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Induction and regulation of CD8+ cytolytic T cells in human tuberculosis and HIV infection

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ABSTRACT

Tuberculosis and HIV continue to be the world-leading killers among infectious diseases, primarily affecting poor people in many developing countries. Despite differences in the immunopathogenesis of human infection with tuberculosis and HIV, experimental evidence from clinical studies and relevant animal models can be used to reflect on the cellular mechanisms responsible for an increased risk of active tuberculosis among HIV-infected individuals. In this review, we will discuss the molecular features and regulation of cytolytic T cells and how deficient cytolytic T cell responses contribute to the pathogenesis of TB and HIV infection as well as TB/HIV co-infection.

1. Introduction

Mycobacterium tuberculosis (Mtb) and HIV are two intracellular pathogens that successfully manipulate the immune system in order to generate persistent infections (Table 1). Mtb is the causative agent of tuberculosis (TB) that replicates and hides within host macrophages ultimately resulting in a chronic granulomatous infection, primarily in the pulmonary tract. Instead, the HIV virus replicates vigorously in activated CD4+ T cells and in dendritic cells (DCs), first in the genital and gut mucosa, respectively, and then subsequently throughout the lymphatic tissue leading to systemic and harmful immune activation. TB and HIV are often called "*the cursed duet*" or "*the evil couple*", given the fact that HIV-infected individuals are highly susceptible, i.e., 40–80 time more likely, to be infected with or reactive latent TB. TB/HIV co-infection has become a major global health problem and about 25% of TB deaths occur among HIV patients, also making TB the leading killer in HIV-positive patients [1]. Even though TB and HIV are two human infections with large differences in pathogenesis, there are a number of common denominators that are important to consider. Immunological control of intracellular infections such as TB and HIV involves both innate and adaptive antimicrobial effector pathways, although complete eradication of infection is rare. Importantly, cell mediated immunity which is critical for TB protection, is gradually destroyed in HIV infection. T cell mediated control of HIV fails due to severe depletion of activated CD4+ T cells, including Th1 and Th17 cells, a rapid induction and expansion of regulatory T cells (Treg) and an impaired maturation and activity of cytolytic CD8+ T cells (CTLs). Impairment of CTL responses is used as an evasion mechanism by these intracellular pathogens to avoid killing of infected host cells by granule-associated effector molecules in activated CTLs. Importantly, protective immunity in intracellular chronic infections is constituted by independent as well as redundant or interdependent events, whereby each piece of the immunological puzzle contributes to a network of polyfunctional immune cells. CTLs are an important link in this chain of events, but we need to reflect on the global immune response in order to better understand the relative contribution of CTL activity in protective immune responses against TB and HIV.

2. Characterization of protective immunity and CTL responses in TB and HIV infection

Complex chronic infections such as TB and HIV are regulated by a combination of effector mechanisms and the synergistic effects of innate and adaptive immune responses. Innate immune effector mechanisms in TB and HIV include local induction and action of antimicrobial peptides (AMPs) such as human defensins and cathelicidin, LL-37 [2,3]. AMPs are small cationic molecules, with the potential to kill microbes through osmotic lysis and are primarily

Abbreviations: Mtb, *Mycobacterium tuberculosis*; TB, tuberculosis; AMPs, antimicrobial peptides; NO, nitric oxide; APCs, antigen-presenting cells; DCs, dentritic cells; CTLs, cytolytic T cells; Treg cells, regulatory T cells.

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Table 1
Comparison of clinical features and hallmark of disease in TB vs. HIV/AIDS.

	Tuberculosis	HIV/AIDS
Causative agent	*Mycobacterium tuberculosis*	HIV-1 or -2 (virus)
Primary site of entry	Respiratory tract	Urogenital mucosa
Primary site of infection	Lung and lymph nodes	Secondary lymphoid tissue
Primary target cell(s)	Alveolar macrophages	Activated CD4+ T cells and dendritic cells
Clinical disease	Active or latent disease	Progressive or non-progressive disease
Histopathology	Granulomatous inflammation	Non-specific inflammation
Innate effector pathways	AMPs and nitric oxide	AMPs and IFN-α
Adaptive effector pathways	Th1/Th17 responses and CTLs (perforin/granulysin)	Th1/Th17 responses and CTLs (perforin/granzymes)

