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REVIEW

HIV-1 and the *Mycobacterium tuberculosis* granuloma: A systematic review and meta-analysis

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SUMMARY

Infection with HIV-1 greatly increases the risk of active tuberculosis (TB). Although hypotheses suggest HIV-1 disrupts *Mycobacterium tuberculosis* (Mtb) granuloma function, few studies have examined this directly. The objective of this study was to determine what evidence exists about the effect HIV-1 co-infection has upon Mtb granulomas. A systematic search of PubMed, Web of Science, and Medline up to 20 March 2015 was conducted, to identify studies comparing Mtb-infected tissue from HIV-1 infected and uninfected persons, or HIV-1 infected persons with stratified peripheral CD4 T cell (pCD4) counts. We summarized findings that focused on how HIV-1 changes granuloma formation, bacterial presence, cellular composition, and cytokine production. Nineteen studies with a combined sample size of 899 persons were included. Although studies frequently were limited by variable or inadequately described definitions of outcomes and analytical methods, HIV-1 was found to be associated with increased bacillary load within Mtb-infected tissue. Reductions in pCD4 counts within co-infected persons associated with both poorer granuloma formation and higher bacterial load. The high degree of heterogeneity among studies combined with experimental limitations made it difficult to conclusively support previously published and prevalent hypotheses about HIV-1/Mtb co-infection granulomas. To elucidate the validity of these hypotheses we have described areas that can be improved in future studies in order to clarify the influence HIV-1 co-infection has upon the Mtb granuloma.

1. Introduction

Tuberculosis (TB) and HIV-1 are two of the leading infectious causes of death worldwide and TB is the leading cause of death among HIV-1 infected persons [1]. Once infected with *Mycobacterium tuberculosis* (Mtb), HIV-1 infected persons have increased morbidity and mortality due to TB compared to HIV-1 uninfected persons [2]. As peripheral CD4 T cell (pCD4) counts fall, susceptibility to active and disseminated TB increase, however, HIV-1 infected persons with relatively preserved pCD4 counts are also at increased risk [3]. It has been hypothesized that the primary cause for increased TB susceptibility in HIV-1 infected persons is due to immunological disruptions of the Mtb granuloma [4,5].

The granuloma is the hallmark of TB. Granulomas consist of a collection of organized immunological cells that form in response to Mtb infection [6]. Granulomas commonly consist of infected and recruited macrophages, differentiated epithelioid cells, all surrounded by a lymphocyte layer. The relationship between the granuloma and TB is complex and not fully understood because granulomas can prevent dissemination and kill Mtb, but also allow persistence of Mtb and even be permissive to its growth [7,8]. This illustrates the delicate balance between bacterial growth and death within the microenvironment of the granuloma and that granulomas form an incompletely effective or even bacterium-permissive immunological response [7,9]. It is hypothesized that HIV-1 disrupts this balance by causing granulomas to be more disorganized, killing resident CD4 T cells, and dysregulating normal T cell, and macrophage function (Table 1) [4,5,10–14], leading to an

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Table 1
Hypotheses how HIV-1 manipulates Mtb granulomas.

Review (first author, year published)	Main hypotheses regarding granulomas in HIV-1/Mtb co-infected persons
Ledru, 1999 [10]	HIV-1's ability to manipulate cytokine and nitric oxide production that are recruited to the granuloma may play an important role in increasing bacterial dissemination.
Bocchino, 2000 [11]	Poor granuloma formation within co-infected persons most likely results from a disruption in the pro- and anti-inflammatory production of cytokines and an increase in cell death of CD4 T cells.
Lawn, 2002 [5]	Granulomas within co-infected persons will be poorly formed and contain increased bacterial growth through the impairment of cellular recruitment and cell-mediated granulomatous response.
Diedrich, 2011 [4]	Granulomas within co-infected persons will have impaired architecture, reduced CD4 T cell counts, impaired T cell and macrophage function, and increased cell death.
Kwan, 2011 [12]	HIV-1 replication may be induced by Mtb-infected macrophages that will indeed lead to HIV-1 infection of adjacent macrophages and CD4 T cells and activated CD4 T cells will increase HIV-1 replication
Geldmacher, 2012 [13]	Preferential depletion of Mtb-specific and total CD4 T cells may play a significant role in granuloma disruption within HIV-1 infected persons
Ansari, 2013 [14]	HIV-1 enters granulomas and causes CD4 T cell apoptosis, depletion, and disrupted recruitment of T cells, which leads to granuloma disorganization.

increase in susceptibility to both active and disseminated TB disease.

Many studies in humans that support these hypotheses have measured immunological responses within non-tissue resident cells: peripheral blood mononuclear cells (PBMC), bronchoalveolar fluid (BALF), and pericardial fluid (PCF) [15–18]. These studies have demonstrated impaired Mtb-specific T cell activity [15,16,18] and killing of Mtb-specific peripheral CD4 T cells [19], and total BAL CD4 T cells [17] in persons infected with HIV-1. Although these data are convincing, there are significant variations in cellular composition and Mtb-specific immunological responses within PBMC, BALF, PCF, and granulomas [20,21]. Extrapolating data from non-tissue resident cells to what is occurring within granulomas may not be appropriate [22]. This illuminates a need to study human granulomas directly in HIV-1/Mtb co-infected persons to better understand how HIV-1 manipulates the TB granuloma. Understanding how HIV-1 manipulates granulomas directly will elucidate the mechanistic cause HIV-1 exploits that increases TB susceptibility.

We systematically reviewed HIV-1/Mtb co-infection literature that examined Mtb granulomas directly. The objective of this systematic review and meta-analysis was to elucidate how Mtb-infected tissues differ between HIV-1 infected and uninfected persons. We focused on studies that reported how HIV-1 changed: granuloma formation [23–29], granuloma organization [23,26–28,30–35], granuloma caseation [23,25,26,29–33], Mtb growth [23–25,27,31,32,35,36], cellular populations [23,26,30–33], cytokine expression [30,31,37] and HIV-1 virion presence within excised tissue [33,38]. To help reduce some of the variability observed within this literature our second objective of this review was to illuminate future strategies to study, analyze, and report granuloma-based data.

2. Methods

2.1. Search strategy and selection criteria

PubMed, Web of Science, and Medline were searched using predetermined combinations of terms (Supplemental Table 1) for relevant peer reviewed studies (through 20 March 2015) that reported histological data in TB diseased tissue from HIV-1/Mtb co-infected persons. We reviewed original articles published in all languages. In addition to the database search, we screened citations in the full-text articles reviewed here, published reviews, book chapters, and suggested papers from experts in the field.

The primary objective of this review was to identify how Mtb granulomas from HIV-1/Mtb co-infected persons differed from granulomas obtained from HIV-1 uninfected persons. Studies were eligible for inclusion if they compared the histology of Mtb infected tissue from HIV-1 infected and uninfected persons, or from HIV-1 infected persons with stratified pCD4 counts. Studies were required to include an acceptable means of defining HIV-1-infected and uninfected groups (either HIV-1 serology or a documented past history of HIV-1 infection, or for studies prior to 1990, an acceptable HIV/AIDS diagnosis by World Health Organization criteria at the time of publication) and confirming TB diagnosis (microbiology or histology consistent with TB, with or without a consistent clinical picture including course of illness and response to treatment). Studies were excluded if the method of biopsy was only fine-needle aspiration (FNA) as this method of excision was unlikely to preserve granuloma architecture and may not capture entire granulomas within the target tissue. Where studies reported or appeared to report on the same or overlapping persons, the results from the earlier study were excluded. Reviewers independently assessed the eligible articles for inclusion and exclusion criteria; disagreements were resolved by consensus. The included studies were assessed for quality of study design and potential limitations to findings.

