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Mammalian cell cultures as models for *Mycobacterium tuberculosis*–human immunodeficiency virus (HIV) interaction studies: A review

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ABSTRACT

Mycobacterium tuberculosis and human immunodeficiency virus (HIV) co-infections have remained a major public health concern worldwide, particularly in Southern Africa. Yet our understanding of the molecular interactions between the pathogens has remained poor due to lack of suitable preclinical models for such studies. We reviewed the use, this far, of mammalian cell culture models in HIV–MTB interaction studies. Studies have described the use of primary human cell cultures, including (1) monocyte-derived macrophage (MDM) fractions of peripheral blood mononuclear cell (PBMC), alveolar macrophages (AM), (2) cell lines such as the monocyte-derived macrophage cell line (U937), T lymphocyte cell lines (CEMx174, ESAT-6-specific $CD4^+$ T-cells) and an alveolar epithelial cell line (A549) and (3) special models such as stem cells, three dimensional (3D) or organoid cell models (including a blood–brain barrier cell model) in HIV–MTB interaction studies. The use of cell cultures from other mammals, including: mouse cell lines [macrophage cell lines RAW 264.7 and J774.2, fibroblast cell lines (NIH 3T3, C3H clones), embryonic fibroblast cell lines and T-lymphoma cell lines (S1A.TB, TIMI.4 and R1.1)]; rat (T cells: Rat2, RGE, XC and HH16, and alveolar cells: NR8383) and primary guinea pigs derived AMs, in HIV–MTB studies is also described. Given the spectrum of the models available, cell cultures offer great potential for host-HIV–MTB interactions studies.

1. Introduction

Tuberculosis (TB) and HIV/AIDS remain the two most important human communicable diseases globally, especially in Southern Africa. In 2012 alone, an estimated 12 million people worldwide reportedly suffered from active TB and 36.9 million people were living with HIV [1]. At least 30% of the people living with HIV worldwide are reportedly infected with *Mycobacterium tuberculosis* (*M. tuberculosis*, MTB) [2]. An estimated 9.6 million new cases of TB were recorded worldwide in 2014 alone, of which 1.5 million died and 0.4 million were co-infected with HIV [1]. Co-infection with HIV and MTB reportedly contributes to the transition of the latter from latency into active TB disease [3]. Active MTB infections reportedly contribute to HIV pathogenesis by promoting increased immune activation and an associated immune exhaustion, which ultimately leads to immune collapse [4]. Furthermore, co-infection of an individual with MTB and HIV is known to complicate the diagnosis and treatment of the two diseases [5].

Co-infection studies are complex as they superimpose multi-factorial pathogenic pathways associated with each disease.

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When studying a co-infection, it is important to delineate the contributions of each co-infecting pathogen and to assess the summative and synergistic effect of their interactions. In co-infection studies, three major levels of investigation can be used. Level one involves examining responses of cells from mono-infected patients in comparison with those infected with the other pathogen [6]. The second level involves the examination of infected cells from patients (or animal hosts) infected with one disease against the effects of the other causative agent. Level three involves the infection of cell culture model systems with one or both pathogens. The third approach, which is the focus of this review, is the one that provides information on the mechanisms that are involved in mono- and co-infections.

The use of cell culture models has, this far, furthered our understanding of interactions between the human host and the two pathogens (HIV and/or MTB) at the level of the cell, and contributed immensely to drug discovery. However, crucial gaps remain in our understanding of the host-HIV–MTB interactions at the molecular level. This may be attributed, in part, to the lack of robust *in vitro* cell models for such studies, since model systems that allow entry and propagation of the pathogens remain largely unappraised. The lack of a robust cell culture system for the two pathogens is a result of the fact that the pathogens are frequently studied separately in cell and animal models. This article therefore assesses, based on studies this far, the potential of cell cultures as models to study HIV–MTB co-infections.