produced by epithelial cells and phagocytic cells, i.e., neutrophils and macrophages at mucosal surfaces [4]. Importantly, AMPs also mediate recruitment of CD4+ T cells to the site of infection and the subsequent up-regulation of AMPs by macrocophages and epithelial cells are of importance for preventing initial uptake of mycobacteria as well as HIV virus at mucosal sites. It has been shown that vitamin D3 and LL-37 confer protection against Mtb infection by the induction of autophagy and enhanced antimicrobial activity in macrophages [5]. In addition, production of nitric oxide (NO) by infected macrophages represents an important innate effector mechanism in TB whereas production of IFN-α and several β-chemokines (MIP-1α, MIP-1β, and RANTES) by activated DC is known to initially restrict intracellular HIV replication. Collectively these innate immune effectors play a major role to prevent microbial uptake and to restrict growth early in infection.

2.1. Polyfunctional T cells play a key role in TB and HIV immunity

During adaptive immunity, it is well-known that T cell mediated responses are central in the regulation of specific host–pathogen interactions in both TB and HIV disease. Particularly, the establishment of polyfunctional T cell responses, characterized by a coordinated expression of multiple effector functions including Th1 cytokines, chemokine release and cytolytic effector molecules, are believed to be crucial to control intracellular infection and to provide long-term host protection. Peptide and lipid antigen mediated activation of MHC- as well as CD1-restricted T cells induce two major effector mechanisms: the production of Th1 cytokines (IL-12, IL-18, IL-23, IL-2, IFN-γ, TNF-α) and cytolytic effector functions in different populations of T cells. The most studied T cell effector mechanism is the secretion of cytokines, including cytokines with both antimicrobial and anti-inflammatory properties. Early in the infection, IL-12 is mainly produced by antigen-presenting cells (APCs) to promote human Th1 responses, and an impairment of IL-12 [6] and subsequent IFN-γ [7] signaling has been associated with progressive disease in both TB and HIV infection. Moreover, IFN-γ [7] and TNF-α [8] have been appointed a frontline position in the induction of a protective response in TB. These cytokines act in a synergistic manner to activate macrophages and T cell subsets and are instrumental in the organization of the granulomatous immune response in TB. Activated macrophages initiate the development of characteristic epithelioid granulomas involving recruitment of T cells that isolate the infection and prevent systemic spreading of bacilli. The granuloma contains live Mtb in a state of dormancy that can persist for decades. Development of active TB disease in 5–10% of infected individuals occurs when the balance in host adaptive cellular immunity fails. In advanced stages of the infectious process, induction of a Th2 (IL-4, IL-13) [9] response and an expansion of CD4+CD25+FoxP3+ Treg cells [10,11], further antagonize the important Th1 response and suppress subsequent CTL activity. However, the initial cellular immune mechanisms that lead to containment of the bacilli or progression of disease have not been fully delineated.

In HIV, a hallmark of protective immune responses is the presence of polyfunctional T cells characterized primarily by the production of IL-2, TNF-α, IFN-γ but also β-chemokines, i.e., MIP-1β and markers for degranulation [12]. Evaluation of the timing, quality and magnitude of HIV-specific T cell responses are of great importance to understand HIV immunopathogenesis. Whereas progression of HIV is strongly associated with chronic immune activation involving activation of immune cells and excess production of proinflammatory cytokines [13], the consequence of such over activation is not immune control but rather escalated immunopathology. This includes persistent viral replication, chronic proinflammatory and IFN-α responses [14], poor Th1 responses and depletion and/or inhibition of particularly Th17 cells at mucosal sites [15] as well as dysfunctional CD8+ CTL with reduced cytolytic killing capacity [16,17].