2.2. Data extraction

Results from the individual studies were categorized into the following outcomes for comparison between HIV-1/Mtb co-infected and HIV-1 uninfected persons, or HIV-1-infected with stratified pCD4 counts: 1) proportion of biopsied samples with granulomas present, 2) quality of granuloma formation (quality of granuloma formation was defined independently within each study), 3) cellular and cytokine presence, 4) proportion of biopsied samples containing Mtb (acid fast bacilli [AFB] or culture positivity [CFU], 5) bacillary load within AFB+ samples, and 6) HIV-1 virion presence.

2.3. Statistical analysis

For outcomes where studies reported individual quantitative or semi-quantitative results for the different outcomes HIV-1/Mtb co-infected and Mtb-only infected persons, a meta-analysis was performed using the Cochrane Database's RevMan program. We calculated summative risk ratios for changes in 1) granuloma presence, 2) quality of granuloma formation, 3) AFB presence 4) CFU in HIV-1/Mtb-co-infected versus Mtb-only infected persons and 5) AFB load. Where results were categorically scored and not simply dichotomous (for quality of granuloma formation and AFB load), the proportions of persons in each group with the highest scores (for well-formed granulomas and AFB load) or lowest scores (for poorly formed granulomas) were used in meta-analyses. Data sets were treated as dichotomous and risk ratio with 95% CIs were

calculated using Mantel–Haenszel method with a random effects analysis model. Heterogeneity was assessed with I^2 statistics and defined as low ($I^2 \leq 25\%$), moderate ($25\% < I^2 \leq 75\%$), high ($I^2 > 75\%$).

3. Results

Our initial search yielded 3645 abstracts and titles (Figure 1). After review of these abstracts, titles, and where appropriate, full studies, 60 studies were identified that examined the histopathology of Mtb infection in persons co-infected with HIV-1. Eight studies were excluded because, while they described the findings in tissue from HIV-1/Mtb co-infected persons, no comparison was made to either HIV-1 uninfected persons, or between different HIV-1 persons at different stages of HIV-1 infection or pCD4 count [39–46]. Sixteen studies were excluded, because their results were based on FNA [47–62]. Four studies did not adequately define HIV-1 [63–66] and one did not adequately define HIV-1 or TB status [67]. Four studies did not differentiate results between Mtb and other mycobacteria [68–71]. One study may have been biased by persons having co-morbidities [72]. Two studies [31,33] reanalyzed, at least in part, some results from persons in previous studies [73,74]. For these overlapping studies, results from the later published study were included [31,33]. Five studies did not sufficiently examine tissue samples [75–79].

Nineteen unique studies [23–38,80–82] were included in this review (Table 2). A total of 413 Mtb-only infected persons (with a range between 3 and 108 persons, median n = 14) and 486 HIV-1/Mtb co-infected persons (range: 5–109, n = 13). The site of TB disease within each study was varied: five examined lymph node [26,31,32,80,83], seven examined pleura [23,27,29,35–37,82], three examined lung [24,28,30], one each examined spine [33], pericardium [25], brain [81], and one study analyzed multiple tissues from different persons (lymph node, lung, pleura, bone marrow) [34]. pCD4 counts were stratified for comparison in HIV-1/Mtb co-infected persons in five studies [23,28,31,34,38]. One study described stratified pCD4 counts without presenting individual data [35]. Two of the pCD4-stratified studies examined only HIV-1/Mtb co-

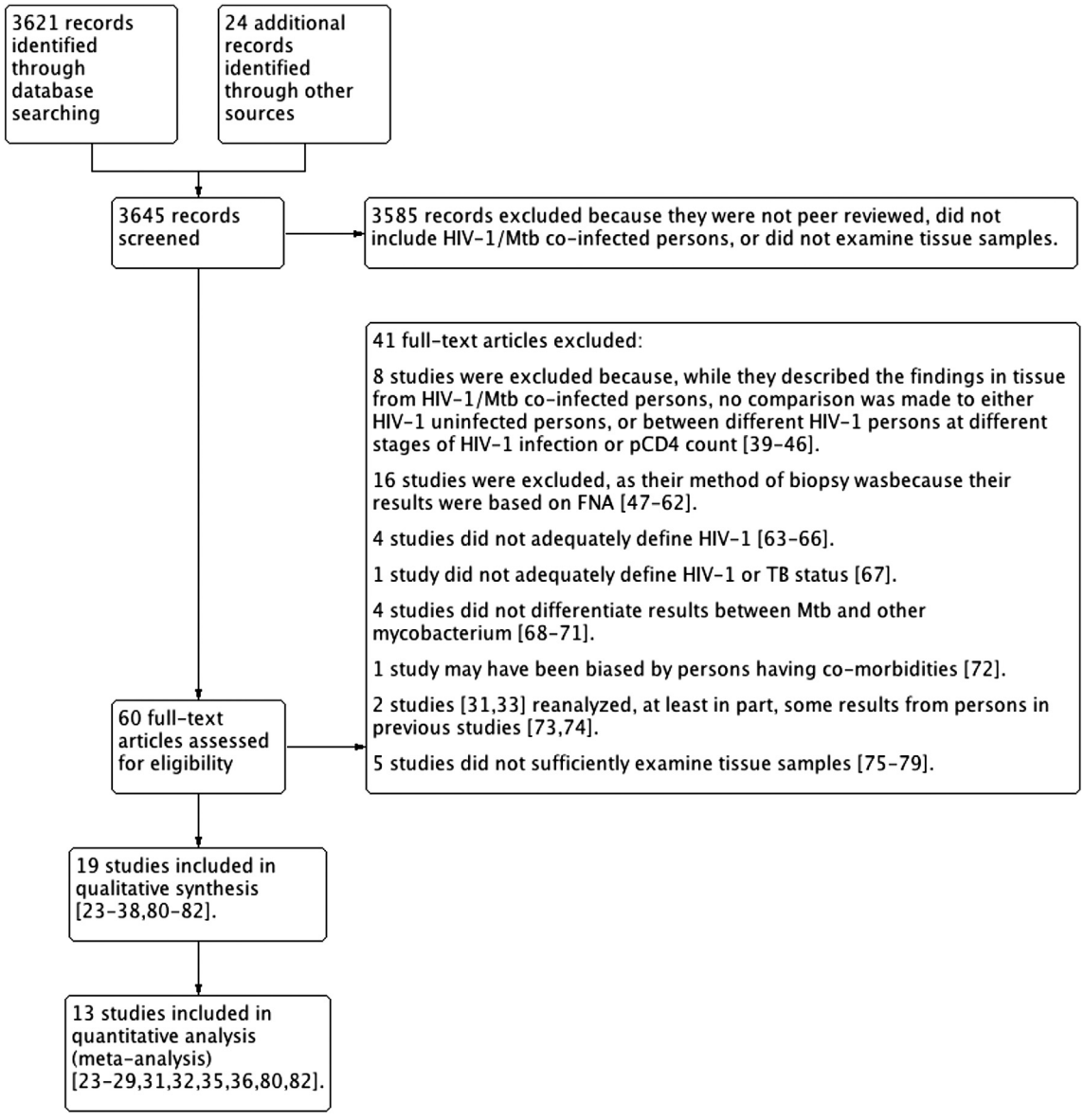

Figure 1. Study selection.

