations' (UN) Sustainable Development Goal (SDG) 3 seeks to end the TB epidemic altogether by 2030. Although there is a decline in the incidence of TB, the decline has been slow, because gaps in preventing, diagnosing and treating TB remain. Without new tools and strategies, the UN targets are unlikely to be met even by 2050. We, therefore, need to completely understand at the molecular level the infection and coinfection processes to form effective strategies of treatment, and also to identify newer targets for drug development [2].

Mtb infection

Mtb is a rod-shaped bacillus with a diameter of about 300 nm (Figure 1). It is an extremely slowly growing bacterium (doubling time 18 – 24 hours compared to 20 minutes for E.coli),requires oxygen, and contains an unusual cell wall rich in mycolic acid. This special cell wall is less permeable to drug molecules, and it also makes detection of the bacterium more difficult.The DNA genome of Mtb (H37Rv) consists of 4.4 × 106 base pairs with approximately 4,000 genes, and the gene make-up equips the bacterium for survival in glucose-deficient and fatty-acid rich environment. The genome has evolved to encode proteins that help the bacterium evade the human immune response. The tubercle bacillus spreads from person-to-person almost exclusively by aerosolized particles contained in aerosol droplets, and one to five bacilli may suffice to transmit the infection by air. Macrophages, dendritic cells, and neutrophils are the predominant phagocytic cells that Mtb can infect using a variety of receptors including ten different Toll-Like-Receptors (TLRs).Each of these receptors activates different signaling pathways in the bound cell. Bacterial activation of surface toll-like receptors on phagocytes induces TNF, IFN-γ, IL-1β, IL-6, IL-12, IL-10 and TGF-β, activating phagocyte and recruited T-cell functions. Though TB is primarily a pulmonary disease, it has other variants where the bone, the central nervous system, and other organ systems are affected, especially when co-infected with HIV.

Once the presence of MTB in the alveolar space is detected by the patrolling dendritic cells, the innate immunity comprising of phagocytic immune cells tries to clear the infecting pathogen.The efficacy of the immune response, however, depends on the genetic make-up of both the host and the pathogen. For example, Mtb utilizes several tricks to derail pathogen destruction through the fusion of phagosome with the lysosome. When all infecting Mtbs are not cleared, Mtb is said to have infected the host (Figure 2).TB disease is of two different types – latent TB and active TB. In the latent TB, the bug is dormant, and there are no symptoms of infection. In the active TB, on the other hand, the bug can spread within the host, and there are symptoms like cough with sputum and blood, chest pains, weakness, weight loss, fever and night sweats.It is observed that only 5 - 10% of total Mtb infections are in the active TB category. The adaptive or acquired immunity tries to prevent dissemination of infection within the body.

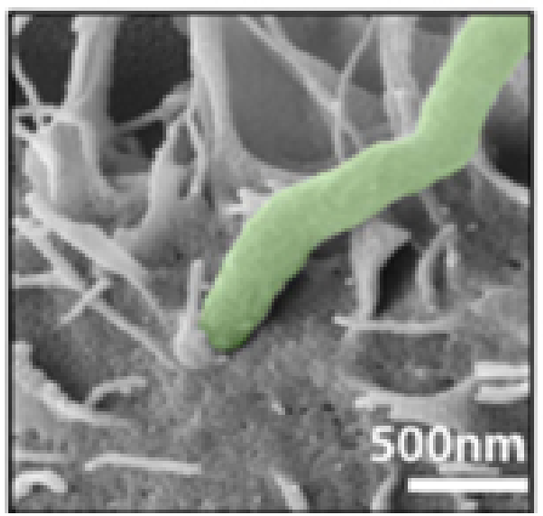

Figure 1. Electron micrograph showing the rod-shaped mycobacterium tuberculosis.