It is believed that Th17 cells can regulate the production of AMPs at mucosal surfaces. In acute HIV infection there is early and rapid onset of a Treg response preventing the differentiation and activation of Th17 CD4+ T cells and subsequently the production of AMPs at the site of infection. Impaired mucosal induction of AMPs contributes to the chronic expression of IFN-α because lack of AMP leads to microbial translocation and IFN-α activation when the integrity of the intestinal mucosa is destroyed [15,18]. Furthermore, it has been described that a Th17 response is induced simultaneously with reduced levels of Treg cells in individuals who control HIV infection, suggesting that a high IL-17 to Treg cell ratio correlates with good prognosis [19]. These patients also control microbial translocation and have no elevated IFN-α production in the chronic state. Interestingly, Th17 responses may also be important for induction of protective immunity in TB [20]. Thus, deficient activation of Th1/Th17 responses and subsequent production of AMPs, as well as excess production of inflammatory mediators, and/or the induction and recruitment of Treg cells at the site of infection may have severe clinical consequences in both of these chronic infections.

2.2. CD8+ CTL responses are impaired in TB and HIV infection

It has been determined that properly matured and activated CD8+ CTLs play a crucial role in the elimination of both Mtb- and HIV-infected cells and consequent immune control [21,22]. In a recent study by Stenger et al., it was elegantly illustrated that CD8+CD45RA+CCR7− effector memory T cells expressing high levels of perforin and granulysin, are crucial components of a protective TB response [23]. Patients with latent TB are known to reactivate the disease upon immune therapy with anti-TNF-α antibodies [8] and importantly it was proven that anti-TNF treatment was associated with antibody mediated depletion of the CD8+ effector memory T cell subset expressing perforin and granulysin [23]. Consequently, antimicrobial activity in Mtb-infected monocytes was significantly decreased and resulted in reactivation of TB. A decrease of terminally differentiated Mtb-specific CD8+ T cells has previously been shown in TB patients [24]. These cells

expressed low levels of both IFN-γ and perforin, which recovered after chemotherapy.

In HIV infection, a skewed maturation of memory CD8+ T cells also results in a high proportion of pre-terminally differentiated CD45RA−CCR7− cells as compared to terminally differentiated CD45RA+CCR7− cells [25]. Accordingly, terminally differentiated HIV-specific CD8+ T cells have been found to be more frequent in HIV controllers compared to progressors [26]. Indeed, such dysmature or pre-terminally matured CD8+ T cells express low levels of perforin and possess impaired cytolytic activity in HIV disease [16].

Thus, individuals with active, progressive TB as well as HIV disease, most likely have an inadequate up-regulation of cytolytic effector molecules in pathogen-specific CTLs, resulting in insufficient killing of infected cells at local sites of infection. In this regard, our group has used clinical tissue samples and *in situ* computerized image analysis to describe that both primary and chronic HIV infection in adults was associated with a lack of perforin expression in improperly matured HIV-specific CD8+ effector T cells [27,28]. A lack of optimal co-stimulation provided by DCs as well as weak polyfunctional responses provided by CD4+ T cells may partly explain this weak cytolytic response [29–31]. Later, we also determined that patients with a progressive HIV infection had significantly increased levels of FoxP3 mRNA expressing CD4+ T cells [32] that correlated with low levels of perforin in CD8+ CTLs in lymphoid tissue as compared to reversed findings in HIV non-progressors [33]. This accumulation of FoxP3+ Treg cells at the site of viral replication was associated with a redistribution of Treg from the blood to the lymphoid tissues [32]. In line with these important findings, we recently discovered that patients with TB disease had impaired CD8+ T cell expression of the adaptive effector granule molecules, perforin and granulysin, in the tuberculous lesions in tissue at the local site of infection [11,34]. Granulysin is a member of the antimicrobial peptide family with potent anti-Mtb activity. We found that there was a compartmentalization of immune responses in TB involving few granule containing CD8+ and CD56+ CTLs but elevated levels of FoxP3+ Treg cells in the lesions, suggesting that active immunosuppression took place in the microenvironment of the granuloma [11]. Whereas CD8+ CTLs expressing perforin and granulysin were absent in the TB lesions, the expression of Mtb-specific antigens in macrophages was high, suggesting that infected cells were enriched in the granuloma. mRNA expression of IL-13 and TGF-β, but not IFN-γ, TNF-α and IL-17, were up-regulated in TB-infected tissue. A Th2/Treg immunosuppressive response could hamper Th1/Th17 cells as well as CTL activity and thus these aberrant immune responses may be part of the pathological alterations in immunity in TB [35] and HIV-infected individuals. Indeed we and others have found that TGF-β was associated with fibrosis and deposition of collagen type I in both TB [11] and HIV [36] infected tissue. Importantly, pre-mature skewing of the immune response to an inappropriate or regulatory phenotype in the acute stage of these infections may prevent the induction of proper CTL and antimicrobial responses and result in progressive disease (Fig. 1).