Table 2
Study summaries.

Study (first author, date, country)	Number of persons with Mtb-only (mean age)	Number of HIV-1 co-infected persons (mean age)	HIV-1 stage and mean pCD4	Biopsy site	Key measurements relevant to review	Main findings (co-infected vs Mtb-only)	Comments
Bezuidenhout 2009 South Africa [37]	6 (38)	6 (34·5)	*Early stage*- qualified by no OI	Pleura	- Granuloma necrosis and counts - IFNγ, TNF, IL12, IL4 mRNA	↑- Granuloma necrosis ↑- TNF mRNA production **?-** IFNγ, TNF, IL12, IL4 mRNA production	- 166 granulomas from 12 patients but analyzed granulomas as independent entities, which may have biased results toward persons that contained more granulomas - Methodology for examining cytokine production was difficult to interpret and compare among other studies - Selection of region of interest (ROI) was not described - Granuloma definitions were not described - No mention of pathologists being blinded
Conde 2003 Brazil [82]	71 (37·2)*	13	Not stated	Pleura	AFB and CFU	↑ AFB smear presence • No significant difference in CFU	- The primary aim of this study was to investigate diagnostic yield of sputum induction in pleural TB. Pleural biopsy tissue, however, was examined as part of the study and the results from this incorporated to our study. - No blinding was described - *Mean age of all persons within study
Danaviah 2013 South Africa [33]	9 (44·4)	13 (26·4)	544·6 +- 315	Spine	- Viral load (tissue homogenate and plasma) - CD3, CD4, CD8 markers - giant cell (GC) counts	• Same granuloma organization • Same CD3% ↓ CD4% ↑ CD8%	- Further analysis of persons from previously published study [82] - Selection of *intact* granulomas may have biased results toward well-formed granulomas - Single CD4 and CD8 markers used may lead to non T cells being quantified - Selection of ROI not described. - Granuloma definitions were not described - No mention of pathologists being blinded
De Noronha 2007	4	5	Not stated	Lung	- Granuloma organization, architecture and	↑ Granuloma disorganization • Similar necrosis	- Autopsies performed on persons that died of

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Table 2 (*continued*)

Study (first author, date, country)	Number of persons with Mtb-only (mean age)	Number of HIV-1 co-infected persons (mean age)	HIV-1 stage and mean pCD4	Biopsy site	Key measurements relevant to review	Main findings (co-infected vs Mtb-only)	Comments
Brazil [30]					composition (from H&E), necrosis, bacilli load, TNF, TGF-β	↓ TNF production • Similar TGFβ ↑ AFB presence stated (not presented individually)	pulmonary TB may have biased results towards more severe TB disease. - 3 blinded pathologist were used - Selection of ROI was not described - Granuloma definitions were not described
Di Perri 1996 Italy [28]	16 (32·5)	16 (30·7)	>400:3 200–400: 3 100–200: 3 <100: 7	Lung	Granuloma organization, AFB load correlated with stratified pCD4 counts	↓ Granuloma counts ↑ Poorly formed granulomas ↓ Well formed granulomas ↑ AFB presence and load • pCD4 counts indirectly correlate to ↑ bacterial load, ↑ poorly formed granulomas, ↓ well formed granulomas	- All patients were sputum negative prior to bronchoscopy, which may have lead to selection bias. - Method of biopsy – from area of consolidation; or right middle lobe or lingual in case of no focal or only diffused consolidation may have biased results - Multiple pathologist reviewers, however no blinding detailed. - Granuloma and AFB load descriptions were available - Selection of ROI was not described
Elliott 1993 Zambia [36]	5	9	Not stated	Pleura	CFU	• No significant difference in CFU	- Study described clinical and diagnostic differences between HIV-1 infected and uninfected persons with TB. - No blinding was described
Heyderman 1998 Zimbabwe [23]	11-(33)	63 (23)	191 (0–2009) >500 11 200–490: 19 100–190: 6 <100: 14	Pleura	Granuloma counts, poorly formed, caseation, GC, AFB and CFU positive, AFB load, stratified pCD4	• No difference in granuloma counts, caseation, formation, giant cells, AFB+ or CFU+ samples, or scanty AFB ↑ Tissue with numerous AFB+ • pCD4 counts may correlate to increased bacterial load	- 63 persons in co-infected group, - pCD4 stratified to results. - Granuloma formation, scanty and numerous AFB loads were not described. - Selection of ROI was not described. - Blinded pathologists were not described.
Hochedez 2003 France [80]	19 (38·5)	13 (36·5)	Not stated	LN	AFB, CFU, Granuloma presence, Caseation	• No significant difference in granuloma presence, AFB, CFU, or caseation	- Method of selection of ROI not described - Precise granuloma descriptions were not provided
Jones 1993 USA [34]	NA	23	Stratified pCD4/mm^3 from 21 persons: <101: 9	9 LN, 6 lung, 4 pleura, 4 bone marrow	Granulomatous changes stratified to pCD4 counts	• *Granulomatous changes* observed regardless of pCD4 counts.	- Granuloma formation and tissue AFB presence were not primary

Table 2 (*continued*)

Study (first author, date, country)	Number of persons with Mtb-only (mean age)	Number of HIV-1 co-infected persons (mean age)	HIV-1 stage and mean pCD4	Biopsy site	Key measurements relevant to review	Main findings (co-infected vs Mtb-only)	Comments
			101–200: 5 201–299: 3 >300: 4				aim of this study - Granulomatous changes were not defined.
Kennedy 1992 USA [24]	45	67	Not stated (likely many late due to year of study)	Lung	Granuloma counts, AFB and CFU positivity	• No difference in AFB smear or CFU ↓ Granuloma presence	- The primary aim of this study was to assess the utility of bronchoscopy in sputum negative persons in the diagnosis of pulmonary TB in HIV-1 infected patients, rather than to assess granuloma differences - 67 co-infected and 45 HIV-1 uninfected persons were in this study. - Granulomas were not defined - Potential sampling bias associated with bronchoscopic biopsy site selection technique may have occurred - Selection of ROI was not described - Blinded pathologists were not described
Luzze 2001 Uganda [35]	33 (33)	109 (34)	Not stated	Pleura	Granuloma formation, CFU, BACTEC culture, days to culture positivity	↓ Well formed granulomas ↑ CFU positivity • No significant difference in BACTEC culture positivity or days to positivity • Granuloma formation was not different in persons with fewer pCD4 counts	- The primary aim of study was to compare clinical, radiographic and diagnostic methods in pleural TB between HIV infected and uninfected persons - Method of granuloma assessment is not specifically described. - No blinding was described
Muller 1994 Germany [31]	8	8	Individual pCD4/mm^3: 100–200:4 200–300: 2 300-400: 2	Lymph node (LN)	Necrosis, MNGC, Epithelioid cell layer, CD68, lysozyme, α-1-anti-chymotryp., Mac387, Ki-M8, CD43, CD3, CD4, CD8, CD25, HLA-DR, Ki-67, CD22, IL-1α, IL-1β, IL-6, TNF, IFN-α, IFN-β. Stratified to pCD4 counts	↓ well formed epithelioid layer within granulomas ↑* Neutrophil ↓GC, marginal fibrosis and angiogenesis • Same necrosis and all other cellular markers ↑* TNF, IFNα, IFNβ, IL6, IL1α, IL1β as pCD4 counts reduce	- All results except cytokine production appeared to overlap with a previously published paper [83] - Examined 22 histological outcomes and stratified pCD4 counts - All measurements were categorical without detailed descriptions - Specific LN type is not discussed - No blinding or selection of ROI described

