UNIVERSIDADE FEDERAL DA BAHIA INSTITUTO DE CIÊNCIAS DA SAÚDE PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA

ELISABETE LOPES CONCEIÇÃO

AVALIAÇÃO DE FATORES GENÉTICOS E IMUNOLÓGICOS RELACIONADOS À IMUNOPATOGÊNESE DA TUBERCULOSE E CO-INFECÇÃO TB-HIV

Salvador, BA

2016

ELISABETE LOPES CONCEIÇÃO

AVALIAÇÃO DE FATORES GENÉTICOS E IMUNOLÓGICOS RELACIONADOS À IMUNOPATOGÊNESE DA TUBERCULOSE E CO-INFECÇÃO TB-HIV

Tese apresentada ao Programa de Pós-graduação em Imunologia, da Universidade Federal da Bahia como requisito parcial para obtenção do título de Doutor em Imunologia.

Orientador: Profa. Dra. Theolis Barbosa Bessa
Co-orientador: Prof. Dr. Jamocyr Moura Marinho

Salvador, BA
2016

Dados Internacionais de Catalogação na Publicação (CIP)
Processamento Técnico, Biblioteca Universitária de Saúde,
Sistema de Bibliotecas da UFBA

C744 Conceição, Elisabete Lopes.

Avaliação de fatores genéticos e imunológicos relacionados à imunopatogênese da tuberculose e co-infecção TB-HIV / Elisabete Lopes Conceição. - Salvador, 2016.

132 f.: il.

Orientador: Prof. Theolis Barbosa Bessa.

Coorientador: Prof. Jamocyr Moura Marinho.

Tese (doutorado) - Universidade Federal da Bahia. Instituto de Ciências da Saúde. Programa de Pós-Graduação em Imunologia, Salvador, 2016.

Área de concentração: Imunologia.

Linha de pesquisa: Imunologia das doenças Infecciosas e parasitárias.

1. Tuberculose. 2. Infecções por HIV. 3. Coinfecção. 4. Polimorfismo genético. 5. Apoptose. 6. Imunogenética. I. Bessa, Theolis Barbosa. II. Marinho, Jamocyr, Moura. III. Universidade Federal da Bahia. Instituto de Ciências da Saúde. Programa de Pós-Graduação em Imunologia. IV. Título.

CDU: 577.7:616.24-002.5

AGRADECIMENTOS

A minha orientadora, Theolis Barbosa Bessa, pelo exemplo profissional, ensinamentos, confiança, disponibilidade, atenção e paciência. Muito Obrigada!

Ao Dr. Jamocyr Marinho, pela co-orintação, parceria e disponibilidade para discussão e traçar estratégias para o bom desenvolvimento do trabalho.

Ao estudantes de Iniciação Científica Felipe Leite, Carolina Amoedo, Valdineia Santos, Silas Gabriel, Gabriel Portela, Aline Taranto, Lucas Adôrno, Lais Pereira, Iza Cristina, Larissa, Liziane, Ana Paula Torres e Paulo Sérgio. Estudantes de Pós-Graduação Michael Rocha e Cleidiane Daltro que me ajudaram no campo, no laboratório e nas análises. Sem a ajuda de vocês jamais conseguiria a realização deste trabalho.

Aos pacientes e voluntários que aceitaram participar do trabalho. Obrigada pela contribuição, sem a qual, este trabalho jamais seria realizado.

A equipe médica e técnica do Hospital Especializado Octávio Mangabeira, Serviço Municipal de Assistência Especializada e do Centro Especializado em Diagnóstico e Pesquisa do Estado, pelo apoio e colaboração na captação dos pacientes.

Ao Dr. Daniel Barber, meu orientador nos Estados Unidos, me deu a oportunidade de trabalhar e aprender as técnicas de citometria em seu laboratório.

Aos meus colegas e professores de curso pela rica troca de experiências.

Às Secretárias da Pós-Graduação e do LIMI por sempre serem gentis e prontas a ajudar qualquer aluno.

A minha mãe pelo apoio e carinho incondicional.

A Deus por ter colocado as pessoas certas no meu caminho.

A todos que, de alguma forma, contribuíram para esta construção**.**

“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota."

(Madre Teresa de Calcutá)

CONCEIÇÃO, Elisabete Lopes. Avaliação de fatores genéticos e imunológicos relacionados à imunopatogênese da tuberculose e co-infecção TB-HIV. 187 f. il. Tese (Doutorado)- Universidade Federal da Bahia, Salvador, 2016.