3. Molecular mechanisms of CTL killing function

CTL activity is mainly mediated by two different molecular mechanisms: (1) granule-mediated release of cytolytic and antimicrobial effector molecules including perforin, granzymes and granulysin, (2) apoptosis induced by death-receptor ligation including Fas–FasL and mTNF–TNFR, PD1–PD1L and DR5–TRAIL interactions. Most experimental evidence suggests that cytolytic T and NK cells using the granule-mediated exocytosis pathway of target cell killing, are most efficient in decreasing the viability of intracellular Mtb bacteria [37] as well as HIV-infected target cells [38]. The killer activity is mediated by cytolytic (i.e., perforin and granzymes) and antimicrobial (i.e., granulysin) effector molecules that are released from granules into the immunological synapse between the CTL and the infected target cell. Hence, lysis of Mtb-infected cells has been shown to be primarily executed by CTLs expressing perforin and granulysin, whereas death-receptor ligation may be more important in the homeostatic regulation of clonal T cell responses induced upon TB or HIV infection (Fig. 1). Death-receptor induced apoptosis of infected target cells may deprive the bacteria of their natural habitat, but fail to attack the microbes and induce direct killing of the bacterial cells [37]. Live bacteria released in the apoptotic process may infect bystander macrophages and continue to propagate the infection. Instead,

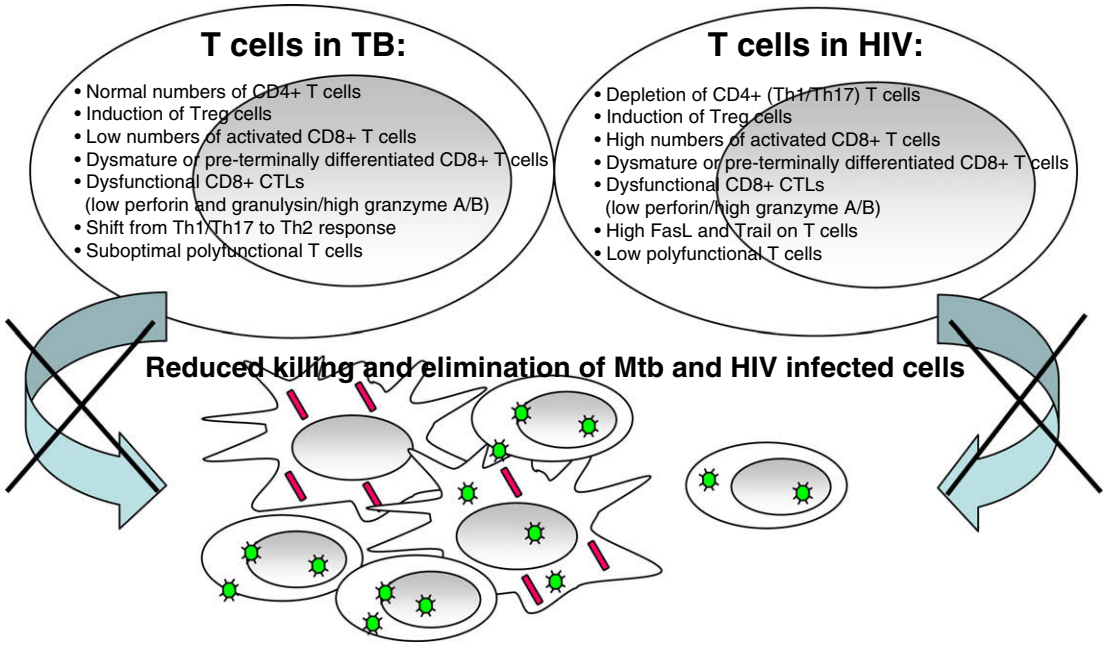

Fig. 1. Schematic illustration of T cells defects associated with TB and HIV infection, respectively. Red microbe: *Mycobacterium tuberculosis*; green microbe: HIV virus. Single- and/or co-infected macrophages and T cells are shown.