Use or failure to use one cell culture model or another for co-infection studies is dependent on the ability of the two pathogens to infect and replicate in the cells that make up the supposed model. *M. tuberculosis* has a tropism for phagocytic cells, particularly alveolar macrophages (AMs) and lung dendritic cells (DC). These cell types constitute the majority of lung antigen presenting cells (APC) and defence against pulmonary infection by phagocytosing foreign particles and presenting these antigens to immune cells [7]. While the macrophage is generally considered a primary target cell for MTB, AMs are most important in the case of pulmonary TB. In fact, attachment of MTB cells to AMs represents the earliest phase of primary infection in pulmonary TB [7].

In HIV-1 infection, the $CD4^+$ T lymphocyte is the primary target cell [8]. However, the virus is also able to infect macrophages, monocytes, DC, epithelial cells (especially the anogenital mucosa) and microglial cells [8]. While loss of $CD4^+$ T cells and systemic immune hyperactivation are the hallmarks of primary HIV infection, the end of the acute infection is associated with the emergence of specific $CD4^+$ and $CD8^+$ T cell responses and the establishment of a chronic phase infection [9]. While other cells may harbour HIV infectious agents, especially DCs, such cells may not generate productive infections [10]. Macrophages, which are located at various systemic sites, especially the gastrointestinal mucosa, constitute a major cellular reservoir for the virus [11].

During the early years of the epidemic, HIV strains were characterized as macrophage tropic (M-tropic), $CD4^+$ T cell tropic (T-tropic) or both (dual tropic) [12]. The tropism for HIV is determined by the expression of both CD4 receptors and C−C chemokine receptor type 5 (CCR5)/alpha-chemokine (CXCR4) co-receptors (R5 and X4 viruses, respectively) [13]. Notably, the HIV particles that are transmitted between individuals, referred to as transmitted or founder viruses are R5 strains [14] that are minimally tropic for macrophages and DCs – implying that the infection of macrophages occurs at a later stage after viral transmission [15]. Vaginal and urethral macrophages, but not intestinal macrophages, have indeed been shown to be the initial cell targets for R5 HIV-1 after the translocation of the virus across the epithelium [15]. It has been shown that HIV-1-specific antibodies and $CD8^+$ cytotoxic T cells are detected in most HIV-1-infected people, yet HIV-1 infection is not eradicated. This is a result of the failure of DCs to sense HIV-1 during acute infection and hence, failure to effectively activate naive HIV-1-specific T cells [16]. DCs play an important role in transporting virus from initial infection sites (usually the genital mucosa) to the draining lymph nodes.

Furthermore, the progression of HIV or MTB within a host is controlled by an interplay between the interacting pathogens and a number of host-specific factors, especially those that are associated with cellular immunity [17]. Both HIV and MTB are known to modulate the function of many immune cells, including macrophages, DCs and $CD4^+$ and $CD8^+$ T cells in humans [17]. While alveolar macrophages are the initial target for MTB [18], they can also be infected with HIV [17]. The pathways by which MTB enhances HIV replication in co-infected macrophages, as shown so far, include the modulation of an enhancer binding protein beta transcription factor (CCAAT) [19], interleukins 1β(IL-1β), IL-6 and IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) production [20] and the activation of the NF-κB pathway [21].

In short, both HIV and MTB show variable tropisms for specific cells, particularly alveolar macrophages [22], depending on the receptors that are expressed on the surfaces of recipient cells. While the mechanisms that underlie the interactions between HIV or MTB and the host cell are starting to emerge, there remains a need to identify the full complement of receptors and cell-based immune factors that influence HIV-1/MTB co-infections. Cell culture models, especially those of human origin, can offer a tractable system to understand host–pathogen interactions without the need to use animal or human subjects.