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Table 2 (*continued*)

Study (first author, date, country)	Number of persons with Mtb-only (mean age)	Number of HIV-1 co-infected persons (mean age)	HIV-1 stage and mean pCD4	Biopsy site	Key measurements relevant to review	Main findings (co-infected vs Mtb-only)	Comments
							* Some conclusions were difficult to determine because of qualitative nature of measurements
Ngilimana 1996 Rwanda [32]	3 (27)	12 (32)	Not stated	LN	Langhan cells, Epithelioid cells, lymphocytic cuff, foamy macrophages, necrosis type, AFB load, epithelioid venule, plasmocytes, granuloma formation	↓* Well formed granulomas ↑ AFB load ↑* Epithelioid macrophages ↓*Lymphocytes and Lanhan cells	- Description of granulomas was detailed in previously published study [51] - Examined a 9 histological outcomes in 12 co-infected persons and only 3 Mtb only persons-Specific LN type was not discussed, - Blinding and ROI selected were not described * All measurements were categorical
Perfura-Yone 2011 Cameroon [29]	108 with pleural biopsy	81 with pleural biopsy	Not stated	Pleura	Granuloma counts and caseum.	• No difference in granuloma presence or necrosis ↑ Necrosis within persons with <200 pCD4/mm^3	- Study focused on clinical, radiological, hematological as well as histological picture in a high number of persons with tuberculous pleural effusion. - 108 Mtb-only and 81 co-infected persons were examined in this study. - The method for selection of ROI not described - There was not mention of blinding of assessing pathologists
Reuter 2006 South Africa [25]	20	5	Not stated	Pericardium	Granuloma counts, central necrosis, CFU positivity	• No significant difference in granuloma counts, necrosis or AFB presence	- Sub dividing 25 persons into 4 groups and 7 histopathological outcomes resulted in small 8 outcomes containing only 1 person. - Blinding and ROI selected were not described - Granulomas were not defined
Shen 1988 USA [26]	3	5	AIDS by CDC 1985 criteria (OI)	LN	AFB, brief granuloma description, epithelioid macrophage counts, CD3, CD4, CD8, CD26, HLA-DR, CD14 percentages	• No difference in granuloma formation ↑ AFB load stated without data ↓ CD3 and CD4% • No difference in CD8%, but CD8 T cells are distributed throughout granulomas • No difference in other markers	- Only 3 persons in Mtb-only group - Problematic definition for 2 persons in HIV-1 group because they were defined as risk factors and symptoms indicative of AIDS related complex without defining what satisfied this – these patients

Table 2 (*continued*)

Study (first author, date, country)	Number of persons with Mtb-only (mean age)	Number of HIV-1 co-infected persons (mean age)	HIV-1 stage and mean pCD4	Biopsy site	Key measurements relevant to review	Main findings (co-infected vs Mtb-only)	Comments
							were deleted from our analysis. - 2 independent observers were used - ROI and blinding were not described - Precise granuloma descriptions were not provided.
Trajman 1997 Brazil [27]	27	9	Not specified	Pleura	Granuloma presence, formation, caseous necrosis, epithelioid cells, GC	• No difference in granuloma counts or formation • No difference in caseous necrosis, epithelioid cells or GC presence	- Outcomes assessed by impression of one pathologist, but with no clearly described criteria for outcomes. - ROI and blinding were not described - Precise granuloma descriptions were not provided.
Tripathi 2014 India [81]	5 (27·8)	5 (31·2)	Not stated	Brain	Granuloma formation	↓ Well formed granulomas in HIV-1 co-infected persons	- Granuloma descriptions were not a primary goal of study - Autopsies were performed between 2 and 13·45 h post mortem, which may have biased results - Assessment of IL-6, MIP-1α, IL-8, and TNF-α described in methods but not reported in results.
Van Der Ende 1999 Germany [38]	NA	9	Group 1: >150 pCD4/mm^3 Group 2 <50 pCD4/mm^3	LN	HIV-1 RNA, granuloma presence and formation from stratified pCD4 counts	• Group 1-inflammation was disorganized with no well-formed granuloma ↑ HIV-1 RNA in and near granulomas than non-granulomatous areas (data not presented in study). • CD4 T cells remained the main source of HIV RNA in both groups.	- Specific LN excised not identified - Compared co-infected patients at different peripheral CD4 counts. - Only study to directly examine HIV-1 presence within granuloma at different pCD4 counts - 2 persons with <50 pCD4/mm^3 and *M. avium* complex were excluded from our analysis - ROI was not described - Precise granuloma descriptions were not provided

infected persons [34,38]. These studies typically included few persons and techniques for defining and assessing outcomes such as granuloma presence and formation, AFB load and cellular presence were variable and sometimes not defined at all. Histological analyses were also not always performed in a blinded fashion and the methods for identifying regions of interest (ROI) examined were not defined. These limitations may have biased results within each of the studies.

3.1. Does HIV-1 co-infection reduce the granuloma presence in Mtb-infected persons?

HIV-1 has been hypothesized to reduce Mtb granuloma formation [5]. If an infected person cannot form granulomas they may be more susceptible to Mtb dissemination. To determine if this hypothesis was true we examined the proportion of Mtb-infected persons with granulomas visible in excised tissue was identified

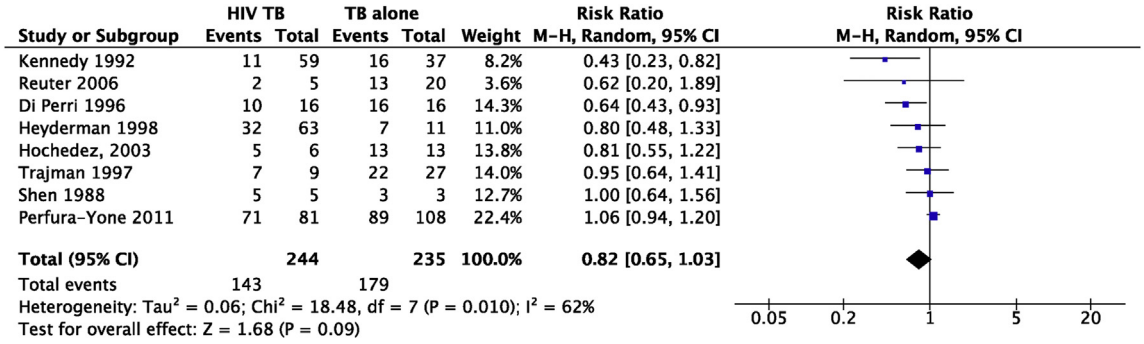

Figure 2. Mantel–Haenszel random effects risk ratio for Mtb granuloma presence in HIV-1 co-infected persons.

and compared between HIV-1-infected and -uninfected persons in eight studies [23–29,80]. Granuloma presence was calculated using the proportion of excised tissue containing at least one granuloma. One study was excluded from this analysis as granuloma presence was a criteria for tissue selection [33] while another was excluded because it reported total Mtb granulomas observed [37] and not granulomas per excised tissue. The lowest granuloma score in one study represented non-organized inflammatory patterns [28], which were not counted as granulomas. For all these studies, granuloma presence was a dichotomous variable, without quantifying granulomas in each excised sample.