Fas/Fas ligand dependent apoptosis of Th1 cells has shown to be induced by Mtb-infected foamy macrophages (high vacuole content) [39], which suggests that death-receptor induced cell death down-regulates the T cell response instead of killing Mtb-infected cells. Foamy macrophages are also an important source of TGF-β, which further results in suppression of important Th1 responses [35]. Similar to TB, lytic granule loading of CD8+ CTLs is required for effective elimination of HIV-infected cells and subsequent immune control [17]. HIV-specific CTLs kill infected cells through perforin-dependent granule exocytosis whereas high levels of IFN-α will up-regulate Fas–FasL [40] and DR5–Trail [41] death-receptors responsible for substantial killing of uninfected bystander CD4+ T cells.

3.1. Optimal activity of fully matured CD8+ CTLs require a coordinated expression of cytolytic effectors molecules

It was recently found that among human PBMCs, the majority of cytolytic effector molecules, i.e., perforin and granzymes, were expressed in CD8+ T cells [42]. Importantly, a high content of cytolytic effectors were tightly linked to cellular maturity as determined by cellular expression of CD57, which suggests a close relationship between cytolytic effector content and cellular differentiation. Accordingly, only highly differentiated effector or memory CD8+CD57^{bright} T cells, correlated strongly with simultaneous expression of granzymes and perforin, providing evidence that co-expression of cytolytic effector molecules in CTLs is necessary to mediate optimal cytolytic activity. In general, the common dogma is that granzymes and granulysin cooperate with the pore-forming protein perforin in order to enter cells and intracellular compartments. Permeabilization of cell membranes is reversible and allows low-molecular weight molecules to enter the target cell. A recent discovery also demonstrates that perforin may facilitate granzyme uptake by activating endocytosis and moving the cytolytic contents to the endosomal pathway [43]. Granzymes A and B induce apoptosis either by activation of the caspase cascade or by targeting the nucleus [44]. The antimicrobial function of granulysin is similar to AMPs of the innate immune system, involving attack of intracellular microbes and osmotic lysis of bacteria [45]. Granulysin creates lesions in the cell wall and has been shown to reduce Mtb-growth in a perforin-dependent manner [45]. It has also been shown that granulysin possess hemolytic properties and thus can trigger apoptosis of infected or self-altered target cells [46]. While granzymes and granulysin are likely necessary in host defense against microbial infection, extracellular deposition of these molecules may also result in tissue injury and necrosis. The perforin-deficiency found in semi-mature CD8+ CTLs of HIV-infected patients is selective, since the induction of granzyme A/B is similar in HIV and control groups [27,42]. Interestingly, it was recently reported that perforin(−)/granzymeA(+) T cells hardly or very weakly killed their target cells, indicating that these T cells represent a more early, pre-mature T cell subset and not fully differentiated effector CTLs [47]. Importantly, enhanced tissue damage may be the pathological consequence of deficient perforin expression in dysfunctional CTLs. Evidently, effective CTL responses in both TB and HIV infection, require a coordinated expression of perforin and granulysin [11,48] or perforin and granzymes [27,42], respectively. Perhaps a minor shift in CTL activity involving disturbed proportions of effector molecules in CTLs, is enough to fuel the infectious process and prevent complete eradication of intracellular microbes (Fig. 1)?