RESUMO

O *Mycobacterium tuberculosis* e o vírus da imunodeficiência humana (HIV) agem em sinergia prejudicando a resposta imune para eliminação de ambos os patógenos. Fatores genéticos dos hospedeiros podem ser determinantes para o risco de progressão da tuberculose (TB) e infecção pelo HIV. Algumas variações genéticas têm sido associadas a diferenças no potencial para indução de apoptose e a alterações na produção de citocinas, tais como os polimorfismos de base única (SNPs) *TNF*-308G>A, *DDX39B* -22 G>C e -348C>T. Outra característica que acompanha a evolução da infecção é o estresse oxidativo sistêmico e o aumento da peroxidação. A Heme oxigenase-1 (HO-1) é o principal agente anti-oxidante expresso no tecido pulmonar, uma enzima de resposta ao estresse que degrada moléculas heme para a liberação de íons ferro, monóxido de carbono (CO) e biliverdina (BV). O presente trabalho propôs investigar fatores genéticos e imunológicos relacionados à imunopatogênese da TB e co-infecção TB-HIV. Para a realização do trabalho foram realizados: 1) uma revisão sistemática da literatura sobre os polimorfismos genéticos envolvidos nas vias e morte e associados com TB, 2) um estudo observacional de corte transversal para determinar a frequência de polimorfismos em voluntários monoinfectados com TB latente ou ativa e co-infectados com TB-HIV. Para este último foram recrutados 109 pacientes com tuberculose pulmonar (PTB), 60 pacientes coinfectados com HIV (TB-HIV) e 74 indivíduos com infecção tuberculosa latente (LTBI), e 3) Uma coorte avaliando os níveis de HO-1 e MMP-1 em pacientes com TB. Na revisão sistemática os polimorfismos dos genes do TNF, TNFR, IL-1 e P2RX7 estavam associados com tuberculose. No estudo de corte transversal a frequência do genótipo TNF-308G foi maior para o grupo LTBI comparado com TB e TB-HIV. A produção de TNF foi maior

entre os pacientes com PTB portadores do genótipo TNF -308GG. Os níveis de IL-1α e IL-1β também foram mais elevados entre os pacientes com PTB portadores dos genótipos DDX39B -22CC e DDX39B -348CC. Não houve relação entre a produção de citocinas e a extensão da doença. Na coorte, os pacientes com TB apresentaram uma dicotomia na resposta de HO-1 MMP-1 com dois fenótipos, HO-1^{hi}MMP-1^{lo} e MMP-1 HO-1^{lo}MMP-1^{hi}. Nosso estudo sugere que polimorfismos envolvidos na via de morte podem estar associados com susceptibilidade para o desenvolvimento da tuberculose, contudo, a frequência dos alelos e genótipos para os polimorfismos estudados não diferiram na co-infecção pelo HIV. O mecanismo entre o estresse oxidativo e remodelamento do tecido pode ter aplicabilidade clínica nos estágios da progressão da TB.

Palavras-chave: Tuberculose. TB-HIV. Polimorfismos. Apoptose. HO-1

CONCEIÇÃO, Elisabete Lopes. Genetic and immunological factors related to immunopathogenesis of TB and co-infection TB-HIV. 187 il. pages. Thesis (Doctoral)- Universidade Federal da Bahia, Salvador, 2016.

ABSTRACT

Mycobacterium tuberculosis and human immunodeficiency virus (HIV) act synergistically damaging immune response to eliminate both pathogens. Genetic factors of the host can be decisive for the risk of progression of tuberculosis (TB) and HIV infection. Some genetic changes have been associated with differences in potential for induction of apoptosis and changes in cytokine production such as single nucleotide polymorphisms (SNPs) TNF-308G> A, DDX39B -22 G> C and -348C> T. Another feature that monitors the infection is systemic oxidative stress and increased peroxidation. The heme oxygenase-1 (HO-1) is the primary anti-oxidant expressed in lung tissue, a response of the enzyme to stress that degrades heme molecules to the release of iron ions, carbon monoxide (CO), and biliverdin (BV) . This study proposed to investigate genetic and immunological factors related to TB immunopathogenesis and co-infection TB-HIV. To carry out the work were performed: 1) a systematic review of the literature on genetic polymorphisms involved in the pathways and death associated with TB, 2) an observational cross-sectional study to determine the frequency of polymorphisms in volunteers monoinfected with latent TB or active and co-infected with TB-HIV. For the latter were recruited 109 patients with pulmonary TB (PTB), 60 patients co-infected with HIV (HIV-TB) and 74 individuals with latent tuberculosis infection (LTBI), and 3) a cohort evaluating the levels of HO-1 and MMP-1 in patients with TB. In the systematic review of TNF polymorphisms of genes, TNFR, IL-1 and P2RX7 were associated with tuberculosis. In the cross-sectional study the frequency of TNF-308G genotype was higher for LTBI group compared with TB and TB-HIV. The production of TNF was higher among patients with PTB patients TNF -308GG genotype. IL-1α and IL-1β were also higher among patients