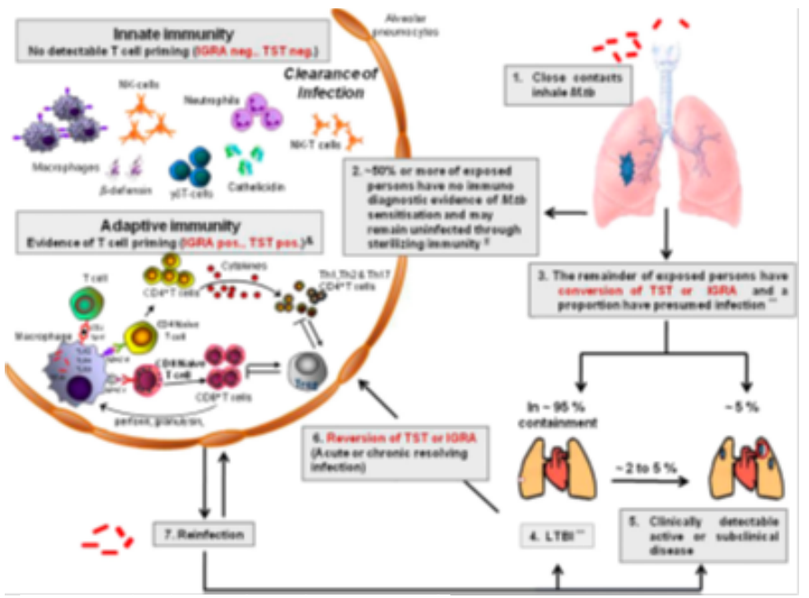

Figure 2. Mtb life cycle[13]

The outcome of a successful adaptive immune response from the host is the formation of what are called granulomas, which are organized immunological structures composed of T cells macrophages, B cells, NK cells, dendritic cells and other immune cells that surround Mtb to prevent Mtb dissemination.Formation of the Mtb granuloma during latent infection is associated with a strong localized and systemic proinflammatory response. The pathogen can stay in a latent state for many years but can be reactivated over a lifetime to cause disease and become transmissible.Disrupting the integrity of the granuloma is one way of shifting the infection from a latent state to an active disease state. Results of recent positron emission tomography and computer tomography

experiments on SIV/Mtb co-infection reveal a correlation between alterations in the number and nature of granulomas and reactivation of the disease, leading to the model shown in Figure 3 [3]. Similar synergy likely prevails during Mtb/HIV coinfection.