4. Regulation of CTL responses by CD4+ T cells, Treg cells and dendritic cells

Maturation and activation into functional CTLs require support from other immune cells and thus improper activation of naïve CD8+ T cells leading to semi-mature and/or dysfunctional CTLs may result from weak help primarily from CD4+ T cells or APCs. Professional APCs such as DCs serve a crucial link to trigger adaptive immune responses including proper activation of CTLs. DCs are the only APC able to stimulate naïve T cells which provide them with a unique property to regulate the initiation of specific immune responses. The level and type of DC maturation will in great essence regulate the quality and magnitude of the following T cells response. Insufficient co-stimulation provided by mature DC may cause a weak cytokine/chemokine or cytolytic immune response. Several interesting findings regarding the regulation of DC maturation and function have been described in the research areas of cancer and infection. It was recently shown that type 1-polarized DCs matured in the presence of IFNs and TLR ligands, were required to induce CTL activity and peripheral tissue-type chemokine responsiveness [49]. Furthermore, IFN-γ and TNF-α producing CD8+ T cells can cooperate with CD40L expressing CD4+ T cells to polarize maturation of DCs capable of producing high levels of bioactive IL-12p70, showing how important the mutual and complementary communication between different immune cells are in the induction of efficient Th1 responses [50]. Importantly, IL-12 will mainly be produced by DCs and sometimes macrophages, which are cells that may be reduced in numbers [51] or dysfunctional in chronic infections such as TB and HIV [52,53].

CD4+ T cells are central for the optimal induction of CD8+ T cell function and maturation as well as the activity of DCs. Although it is known that CD4+ T cells producing IFN-γ are essential for protective immunity in TB, the molecular relationship between CD4+ and CD8+ T cells in TB is poorly described. More is known in HIV, where selective depletion or functional inactivation of CD4+ T cells in acute and chronic HIV disease [54] unequivocally results in poor CD4+ T cell help required to induce crucial antibody and CTL responses. The rate of activation-induced apoptosis of both HIV-infected and uninfected CD4+ T cells is high, resulting in rapid and severe depletion of CD4+ T cells, especially in the gastrointestinal tract but also in secondary lymphoid organs and blood. Functional impairment of the remaining CD4+ T cell pool may involve either induction of anergy or active immunosuppression mediated by Treg cells. Anergy induction in HIV, has been shown to involve inhibition of IL-2 production and expression of the high-affinity IL-2R [55], which will result in a down-regulation of CD4 co-receptors and a decreased induction of CD40L on T cells [56]. In addition, expression of MHCII and the co-stimulatory molecules CD80 and CD86 on APCs may not be properly up-regulated [29]. A following reduction in IL-12 secretion in combination with HIV-induced expression of IL-10 in monocytes or macrophages may result in decreased proliferation of human PBMCs [57].

4.1. Treg cells can down-regulate imperative T cell responses in chronic infections

Natural or induced Treg cells are a heterogenous population of CD4+ T cells that control autoimmunity and excessive immune responses to infection. Expansion and enrichment of Treg cells can be induced to suppress important immune responses at the site of microbial replication and may affect CTL activity in several different ways: (1) by suppressing CD4+ T cells producing Th1 cytokines that are necessary for the activation and function of antigen-specific CTLs, (2) by down-regulating the antigen-presenting capacity and expression of co-stimulatory molecules on APCs or (3) by inhibiting the cytolytic function of CTLs. Suppressive activity can either be induced through cell–cell contact dependent mechanisms such as ligation and signaling of inhibitory receptors CTLA-4, GITR and PD1L, or secretion of soluble factors, primarily the anti-inflammatory cytokines IL-10 and TGF-β. Several reports using various disease models have been able to prove that Treg cells, directly

or indirectly, can mediate suppression of antigen-specific CTL function, which may considerably affect the ability to control microbial growth and clearance *in vivo*. Treg cell mediated suppression has been characterized by reduced proliferation and secretion of soluble factors but also by an inability to produce granzymes and to degranulate cytotoxic molecules, ultimately resulting in decreased cytolytic function and persistence of the pathogen [58,59]. Furthermore, it has been demonstrated that CD25+FoxP3+ Treg cells isolated from lymph nodes of HIV-infected patients, were particularly potent to suppress CTL function, especially in patients with high levels of plasma viremia [60].

Interestingly, a subset of mature DC can down-regulate T cell activation through induced expression of indoleamine-2,3-dioxygenase (IDO) protein and IDO enzyme activity [61]. It is believed that IDO exerts immunosuppressive effects by degrading the essential amino acid tryptophan, thereby down-regulating important T cell functions. Of clinical relevance it has been shown that an inflammatory infiltrate of IDO- and FoxP3-positive cells were introduced at the site of DC-based vaccination of melanoma patients, suggesting an IDO-mediated induction of regulatory T cells [62]. Moreover, it was recently shown that HIV-induced IDO can inhibit both CD4+ and CD8+ T cell responses [63].