2. Immortalised and primary cell culture models in host–pathogen interaction studies

Immortalised cell lines and primary cell cultures, both of which are often used in host–pathogen interaction studies, offer several advantages over patient-based studies. These include the fact that they are cost effective, easy to use and are often ethically acceptable alternative models of human disease [23]. Immortalisation makes these cells less physiologically relevant than primary cells as models for host–pathogen interaction studies. However, a few immortalised cell cultures including a mouse AM cell line (MH-S) and a human ovarian surface epithelium cell line (HOSE1 line) are known to behave like primary cells [24]. The immortalised cells offer exclusive advantages over other cell cultures in that they are readily infected with both pathogens, have high proliferation rates and long lifespans, maintain their phenotype in culture, provide enough experimental source materials for extensive study and produce homogenous cell populations which ensure consistency and reproducibility of findings [23]. For these reasons, immortalised cell cultures have become a standard *in vitro* tool for host–pathogen interaction studies despite the inherent failure of these models to fully simulate human diseases.

Successful use of primary and immortalised cell cultures in HIV or MTB studies has been demonstrated [25]. Primary cell cultures offer an added advantage over immortalised cells in that they closely mimic the environment of pathogens *in vivo* [26]. However, the use of primary or immortalised cultured cells is limited by a number of inherent weaknesses, including the fact that (i) most immortalised cell lines cannot fully mimic the *in vivo* cell conditions, [27] (ii) structures on the surface of cultured cells, including receptors, are increasingly modified with increase in the passage number, [28] (iii) cultured cells lack access to the native environment that typically modulates their phenotype *in vivo* [28] and (iv) the cells do not retain *in vivo* functionality and morphology [29]. Primary cell culture models present an additional limitation in that they are practically difficult to propagate to sufficient numbers for host–pathogen interaction experiments [30]. Hence, primary cells and immortalised lines are not optimal models for highly specialized cell types that characterise diseased tissue *in vivo*. To circumvent challenges posed by immortalised and primary cell cultures in host–pathogen interactions, stem cell culture models have emerged, which include pluripotent cells.

3. Cell culture models for HIV–MTB co-infection studies

3.1. The potential of human-derived primary cell cultures as models for HIV–MTB co-infection studies

3.1.1. Human peripheral blood mononuclear cells (PBMCs)

The use of PBMCs, including lymphocytes and monocytes in HIV–MTB co-infection studies has also been described. The successful use of PBMCs in host–pathogen interaction studies has permitted the elucidation of basic pathogenic mechanisms, including the demonstration of the enhancement effect of MTB on HIV replication, relying on the modulating function of monocytes, tumour necrosis factor alpha (TNF-α) and monocyte chemo-attractant protein-1 (MCP-1) cytokine pathways [31]. A recent study by Wilkinson *et al.* [32] demonstrated the occurrence of tuberculosis-associated immune reconstitution inflammatory syndrome among HIV–TB-co-infected patients commencing antiretroviral therapy (ART) using an *ex vivo* culture of PBMCs. In the same study, Wilkinson *et al.* [31] demonstrated that infection with MTB and ART influences gene expression in freshly isolated and cultured PBMCs from HIV-infected individuals. Vijayakumar *et al.* [25] described the use of an *in vitro* cell model comprising PBMCs and monocyte-derived macrophages (MDM) in a study on interactions between *Mycobacterium bovis* (*M. bovis*) BCG and HIV-1, as well as in drug toxicity and activity studies in a co-infection. Subsets of the PBMCs, especially monocytes and T cells, have provided important information on the attachment, entry and replication of MTB and HIV, making such cells a useful platform for complex host-HIV–MTB interaction studies [25].

3.1.2. Human ex vivo monocyte derived macrophages (MDM)

The use of *ex vivo* MDM cultures, a fraction of the PBMC, in TB and HIV research has also been described [33]. Campbell *et al.* [34] showed a link between autophagy in human MDM and vitamin D3-induced innate immunity, and consequently demonstrated the potential of vitamin D3 as therapy for HIV–MTB infections. Furthermore, MDM have been used as a model for transcriptomic and proteomic profiling of HIV or MTB in host macrophages [35,36]. Chertova *et al.* [35] detected at least 250 host proteins and at least 29 viral proteins in HIV infected human MDM. Ragno *et al.* [36] demonstrated the modulation of a number of TB related factors in MDM. The fact that macrophages constitute a reservoir for both HIV and MTB [11,37] and that these cells specialise in internalising intracellular pathogens, makes these cells a convenient tool to study HIV–MTB co-infections.