HIV-1 did not change the likelihood of excised tissue containing granulomas. The proportion of persons with tissue that contained granulomas varied widely for both groups across the studies. In tissue from HIV-1 infected persons, Mtb granuloma presence ranged from 19% [24] to 100% [26], and from HIV-1 uninfected persons from 43% [24] to 100% [26]. All studies apart from two [26], reported at least a slight reduction in granuloma presence within HIV-1 infected persons. However, only one study reached statistical significance [24]. The probability ratio calculated based on forest plot did not identify a significant difference for granuloma presence between the two groups (Figure 2, RR: 0.82, 0.65–1.03). Varying pCD4 counts correlated to a difference in granuloma presence within HIV-1/Mtb co-infected persons in one study [28] but not in another [23].

3.2. Does HIV-1 co-infection change the quality of Mtb granuloma formation?

The ability of granulomas to kill Mtb and prevent its dissemination, in part, relies on how well they are organized [6]. It has been hypothesized that HIV-1 disrupts normal granuloma formation [4,5], which could increase TB susceptibility in HIV-1 infected persons. To determine if HIV-1 changes Mtb granuloma formation quality we identified eight studies that reported the presence of well- or poorly-formed granulomas [23,26–28,30–32,35]. Techniques used to score granuloma organization were highly variable and included: an assessment of the presence of epithelioid cells, lymphocytes and caseation [28,30], or focused on the organization of the epithelioid cells alone [31]1. The remaining studies relied on pathologist impressions only without providing a description [23,26,27,32,35].

No quantifiable difference in the appearance of well- or poorly-formed Mtb granulomas were observed in HIV-1 co-infected and HIV-1 uninfected persons. Four studies presented findings for well- and poorly-formed granulomas [27–29,32], while one presented results for poorly-formed granulomas only [23] or well-formed granulomas only [35] in HIV-1 co-infected and uninfected persons. Four of the five studies that examined the presence of well-formed granulomas identified a non-statistically significant [28,31,32] or significant [35] reduction in HIV-1 infected persons, while the remaining study identified no difference [27]. Similarly, three of five studies that quantified poorly-formed granulomas identified a non-statistically significant increase within co-infected persons [28,31,32], while the remaining two identified no difference [23,27]. Forest plots for both well- and poorly-formed granulomas did not meet statistical significance (Figure 3A: well-formed: RR 0.33 [0.08, 1.26], 3B: poorly-formed: RR 2.63 [0.24, 28.91]).

Qualitative assessments of Mtb granuloma formation were highly variable and inconclusive in both HIV-1 infected and HIV-uninfected persons. Other studies described granuloma formation quality between the two groups without presenting results from individual persons [26,30,33,81]. Shen et al. [26], made an overall statement that all five persons with AIDS and TB lymphadenitis contained caseous granulomas similar to those in the HIV-1 uninfected persons. Danaviah et al. [33], also reported no difference in granuloma formation between co-infected and singly infected persons with spinal TB, but their method stated *intact* granulomas were chosen for examination, a strategy that may have biased results toward well-formed granulomas. Conversely, De Noronha et al. [30], reported three well-defined zones (caseation, epithelioid cell layer and lymphocytic cuff) within the granulomas of persons with pulmonary TB without HIV-1 compared to atypical disorganized arrangement of the granulomas located in co-infected persons. Tripathi et al. [81], described the presence of well-formed granulomas in the brain of HIV-1 uninfected persons with TB meningitis but noted their absence in HIV-1 co-infected persons. While it is widely believed the increased susceptibility of HIV-1 infected persons to disseminated TB is caused by impaired granuloma formation, we found the results reported in this area to be conflicting and thus the evidence to support this hypothesis lacking.

HIV-1 did not change the presence of caseous granulomas. A hallmark of TB is the caseous granuloma [6], and to determine if HIV-1 changes caseation necrosis we identified studies that qualitatively [30,31,33] or quantitatively [23,25,26,29,32,37] reported caseous granuloma presence in co-infected and singly infected persons. Most studies identified no difference in granulomatous necrosis or caseation between co-infected and singly infected persons [23,26,29,31]. Two studies reported less necrosis in co-infected persons without statistical analysis [25,32]. Only one study reported a statistically significant increase in necrotic lung granulomas in co-infected persons [37]. These data suggested that HIV-1 co-infection did not change the likelihood of granulomas becoming caseous.

pCD4 depletion associated with poorly formed Mtb granulomas in HIV-1 co-infected persons. To determine if HIV-1 progression

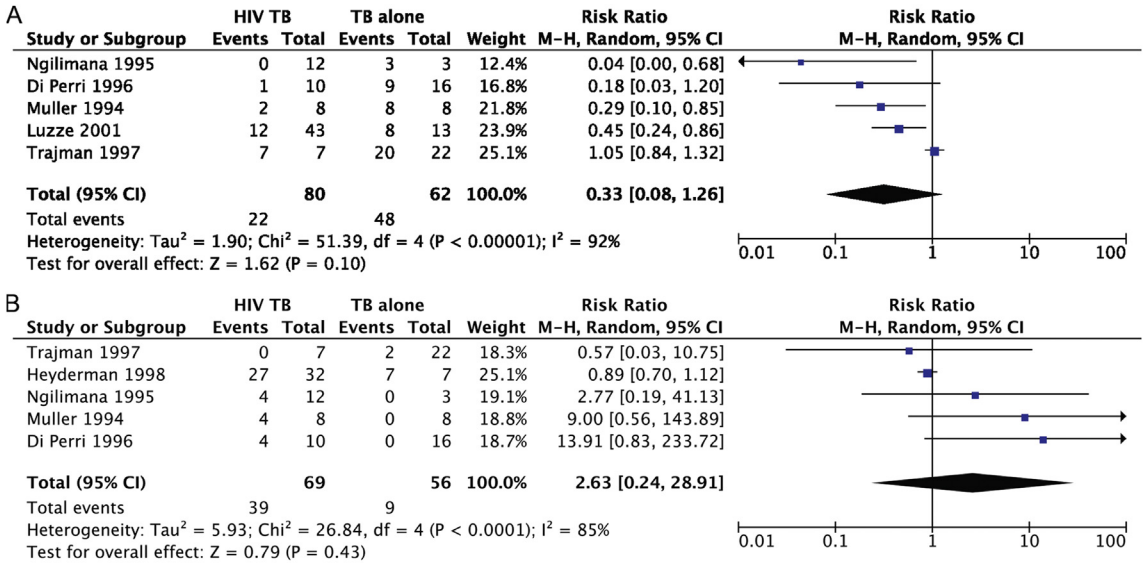

Figure 3. Mantel–Haenszel random effects risk ratio for well-formed- (A) and poorly-formed (B) granulomas in HIV-1 co-infected persons.