5. Regulation of cellular immunity and CTL responses in TB/HIV co-infected patients

In HIV-infected individuals, the immunological balance is tipped in favor of the pathogen and this will sometimes result in active replication and spreading of bacteria and progression of TB disease. Several factors may contribute to the increased risk of active TB among HIV-positive patients and this increased risk occurs already from the early onset of HIV infection, even long before virus-induced immunosuppression is manifested (Fig. 1). Reduced expression of innate effector molecules such as AMPs at mucosal surfaces may be of relevance. Nevertheless, depletion of Mtb-specific CD4+ T cells in HIV-infected patients is most certainly of great importance. Intercellular spread and propagation of HIV virus in activated CD4+ T cells may ultimately lead to immune-mediated lysis of HIV- as well as Mtb-specific T cells. Therefore, not only HIV-specific immunity is affected by the loss of CD4+ T cells, HIV also blocks the ability of the host to mount an effective Mtb-specific T cell response. It is well-known that immunity to non-HIV microbes is restored after initiation of HAART therapy. However, more recent clinical studies demonstrate that Mtb-specific CD4+ T cells from patients with chronic HIV, maintain an impaired capacity to secrete IFN-γ, despite a recovery in CD4+ T cell counts after long-term treatment with HAART [64]. Previously, it has been determined that reduced IFN-γ production by PBMCs can be used as a marker of severe TB in HIV-negative and especially HIV-positive patients [65]. A low IFN-γ response has also been shown to be associated with reduced induction of NO in activated Mtb-infected macrophages [66]. Since IFN-γ is crucial in providing a protective response in TB, this HIV-associated T cell defect, may contribute to the development of active TB infection in HIV-infected individuals.

The molecular basis for impaired CD8+ CTL responses in TB/HIV co-infected patients has not been thoroughly investigated. CD8+ T cells obtained from PBMCs of TB/HIV co-infected patients were previously shown to express more pre-apoptotic CD95 and less of the co-stimulatory receptor CD28 on the cell surface [67]. These CD8+ T cells also produced significantly lower levels of TNF-α compared to TB single-infection [68]. Whereas the antimicrobial effects of granulysin is of great importance for CTL function in TB, the role of granulysin in control of HIV infection is not known. Interestingly, lack of perforin may hamper granulysin-mediated elimination of Mtb and thus contribute to the molecular defect responsible for development of primary TB infection or reactivation of TB in HIV-infected individuals. Importantly, it seems like a deficiency in perforin is not a common consequence of chronic infection since reduced perforin content have been revealed to be a unique characteristic of HIV-specific CD8+ T cells whereas, i.e., EBV- and CMV-specific cells from HIV donors maintain a high perforin expression [42].

5.1. Up-regulation of Treg responses predisposes HIV-infected patients to TB

Since HIV-infected patients can develop active TB even if their CD4 counts are high, there must be other factors responsible for the increased susceptibility of TB among HIV-infected patients that contribute to the current global spread of TB. Of major importance are probably the clinical findings that CD4+FoxP3+ Treg cells are significantly up-regulated in the lymphoid compartments of patients with an uncontrolled HIV infection [32,33]. A relatively increased number of Treg cells compared to other T cell subsets, especially CTLs and Th17 cells [19], may partly be the cause of HIV disease progression. Since an elevation in CD25+FoxP3+ Treg cells have also been reported in TB [10,11], there may be synergistic immunosuppressive effects of different Treg populations found in TB/HIV co-infected patients that contribute to the persistence of the infection. Interestingly, induction of IDO in both TB [69] and HIV [63] may enhance the induction and accumulation of Treg cells at the site of infection. These regulatory subsets might have a role in suppressing TB- as well as HIV-specific immunity, thus providing the mechanisms that limit pathology but also down-regulate important cellular immune responses at the site of infection.