3.1.3. Human ex vivo alveolar macrophages (AM)

In addition to the human MDM, the use of AM, which are differentiated from monocytes and obtained by bronchiolar lavage, in studies that seek to understand interactions between the host and HIV-1/MTB has also been described [38]. Through the use of human AM, Spira *et al.* [39] demonstrated the importance of the modulation of TNF-α expression in defending the host against tuberculosis. Furthermore, Silver *et al.* [40] showed that infection of an individual with the H37Rv strain of MTB upregulates the expression of IL-23 in the AM but not in PBMC derived macrophages, pointing towards the importance of the cytokine in pulmonary infection with the bacterium. Besides being able to permit the attachment, entry and replication of HIV and MTB, AM also express a range of immune factors, namely Th1 cytokines (IL-2 and IFN-γ)or Th2 cytokines (IL-4 and IL-10) in response to infection with either or both pathogens [41]. These features make AM a convenient model for HIV–MTB co-infections and related drug discovery studies.

3.1.4. Human ex vivo T lymphocytes

While HIV-1 infects various cell types of the immune system, $CD4^+$ T helper cells constitute a major target for the virus. $CD4^+$ T cells express high levels of the HIV-1 receptor CD4 on their surface and are highly permissive to HIV-1 replication [42]. *Ex vivo* human T lymphocytes and other immune cells obtained from HIV–MTB infected and disease free individuals have been used in studies on interactions between the two pathogens and the human host [43]. Protocols to isolate and culture $CD4^+$ and $CD8^+$ T cells from PBMCs are widely described. Primary cultures of T lymphocytes obtained *ex vivo* have provided vital clues on the pathogenesis of HIV and MTB.

Despite the limitations that are associated with these models, the data gathered from primary cells *in vitro* can be readily extrapolated to the *in vivo* disease situation. The fact that human macrophages are a common reservoir for HIV and MTB validates the use of these cells as models for HIV–MTB co-infections.

4. The potential of human-derived cell lines as models for HIV–MTB co-infection studies

Studies have described the use of human derived cell models in HIV and/or MTB research, including the use of (i) U937, [6] (ii) T lymphocytes (CEMx174, ESAT-6-specific $CD4^+$ T-cells), [44,45] and (iii) alveolar epithelial cells (A549) [46,47]. U937 (American Type Culture Collection – ATCC® CRL1593.2™) is a human monocyte-derived macrophage cell line that was established from the pleural effusion of a patient with histiocytic

lymphoma [48]. The U937 cell line expresses receptors CD13, CD15 and CD33, and mRNA for the c-myc oncogene [6]. The use of U937 cells has led to the discovery of a number of MTB proteins, including Rv2004c-6, Rv1510, [49] Rv1268c, [46] and protein 16084, a peptide of Rv2707 [50]. Wang *et al.* [51] used the U937 cell line to demonstrate that phosphotyrosyl phosphatase activator (PtpA), phosphatase, is essential for the pathogenesis of MTB. In the same studies, Wang *et al.* [52] showed that MTB subverts the host innate immunity by co-opting host ubiquitin, a pathway which can be exploited as a drug target. The U937 cell line is therefore a convenient model for HIV–MTB drug discovery and pathogenesis studies.

Interactions between HIV and MTB with their host have also been demonstrated using the A549 cell line [44,52]. The use of A549 in HIV-1 drug discovery studies has also been described [51]. Using this cell line, protein arginine methyltransferase 6 (PRMT6) was shown to methylate the HIV-1 Tat and, hence, to act as a restriction factor for HIV replication [47]. This has shown the importance of the A549 cell line as a convenient model for further studies on MTB/HIV pathogenesis, including vaccine design.