associated with disrupted granuloma formation we examined five studies that correlated pCD4 counts with granuloma formation in co-infected persons [28,31,34,35,38]. Three studies reported more poorly formed granulomas in those with lower pCD4 counts [28,31,34]. One study described no correlation between pCD4 counts and granuloma appearance [35]. Di Perri et al. [28], demonstrated that six persons with the lowest categorically defined granuloma scores (unorganized inflammation) had a mean pCD4 of 49.2 pCD4/mm^3 compared to the remaining ten persons with a mean pCD4 of 238.6 pCD4/mm^3. Similarly, Muller et al. [31], reported well-formed granulomas in two persons with >300 pCD4/mm^3, a combination of well- and poorly-formed granulomas in persons with 200–300 pCD4/mm^3 and only necrosis and foamy macrophages within lymph nodes of persons with <200 pCD4/mm^3. Van Der Ende et al. [38], reported an absence of well-formed granulomas within the two HIV-1/Mtb co-infected persons with <50 pCD4s/mm^3, while the five persons with >170 pCD4/mm^3 contained multiple well-formed granulomas. Granulomatous changes were observed but not defined, in 75% (9/12) of excised tissues from HIV-1 co-infected persons with <101 pCD4/mm^3, although this reduction was not significantly different from other co-infected persons with higher pCD4 counts [34]. Taken together, lower pCD4 counts in HIV-1 infected persons generally correlated to poorer granuloma formation, particularly as pCD4 counts fall below 50 pCD4/mm^3.

3.3. *Does HIV-1 change the cellular composition of Mtb granulomas?*

It is hypothesized that T cells and macrophages, essential components for normal granuloma activity, are preferentially killed or manipulated by HIV-1 at the site of TB disease [4,5,13]. To determine if cellular composition of granulomas from HIV-1 co-infected persons differ from HIV-1 uninfected persons we identified three studies that compared the presence of T cells in two groups. The studies that quantified T cell presence used single CD3, CD4, or CD8 antibodies for identification [26,31,33], so the presence of CD4+CD3- (macrophages, dendritic cells) and CD8+CD3- (NK cells) non-T cells may have biased results by staining false positives.

CD3, CD4, or CD8 T cell counts within granulomas were highly variable. Two studies qualitatively identified fewer lymphocytes within granulomas from co-infected persons [30,32], but did not specifically identify the types of lymphocytes involved because the assessments were made on hematoxylin and eosin stained slides. Findings for CD3 cells were conflicting. Two studies identified no difference in CD3 T cell presence within co-infected granulomas [31,33], while a third reported a reduction [26]. CD8 T cell presence was also variable. One study demonstrated an increase in CD8 T cells within granulomas from co-infected persons [33], while two identified no difference [26,31]. A more detailed examination of CD8 T cell localization within the granuloma found that, although the number of CD8 T cells were the same, they were more widely dispersed throughout granulomas of HIV-1 infected persons, while they were confined to the lymphocytic mantle surrounding the epithelioid cell layer in HIV-1 uninfected persons [26]. Two studies reported a statistically significant reduction in CD4 T cells in granulomas of co-infected persons [26,33] while a third identified fewer CD4 T cells in only three of eight persons with HIV-1 co-infection [31] compared to the HIV-1 uninfected group. The three persons with fewer CD4 T cells all had <200 pCD4/mm^3. The high variability in T cell staining make it difficult to determine how HIV-1 changes cellular composition.

Macrophage presence within Mtb granulomas were highly variable. Macrophages are also an essential component of granulomas, forming the epithelioid cell layer. Findings from studies in this area were also conflicting [23,31–33]. Two studies described a disruption of the epithelioid layer within granulomas in HIV-1 infected persons without defining those disruptions [31,32]. The presence of Langhan's giant cells, which form when macrophages fuse and are commonly seen in Mtb granulomas, were also variable. Results from two studies demonstrated no difference in count [23,33], while another study found fewer Langhan's giant cells in those co-infected with HIV-1 [31]. Muller et al. [31], determined that HIV-1 uninfected persons and those with >200 pCD4/mm^3 contained more Langhan's giant cells within their granulomas than HIV-1 uninfected persons. In contrast, granulomas from those with <200 pCD4/mm^3 primarily contained foamy macrophages. Due to the high variability in macrophage and Langhan giant cells counts,

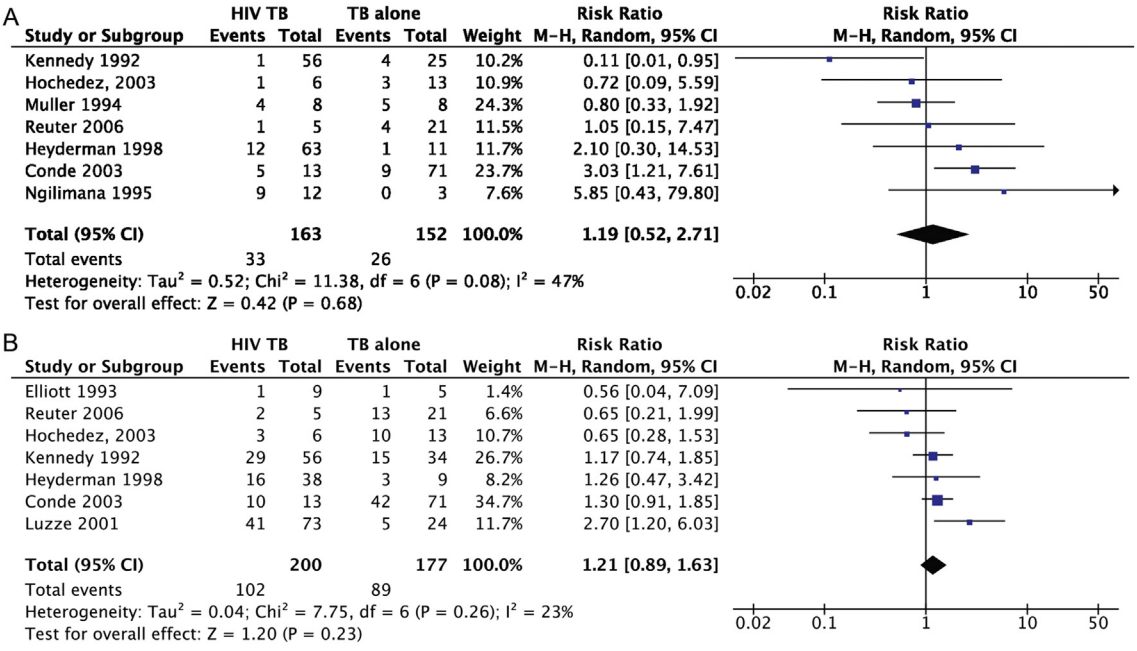

Figure 4. Mantel–Haenszel random effects risk ratio for AFB (A) and CFU (B) presence within excised tissues of HIV-1 co-infected persons.

nothing conclusive can be stated about how HIV-1 changes their presence within granulomas.