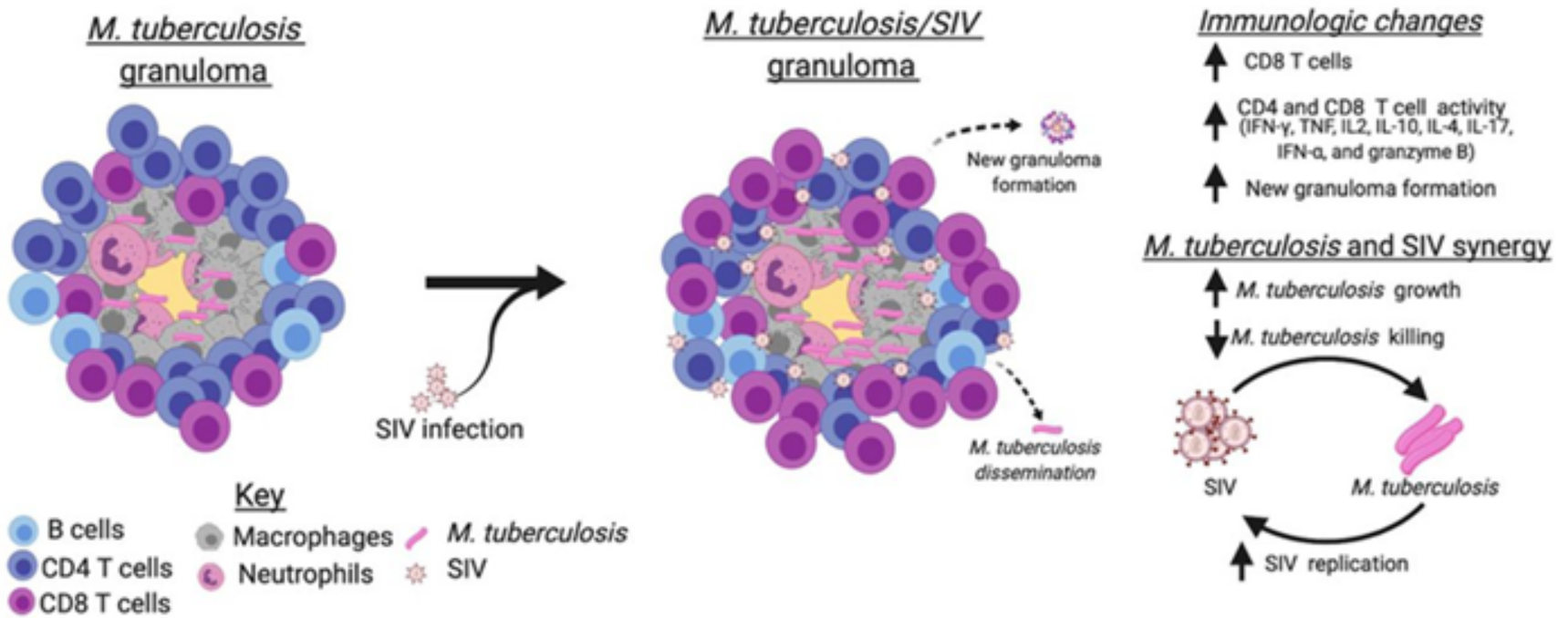

Figure 3. Granuloma in latent TB and synergistic effects of coinfection with SIV [3].

Human Immunodeficiency Virus (HIV)

HIV is an enveloped retrovirus that causes infectious disease HIV/AIDS. This virus was discovered in 1981, and therefore is relatively new compared to MTB bacterium. A most disturbing feature of this virus is that it attacks the human immune cells that help the body to fight infection. These cells include T-lymphocytes (also known as T cells), monocytes, macrophages and dendritic cells that express CD4 protein on their surface. The CD4 count (CD4 cells per millilitre of blood) is used to describe the severity of the disease. A count below 200is associated with full-fledged AIDS when the body immune system is badly damaged.

The glycoprotein, gp120, in the viral envelope binds to CD4 and CD8 proteins on the cell surface. Besides, a co-receptor protein is also required on the cell surface for the virus to gain entry into the cell. There are two known co-receptors, CCR5 and CXCR4, and different strains of HIV use different co-receptors. The virulence of the virus using either co-receptor appears to be different. The life cycle of HIV, shown in Figure 4, comprises of seven stages: 1) receptor-binding, 2) membrane fusion, 3) reverse transcription of the viral RNA genome, 4) integration of viral cDNA into the host chromosome, 5) production of viral RNA and proteins, 6) virus particle assembly and 7) viral budding and release. Any molecule that can effectively interfere with these steps in the viral life cycle would be a potential anti-HIV drug [4]. The viral genome encodes for six proteins Tat, Rev, Nef, Vif, Vpr and Vpu, which play a regulatory role and control the ability of HIV to infect a cell, evade the immune response, multiply and exit to infect other cells. The viral genome also encodes for three enzymes to help in its replication: 1) reverse transcriptase – conversion of the viral RNA genome into cDNA, 2) integrase – incorporation of viral cDNA into the host chromosome, and 3) protease – cleavage of newly translated viral polyproteins into different functional proteins through cleavage of specific peptide bonds in the polyprotein.

Inhibition of these enzymes would be a scientific method of treating HIV/AIDS infection. Indeed, many of the anti-AIDS drugs developed using structural information obtained through crystallography, and used in anti-retroviral-therapy (ART), are members of these three classes of inhibitors [5 -7]. The situation, however, is not so simple. The virus can stay undetected inside the human body in the integrated state (pro-virus) for long periods. These latent-cells, which can potentially be activated to produce active virus, therefore are considered as reservoirs of the virus. These latent-cells can't be killed by the immune surveillance system, and therefore, HIV/AIDS is often described as an incurable disease. The second complication is that of drug-resistance through mutations in viral proteins [8,9].