4.1. Stem cells

To circumvent the limitations that are inherent in immortalised and primary cell cultures as highlighted above, researchers are now opting for stem cell technology as a viable alternative for MTB/HIV pathogenesis/interaction studies. Stem cells are pluripotent and non-specialised cells with the potential to differentiate into other types of specific cells, such as blood, brain, tissue or muscle cells [30]. Newer developments in the field have seen the development of induced pluripotent stem cells (iPSC), which are genetically modified stem cells that behave like embryonic stem cells [53]. Multiple studies have reported a great potential of iPSC on interactions between HIV–MTB with the host cells [54,55] and their use as functional cure for HIV [56–58]. Recent developments have shown that the incorporation of anti-HIV lentiviral vectors in stem cells offers renewed hope for developing safer therapeutic interventions with long-lived efficacy against HIV [56,57]. Stem cells are also a convenient tool for vaccine design against HIV [58]. Stem cell based vaccine design protocols involve the induction of somatic cells to iPSC, expansion of iPSC, genetic or chemical modification to produce immune cells, *in vitro* antigen presentation and processing which culminate in the production of memory B cells that can secrete functional antibodies [58]. The generated cells are then transplanted back into the host as a vaccine [58].

However, despite the recent expansion in the use of stem cells in host–pathogen interactions, their use has not been without limitations. The existing iPSC protocols do not allow for efficient direction of these cells into desired lineages [54]. Additionally, as in the case of primary cell culture models, stem cell yields are frequently inadequate for each study [54]. The use of iPSC, like all other modified cell culture models, has an inherent limitation in that outcomes from the model do not fully mimic those of the disease [54] To circumvent these obstacles, even newer approaches have been developed including the use of three-dimensional (3D) organoid cultures using iPSC, xenotransplantation of human cells into animal models and multiple cell co-culture models that include cells derived from isogenic iPSC.

Nonetheless, the advantages of using stem cells out-weigh the limitations highlighted above. As such, stem cells present a great potential as models for vaccine design, particularly against HIV-1.

4.2. Three dimensional (3D)/organoid models

Recent developments have made it possible to direct the differentiation of human pluripotent stem cells (hPSC) into 3D organoid cultures *in vitro* using a temporal series of growth factor manipulations to mimic embryonic tissue development. Lancaster *et al.* [59] reported the development of an hPSC derived 3D brain organoid culture system (cerebral organoid), which they claimed differentiates into various discrete, though interdependent, brain regions. If validated, such a development can serve as an essential tool to create various tissues that mimic targets for HIV and MTB. Three dimensional (3D) assays allow for the evaluation of host–pathogen interactions in multicellular tissues.

In people infected with MTB, the bacterial cells are 'walled off' in granulomas/tubercles away from the immune system [60]. There is a need to recreate conditions *in vitro* in order to understand the reasons that underlie the long hibernation of MTB in granulomas and the transition of MTB from latency into active TB disease, and discover drugs that target the bacterium in tubercles. The development of granulomas *in vitro* using conventional cell cultures has been described with variable success [61]. Guirado *et al.* [61] demonstrated the development of granulomas from a human PBMC culture. Al Shammari *et al.* [62] also described the development and use of a 3D cell culture model of tuberculosis granuloma formation. So far, the use of 3D iPSC technology in granuloma formation has not been described. We perceive the use of iPSC in 3D cell culture technology, with potential to form granulomas, as a future for HIV–MTB research.

4.3. Human blood–brain barrier (BBB) cell model

HIV-1 is known to cross the BBB to invade the central nervous system during the early stages of the viral infection, which culminates in the impairment of neurons, becoming evident several years later [63]. A number of models that simulate the BBB for HIV research have been described [63]. Persidsky *et al.* [63] describes a model of the BBB consisting of a matrix-coated membrane with brain microvascular endothelial (BMVEC) cells on one side and astrocytes on the other. This model demonstrated the formation of tight junctions between the astrocytes and the monolayer of BMVEC cells, the development of filopodia, lysosomes, and vesicular Golgi complexes, and the upregulation of proinflammatory cytokines (TNF-α, IL-6, and IL-10) [64]. Cell culture models can therefore be useful tools for studying the entry of HIV across the BBB.