3.4. Does HIV-1 change cytokine expression within Mtb granulomas?

Granulomas contain a delicate balance of pro- and anti-inflammatory cytokine responses [84]. If HIV-1 disrupts this balance, then Mtb dissemination may occur. Although HIV-1 reduces IFN-γ and TNF production by Mtb-specific T cells in BAL, PF, and PCF [15–19], it is not known how HIV-1 changes cytokine expression in granulomas.

Cytokine expression in co-infected granulomas was highly variable and inconclusive. Cytokine expression was also analyzed within granulomas of co-infected and singly infected persons [30,31,37] and findings were again conflicting. TNF production within granulomas was highly variable in co-infected persons. More granulomatous cells were positive for TNF mRNA in co-infected persons than HIV-1 uninfected persons [37]. Likewise, a qualitative trend of more overall TNF production in co-infected persons with <160 pCD4/mm^3 was also observed [31]. De Noronha et al. [30] reported less total TNF produced within granulomas of HIV-1 co-infected persons with TB lymphadenitis, without quantification. No difference in expression of IL-1α, IL-1β, IFN-α and IFN-β [31] or the mRNA presence of IFN-γ, IL-4 and IL-12p40 [37] were identified between the two groups. Foamy macrophages that replaced epithelioid macrophages in granulomas of four co-infected persons with <200 pCD4/mm^3, were more intensely stained for IL-1α, IL-1β, IFN-α and IFN-β [31]. The lack of studies and high variability in staining makes it difficult to quantify how HIV-1 changes cytokine production within Mtb granulomas.

3.5. Does HIV-1 increase the Mtb content of granulomas?

The primary function of a granuloma is to kill Mtb and prevent its dissemination. HIV-1 increases the risk of extrapulmonary TB [1]. The increased risk of Mtb dissemination is hypothesized to be caused by reduced Mtb killing within the granulomas. To determine if Mtb granulomas from co-infected persons were more likely to contain Mtb than HIV-1 uninfected persons, we analyzed studies that specifically quantified either AFB+ or CFU+ samples out of the total number of excised tissue from both co-infected and singly infected persons. One study could not be included in this meta-analysis because their lowest categorical score was defined as *no more than a single bacillary unit was seen in ten microscopic fields* [28], which could technically represent zero bacilli within ten fields (AFB-) or one bacilli (AFB+).

pCD4 depletion increases the likelihood of Mtb granulomas containing at least one bacillus, while HIV does not increase bacilli presence. Of the seven studies that reported the proportion of AFB+ biopsied tissue within HIV-1 co-infected and uninfected persons [23–25,31,32,80,82] a high variability of AFB+ rates existed in both groups with HIV-1 co-infected persons ranging from 1.8 [24] to 75% [32] and HIV-1 uninfected persons from 0 [32] to 62.5% [31]. HIV-1 did not change AFB+ presence (Figure 4A. RR 1.19 [0.52, 2.71]). Likewise, the seven studies that identified CFU+ [23–25,35,36,80,82] did not suggest a difference in bacterial content (Figure 4B. RR 1.021 [0.89, 1.63]).

As pCD4 decreased the likelihood of granulomas containing at least one bacillus increased. Two studies found AFB+ presence inversely correlated with pCD4 counts in HIV-1 infected persons [23,28]. Biopsies from co-infected persons with <100 pCD4/mm^3 were more likely to be AFB+ (43%, 6/14) than co-infected persons with >100 pCD4/mm^3 (8%, 3/36) [23]. The depletion of pCD4 T cells led to increases in Mtb presence within granulomas.

HIV-1 increased the amount of Mtb in granulomas. Three studies quantified AFB load in excised tissues in each group [23,28,32]. One study quantified AFB load between a low ≤1 AFB to a high of >10 AFB per 100× ROI [28], another identified the number of tissue samples with either numerous vs. scanty AFB [23], and the final study categorically assigned absent to abundant AFB [32] without a description. The forest plot and subsequent calculated risk ratio for HIV-1 infected persons identified an increased AFB load within AFB+ tissue (Figure 5. RR 4.30 [1.07, 17.18]). Di Perri et al. [28], identified an inverse correlation between pCD4 count and AFB load within excised tissue from co-infected persons.

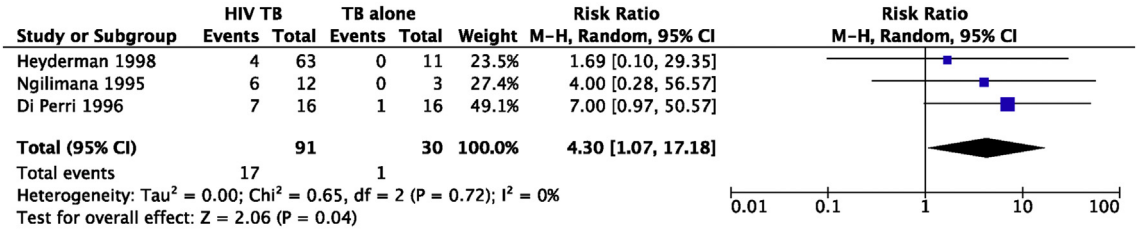

Figure 5. Mantel–Haenszel random effects risk ratio for abundant AFB load in HIV-1 co infected persons.

Conversely, HIV-1 infection status did not change days to positive BACTEC culture in excised pleural tissue [35]. HIV-1 infection appears to increase Mtb abundance within individual granulomas.

3.6. Does HIV-1 preferentially infect TB diseased tissue?

TB granulomas have been hypothesized to be preferential sites of HIV-1 replication (Table 1) [4,5,12]. If HIV-1 preferentially localizes within granulomas then the virus would have a better chance of killing Mtb-specific T cells and Mtb-infected macrophages because of their high prevalence within granulomas. This specific killing could lead to increased Mtb growth.

HIV-1 infects Mtb granulomas. Van der Ende et al. [38] qualitatively examined HIV-1 RNA presence within T cells and macrophages in lymph nodes from five persons with >150 pCD4/mm^3 to two persons with <50 pCD4/mm^3. In the former group, there were well-organized granulomas and the number of HIV-1 RNA+ cells was 10-fold higher in or near granulomas compared to other non-granulomatous areas. In those persons with pCD4 counts <50 pCD4/mm^3, HIV-1 RNA+ cells were distributed more evenly throughout the lymph node, independent of the inflammatory infiltrate. CD4 T cells were the dominant source of HIV-1 RNA within most of the lymph nodes. These findings were based on a small sample size and were only presented as an overall impression rather than by individual findings. HIV-1 viral RNA was also identified with PCR in homogenized spinal tissue that contained granulomas [33], but it was not possible to determine if virus originated in the granuloma or surrounding tissue. HIV-1 does localize within Mtb granulomas, but more studies need to be performed to confirm what cells are preferentially infected.