Another risk factor may involve the experimental findings that Mtb and HIV can act synergistically to manipulate the immune system, by targeting DC-SIGN, a C-type lectin expressed on the surface of DCs. It is believed that DC-SIGN binding to cell wall and envelope proteins of Mtb and HIV, respectively, induce improper DC maturation and immunosuppression which outbalance TLR induced DC activation and inflammation [70]. TLR signaling induces IL-12 production and T cell activation, whereas the interaction of DC-SIGN with Mtb and HIV components, trigger production of anti-inflammatory IL-10. IL-10 could impair the activation of protective T cell responses directed against Mtb and HIV and also act specifically to down-regulate IFN-γ production to minimize immunopathology. Accordingly, the balance between TLR and DC-SIGN signaling may determine the outcome of the immune response in TB/HIV co-infection.

5.2. TB granuloma formation is deficient in HIV-infected patients

If a pathogen is not easily cleared, chronic inflammation upon persistent infection can itself become destructive to the host due to the continuous process of active inflammation, tissue destruction and repair. Whereas Mtb is known to induce a characteristic granulomatous inflammation, HIV induces a non-specific inflammatory reaction. The TB granuloma is characterized by a ball-like organized collection of immune cells with a core composed of tightly clustered Mtb-infected macrophages, which aid microbial containment and prevent systemic spreading of the bacteria. Many types of immune cells and soluble factors, including cytokines, chemokines and cytolytic effector molecules, are involved in the generation and maintenance of the granuloma. It is the immunological balance between the host immune system and bacterial factors that will determine if TB infection will be controlled in the environment of the granuloma or if active TB disease will develop. Therefore it is not surprising that granuloma formation is defective in TB/HIV co-infected patients [71]. Defective granuloma formation is dependent on the degree of immunosuppression in HIV-infected individuals: HIV-positive immunocompetent patients maintain the

capacity to form granulomas whereas patients with moderate immunosuppression or AIDS, gradually loose the ability to maintain cellular integrity and to control bacterial as well as viral replication in the granuloma [72]. Reduced macrophage activation and cellular recruitment, few CD4+ T cells and blunted T cell responses is associated with numerous bacilli and increased viral load in the residual TB granuloma [72]. Similarly, reactivation of latent Mtb in already established granulomas of TB/HIV co-infected individuals, promote disintegration of the granuloma and ultimately dissemination of bacilli as well as HIV-infected cells that have been trafficking into the granuloma. Reduced killing of macrophages co-infected with Mtb and HIV, simultaneously as macrophage function is diminished, may enhance bacterial replication and also result in a sustained increase in local replication of virus. In addition, HIV-induced depletion and/or reduced activation of DCs may result in decreased uptake of apoptotic cells and bacterial components by bystander DC, which could lower cross-presentation of Mtb-antigens and impair subsequent activation of Mtb-specific CTLs.

It has been suggested that the magnitude of Mtb-specific T cell responses enhance local HIV replication and viral pathogenesis at the site of TB infection [73]. Here, Mtb-induced production of excess amounts of MCP-1 and TNF-α, may be partly responsible for the transcriptional activation of HIV [74]. In addition, Mtb infection is associated with a reduced production of HIV inhibitory β-chemokines as well as an up-regulated expression of the HIV-1 co-receptor, CCR5 on T cells that result in increased viral replication in activated CD4+CCR5+ T cells [75]. Excess activation of the immune response may also have deleterious effects and result in immune pathology. Accordingly, it was recently shown that there is a clear association between TNF-α expression and an increased number of necrotic granulomas in HIV-infected patients with pleural TB [76]. Chronic release of proinflammatory cytokines induce tissue necrosis and rupture of granulomas, which will result in systemic release of TB bacteria.

6. Conclusions

Altogether, these data underline the importance of proper cellular immune responses and especially the induction of functional CTLs, in order to execute killing and elimination of HIV and TB-infected cells, that otherwise will nurse the survival of both intracellular pathogens resulting in severe clinical disease. The assessment of TB- and HIV-specific polyfunctional and phenotypic T cell responses will continue to advance our future understanding of these chronic diseases. Exploring the effects of Mtb and HIV related to the *in situ* cellular immune response at local sites is essential to understand the consequences posed on CD8+ CTL function, especially associated with dysfunctional CTL activity.

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