5. The potential of primary murine cell cultures as models for HIV–MTB co-infection studies

5.1. Mouse primary cells and cell lines

The use of mice in HIV research is limited by the lack of infectivity of the mice cells by HIV, lack of factors necessary for HIV-1 replication and the expression factors that interfere with

retroviral replication [63]. However, the use of a number of murine cell lines in HIV research has been described, including the macrophage cell lines RAW 264.7 (ATCC® TIB71™), [64] J774.2 (ATCC® TIB-67™), [65] fibroblast cell lines including NIH 3T3 (ATCC® CRL1658™), [66] C3H clones (ATCC® CCL226™ and ATCC® CRL1411™), embryonic fibroblast cell lines, [67] T-lymphoma cell lines S1A.TB, TIMI.4 and R1.1. Despite the fact that mouse T cells restrict the integration of virus genome during replication, RAW 264.7 cells has frequently been described in HIV research, especially in pathogenesis and drug discovery studies [64]. The use of RAW 264.7 cells in MTB research is also described [68]. Another murine macrophage cell line, J774.2 (ATCC® TIB-67™), has also found a place in HIV and TB research. The use of J774.2 cells has been described in multiple studies, including drug discovery against HIV and MTB [69,70].

5.2. Rattus cell line models for MTB and HIV studies

Only a few rat cell lines have been used in HIV and TB research, especially XC (ATCC® CCL-165™) [71,72]. A study by Goffinet *et al.* [71] showed that rat cells (Rat2, RGE, XC and HH16) complete all of the early steps in the HIV-1 replication cycle, including provirus integration *in vivo*, with high efficiency and elevated HIV gene expression in primary rat T-cells. However, a study by Bieniasz and Cullen [73] showed that HIV-1 has limited entry efficiency in rat cells. Hino *et al.* [72] demonstrated that BCG-infected rat alveolar cells (NR8383) are a potential *in vitro* model for chronic infection with MTB. The fact that rat cell lines have limited permissibility for HIV entry makes them a less preferred model for HIV–MTB research compared to human cell models.

5.3. Cavine cell lines for MTB and HIV studies

Guinea pigs, besides being used as animal models for various diseases including TB and HIV, are also widely used as a source of cell cultures for studies on the pathogenesis of MTB [74]. A protocol to isolate AM from guinea pigs, which includes a bronchoalveolar lavage technique, is widely described [74]. The guinea pig AM cells may be a more ethically acceptable model for host pathogen interactions than human derived cell lines and primary cultures.

6. The potential of cell culture models in high throughput studies of HIV–MTB co-infections

High throughput techniques, which incorporate automation of experiments to allow large scale repetitions in tests including cell-based arrays, have become a new frontier for drug discovery [75]. Christophe et al. [76] described a high-throughput and high-content workflow, which incorporates the use of cell culture and imaging technology in the development of new TB drugs. Jiang et al. [77] also described a high throughput method that uses human embryonic kidney 293 (HEK293T) cells to study gene expression in the presence of HIV. High throughput techniques can be adapted to any cell model that is permissive to attachment, entry or replication of HIV and MTB. The high throughput technique is an essential addition to cell culture modelling, which permits researchers to obtain reliable results faster.

7. Conclusion

Cell culture models, especially those of human origin, have great potential as models to study interactions between HIV and MTB in co-infected individuals, or to study host responses to either or both pathogens. Human macrophages, including AM and MDM, bear an unparalleled potential as models for these co-infections due to their permissiveness for HIV and MTB attachment, entry and replication, and their capacity to elicit host specific cell-based immunity in the presence of either or both pathogens. Rat and mouse cell cultures offer limited potential since they do not efficiently support all stages of HIV replication. The exploitation of stem cell technologies such as iPSC, whose differentiation can be directed as desired and 3D cell culture models are recent developments that have provided a new dimension to the field of host–pathogen interactions. The incorporation of high throughput technologies to cell culture modelling has pushed the frontiers of HIV–MTB co-infection research allowing researchers to have more options for host–pathogen interaction studies. While multiple cell culture models are available for host-HIV–MTB interaction studies, the complexity of such interactions demands the development and validation of more cell culture models.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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