4. Discussion

It has been hypothesized that HIV-1 increases TB susceptibility by reducing the formation and function of Mtb granulomas. Studies that have examined Mtb granulomas directly in HIV-1 co-infected persons have not been collated until now. We performed this systematic review and meta-analysis to determine which hypotheses could be confirmed by the human literature and which ones need more research. Despite the strong epidemiological association between TB and HIV-1s [12], research into how HIV-1 manipulates the Mtb granuloma was conflicting and quite limited.

One outcome with consistent findings and a significant difference between the HIV-1 infected and uninfected groups was bacillary load. In the three studies that examined this outcome, HIV-1 co-infected persons consistently demonstrated greater bacillary load [23,28,32]. This was the only finding that provided some potential explanation for the increased susceptibility to more severe TB disease in HIV-1 infected persons. However, when studies were stratified by pCD4 counts [23,28,31,35,38], results were more consistent. Lower pCD4 counts were associated with increased bacillary presence [23,28], inflammatory cytokine production by foamy macrophages [31], and poorer granuloma formation [28,31,38]. These data suggest that as HIV-1 disease progresses, the ability to maintain normal granuloma function becomes impaired. These findings also illuminate the importance of stratifying pCD4 counts when analyzing outcomes from HIV-1 infected persons to achieve more accurate and consistent findings. Consideration of other clinical characteristics including duration of TB disease or symptoms, gender, age, HIV-1 and TB treatment status, and previous TB disease may also help to improve the consistency of findings.

In all other outcomes, there was high variation between studies, both in terms of methods used to assess outcomes, and also their results. Heterogeneity scores for all measured outcomes were moderate to high, and the summative risk ratios had very wide confidence intervals for the outcomes of granuloma presence, formation quality and AFB presence when comparing TB diseased tissue from HIV-1 infected and HIV-1 uninfected persons. In terms of studies' outcomes such as cellular composition of granulomas and cytokine expression, findings were also conflicting and no trend could be extracted. There was also a surprising lack of data that focused on the effect Mtb bacilli had upon the distribution of HIV-1 transcripts. Studies that only described findings from TB

Table 3
Guidelines for future granuloma-based studies.

Category	Recommendations for future granuloma-based studies
Granuloma descriptions	• Include detailed descriptions of all granuloma scores for individual types of granulomas with reference images (if space is limited then the authors should provide example images upon request). • Detail how scores were tabulated. Were all granulomas counted and averaged? Do certain granulomas skew results because of their size? • Present the range of granuloma scores within each individual as well as each group. • Explain how each granuloma or region of interest was chosen to be scored (random selection?) • Only compare granulomas that reside in the same tissue type among persons to reduce variability.
Study groups	• HIV-1 and TB diagnoses should be included for all patients • HIV-1 studies should include pCD4 counts • Drug treatment should be stated
Study design	• Were the observer's blinded? • Describe how each parameter was specifically quantified instead of using vague subjective terms such as “abundant” or “scanty”. • Were their enough persons to justify conclusions? • Autopsy studies should state how long the subject was dead prior to autopsy and studies that focus on excised tissue should state the time it required to fix the tissue.

Table 4
Questions that still need to be answered within the HIV-1/Mtb co-infection literature.

Category	Questions
Compositional changes	• Does HIV-1 significantly change granuloma formation? • Do HIV-1 and AFB presence correlate in infected tissue? • Does HIV-1 significantly reduce CD4 T cell counts within granulomas?
Functional changes	• Does HIV-1 significantly reduce Mtb-specific T cell functionality (chemokine and cytokine production or cytolytic activity) within granulomas more than within the periphery? • Does pCD4 T cell depletion correlate to increased changes within granulomas? • How do functional T cell responses correlate among PBMC, BALF, PF, and within granulomas and does this correlate to disease status?
HIV-1 and ART focused research	• Does HIV-1 preferentially replicate within granulomas? Which cells are preferentially infected within granulomas and the surrounding tissue? • Do HIV-1 and Mtb co-localize within the same cells or within close proximity inside of granulomas? • Does anti-viral penetration of granulomas correlate to improved granuloma function, organization, reduced viral titers, or patient outcome?
Identifying biomarkers	• Does anti-viral penetration into granulomas correlate to pharmacokinetics observed within the plasma? • What blood biomarkers correlate to significant changes in granuloma function?

diseased tissue from HIV-1 infected persons that did not stratify pCD4 counts or make comparisons to HIV-1 uninfected persons were excluded from our review. However, data from the studies we excluded [39–46] revealed similar variability in findings, particularly in regards to granuloma formation and AFB presence (Supplemental Table 2). Heterogeneity and the high variability among techniques used may have reduced consistency in findings among the studies examined in this review. Some of the variability among these studies may have been reduced if they had been better powered.

High variability in methods used for each study may have limited our findings. As previously mentioned, where semi-quantified scoring systems were used to quantify outcomes such as granuloma formation or AFB load, methods either varied or were not described. The methods scoring outcomes varied considerably: *poorly- or well-formed granulomas* [23,27,31], *well-formed with giant cells* [35], *scanty or numerous AFB* [23], *rare or abundant AFB* [32], *+++ or + AFB* [30] and other semi-quantified scores [28,30–32,81]. These terms were often applied without specific definition and as such were open to subjective interpretation and observer bias [85]. A significant variation likely existed in how individual tissues were scored among the studies and it was difficult to identify the effect this had on our overall results.

A second concern was that for semi-quantified variables, in particular quality of granuloma formation, results were represented by a single score. This score may have failed to represent the range of granulomas or unorganized inflammation that was likely located within a single tissue section. Granulomas are highly variable in terms of size, histological type [6], formation quality [31], cytokine expression [84], and bacterial load [86]. It was not clear in these studies whether this variability was assessed or how it was handled when choosing how to score the tissues. Conceivably, a number of strategies may have been adopted if such variations were present, including scoring by the largest granulomas, the most numerous, a *random* selection within the tissue, or an average of all granulomas present. Without knowing which methods were used to provide an overall score for tissues, the implication for potential bias was difficult to ascertain. The high variability in findings may have been reduced if all granulomas identified within each tissue was represented, along with exactly how each granuloma was selected and scored. Only one study specifically stated that the assessing pathologists were blinded [30], which could have reduced the risk of observer bias [85].

While the studies we reviewed assessed the effect of HIV-1 on Mtb granulomas, we have demonstrated there was little that could be conclusively stated about how HIV-1 changes the site of TB disease. The primary issue we identified was that findings from different studies could not always be compared, despite having examined the same outcomes. To increase comparability of future studies we have developed a basic guideline for research on the pathology of HIV-1/Mtb co-infection (Table 3) because important unanswered questions need further exploration (Table 4).

The objective of our guidelines are to ensure that future studies provide enough detail through a reduced-biased methodology that will allow other researchers to compare results. We also encouraged open access to examined images of Mtb-infected tissues within these studies. This would facilitate researchers' ability to evaluate their peers' analyses of these tissues and minimize redundant research effort. Implementing both these guidelines and open access to pathology images in future work would help build consensus on their criteria needed to properly assess granulomas, even if the number of available samples are small in individual studies. Increasing the cohesiveness of data will heavily contribute to the overall understanding of how HIV-1 alters immunity at the site of TB disease. We hope this will provide a greater understanding of the dynamics of this co-infection at the local level, which in turn may lead to novel interventions and therapies targeting the microenvironment of granulomas.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tube.2016.02.010.

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