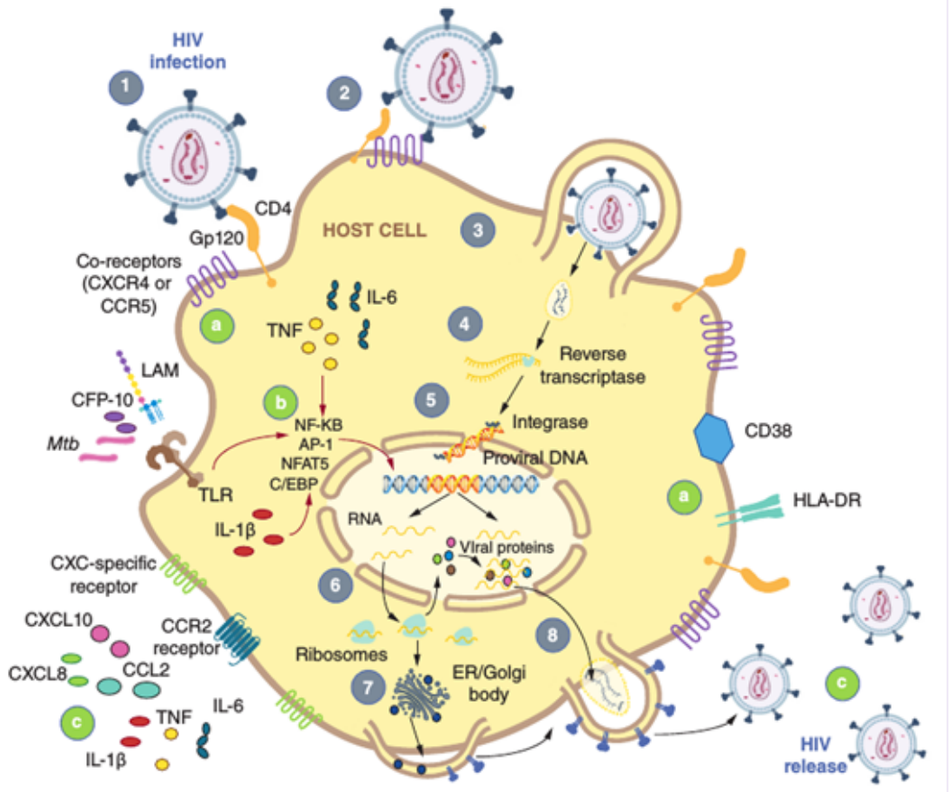

Figure 4. Effect of Mtb infection on the life cycle of HIV in co-infection [10].

The synergy between HIV-Mtb coinfection

There are several bacteria and viruses that successfully establish infections in humans when the human immune system is weakened. Such infections are described as opportunistic infections. HIV infection weakens the immune system by reducing the CD4 T-cell count to values below the normal value of about 500–1,200 cells/mm3. While many different types of bacteria can infect when CD4 count is below 200, Mtb is the only bacterium that can successfully infect an HIV-positive person even when the CD4 count is in the range 200 -500. This makes Mtb the most efficient opportunistic pathogen, and also suggests synergy between HIV and Mtb infections. The synergy is also indicated by the observation that HIV-infected patients have a 5–10% annual risk of developing TB, compared with a risk of 5–15% over the whole life-time for HIV-1-uninfected persons. The central players in this synergy are the functionally impaired T-cells and the cytokines they secrete. TB remains the leading cause of death among people living with HIV. As already pointed out, loss of protective CD4 T cells is not the sole reason for the increased susceptibility of HIV positive people for Mtb infection. The increase can be attributed to at least two mechanisms: the increased reactivation of latent TB or increased susceptibility to exogenous Mtb infection. Different experimental studies indicate the following factors as responsible for the synergistic relationship between Mtb and HIV, as shown in Figure 4 [10,11]:

1. Innate and adaptive Immune response to latent Mtb infection creates an expanded cellular niche (e.g activated CD4+ and CD8+ T-cells) susceptible to HIV-1 infection. Increased CCR5 and CXCR4 surface presentation on Mtb-antigen-specific CD4+ T-cells and increased CD38+/HLA-DR+ T-cells.

2. MTb infection Increases secretion of chemokines and proinflammatory cytokines (e.g., TNF, IL-1, IL-6) and transcription factors such as (NF-κB, AP-1, SP1, C/EBP and NFAT5) causing increases in HIV-1 replication by about 30%, even in bystander Mtb-uninfected cells.Increased secretion of CCL5 enhances replication of X4-tropic HIV-1 which causes much faster progress toward AIDS.

3. Latent TB disorients the host immune system making it easier for HIV-1 to evade the human immune system and spread quickly.

4. Mtb infection reactivates dormant HIV-1 (pro-virus) to multiply and spread in the body and enhances viral reservoir cells. Increased CXCL10 recruitment of HIV-1-infected T-cells to Mtb microenvironment. Enhanced recruitment of Mtb-specific T-cells to the bone marrow would create another niche for viral expansion and replication,

5)HIV-1 infects activated Mtb-specific T-cells, leading to their preferential depletion. HIV infection also causes a progressive loss of Mtb-specific T-cell functions, including T-cell proliferation, cytokine production, and cytotoxic capacity. HIV infects and destroys preferentially those T-cells (polyfunctional T cells) which protect against Mtb infection.

6) This early CD4+ T-cell depletion and increase in virus-containing CD8+ T cells on HIV infection alters the cellular composition of the granulomas surrounding the Mtb-infected macrophage (Figure 3). As a result, the granuloma becomes structurally porous and Mtb bacilli escape and spread the infection to other parts of the body.

7) HIV infection renders T cells dysfunctional by chronic activation, and it also disturbs the desired balance between distinct T-cell populations, such as the proportion between naïve/effector/activated, Th17/regulatory T cells (Treg).

8) HIV-infection promotes Mtb infection and active TB through up-regulation of Mtb entry receptors on macrophages. De novo Mtb reinfection in immunosuppressed HIV-infected individuals causes rapid progression to symptomatic disease with survival times as short as 14 days and fatality percentages over 85%.

9) HIV infection delays the establishment of Mtb antigen-specific immune responses by impairing TNF-mediated macrophage apoptosis.

Treatment of HIV-Mtb coinfection

There is clear evidence that providing antiretroviral therapy to HIV-infected adults during tuberculosis treatment reduces mortality. Treatment should be initiated without any delay as the probability of emergence of drug-resistant Mtb is significantly enhanced by co-infection with human immunodeficiency virus (HIV) [12]. However, the detection of Mtb infection is made more difficult by the co-infection.

Drug resistance

Several drugs, to be taken for 6 – 12 months, have been in use to treat tuberculosis, and the most popular first-line drugs are rifampicin and isoniazid. Rifampicin inhibits elongation of mRNA by binding to the β subunit of the RNA polymerase enzyme. Isoniazid is a prodrug, which once activated by the catalase/peroxidase enzyme of the host, inhibits the production of mycolic acid required for bacterial cell wall synthesis. Mycobacteria develop drug-resistance and cause MDRTB, XDR-TB and totally resistant tuberculosis. Treatment of MDRTB and XDR-TB takes longer, success rates are lower (55% and 30%) and also costs prohibitively more (5 to 6 times more). Duration of survival to death can be as short as two weeks.

Some of the mechanisms of resistance development are: 1) Target alteration – reduce drug binding through mutation of the target, 2) mimicking the target to sequester drugs e.g. MfpA mimics DNA double helix thereby sequestering DNA binding drugs, 3) inactivate drugs through chemical modification, e.g. an acetyltransferase, Eis (enhanced intracellular survival) acetylates multiple amine groups of aminoglycosidic drugs, 4) drug degradation by Mtb enzymes and 5) drug efflux – at least 18 transporters in mycobacteria have been found.

Drug-drug interactions

Treatment of co-infections is also beset with the complexity of drug-drug interactions. Drug interactions between antiretroviral and anti-TB agents are common in the management of patients with HIV and TB. Drugs for HIV and TB don't always work well together. For example, Protease Inhibitor (PI)/ritonavir + rifabutin- the combination is a prescribed treatment for HIV/TB. This treatment regimen has the following problem. ritonavir-boosted PIs markedly increase rifabutin concentrations and reduce its clearance necessitating a reduction in the dose of rifabutin by 50% to 75%. Toxicity (neutropenia, uveitis, hepatoxicity, rash, gastrointestinal symptoms) and suboptimal rifamycin exposures with reduced dose may lead to drug-resistant bacteria. On the other hand rifampicin-induced, cytochrome P450 activity may cause sub-therapeutic levels of PIs. It is in-fact observed that co-treatment for TB and HIV significantly increased the risk for major mutations conferring resistance against PI.

Conclusions

TB remains a prime killer in the world of infectious disease. While it does enter latency in 90% of cases, reactivation is possible, and coinfection with HIV is found to be especially effective in this. HIV has a profound effect on TB, including faster rates of disease progression, higher rates of drug resistance, and increased mortality among patients with MDRTB. The coinfection disturbs the nature and structure of granulomas that isolate Mtb thereby facilitating the dissemination of Mtb. In the coinfection, the immune system is rendered dysfunctional thereby promoting propagation of each pathogen. Therefore, eradication of TB, a stated goal of the UN, becomes dependent on the eradication of HIV. Though the mechanism of HIV replication makes HIV incurable, recent research on latency-reversing agents (LRAs) is an effort to eradicate HIV by the "shock and kill" strategy [2]. If successful, this would help eradicate Mtb as well. Co-infection is also observed to lead to the emergence of MDRTB and XDR-TB, effective treatment of which requires identification of novel targets for drug development.

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