with genotypes of PTB patients DDX39B -22CC and DDX39B -348CC. No relation between the production of cytokines and the extent of disease. In cohort, patients with TB presented a dichotomy in HO-1 MMP-1 response with two phenotypes, HO-1hiMMP-1lo HO-1 and MMP-1loMMP-1hi. Our study suggests that polymorphisms involved in the death pathway may be associated with susceptibility to the development of tuberculosis, however, the frequency of alleles and genotypes for the studied polymorphisms did not differ in co-infection with HIV. The mechanism of oxidative stress and remodeling of the tissue may have clinical applicability in the progression of TB staging.

Keywords: Tuberculosis. TB-HIV. Polymorphisms. Apoptosis. HO-1

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS

ACD - Morte celular acidental

AM - Macrófagos alveolares

APC - Célula apresentadora de antígeno

BAT-1 - Antígeno leucocitário humano -B associado ao transcrito-1

BMI - Índice de massa corpórea

BCG - Bacilo de Calmette-Guérin

BV - Biliverdina

CEDAP - Centro Especializado em Diagnóstico Assistência e Pesquisa

CLRs - Receptores tipo selectina

CO - Monóxido de Carbono

DC - Célula Dendrítica

EBMSP - Escola Bahiana de Medicina e Saúde Pública

FADD - Domínio de morte associado ao Fas

FcRs - Porção Fc das imunoglobulinas

FIOCRUZ - Fundação Oswaldo Cruz

FMs - Macrófagos espumosos

GALT - Tecido linfóide associado ao trato gastrointestinal

GP - Glicoproteína

HC - Controles Saudáveis

HEOM - Hospital Especializado Octávio Mangabeira

HIV - Vírus da imunodeficência humana

HLA - Antígeno leucocitário humano

HO-1 - Heme Oxigenase - 1

HWE - Equilíbrio de Hardy-Weinberg

IL-1α - Interleucina-1 alfa

IL-1β - Interleucina-1 beta

IL-6 - Interleucina-6

IL-8 - Interleucina-8

IL-10 - Interleucina-10

IFN-γ - Interferon-gamma

LOF - Perda de função

LTBI - Infecção tuberculosa latente

MMP - Matriz metaloproteínase

MHC - Complexo principal de histocompatibilidade

Mtb - Mycobacterium tuberculosis

NLRs - Receptores tipo NOD

NO - Óxido Nítrico

OMS - Organização Mundial da Saúde

PBMC- Célula mononuclear do sangue periférico

PCR - Reação em cadeia da polimerase

PRRs - Receptores de reconhecimento padrão

PTB - tuberculose pulmonar

P2X7R - receptor purinérgico P2X

RCD - Morte celular regulada

SEMAE - Serviço Municial de Assistência Especializada

SESAB - Secretaria de Saúde do Estado da Bahia

SIDA - Síndrome da Imunodeficência Adquirida

SLE - Lúpus Eritrematoso Sistêmico

SNP - Polimorfismo de base única

Sp1- Proteína estimulatória-1

SRs - Receptores scavengers

STAT - Fator de Transcrição ativador de sinal

TB - Tuberculose

TB-HIV -Tuberculose- Vírus da imunodeficência humana

TBC - Tuberculose crônica

Th - Célula T helper

TLRs - Receptores tipo Toll

TNF - Fator de Necrose Tumoral

TNFR - Receptor de Fator de Necrose Tumoral

TRAIL - Ligante indutor de apoptose associado ao TNF

TRADD - Domínio de morte associado ao Receptor de TNF tipo 1(TNFRSF1A

TST - Teste tuberculínico cutâneo

SUMÁRIO

1. INTRODUÇÃO

A Tuberculose (TB) é maior ameaça para saúde global e atualmente lidera com a infecção pelo vírus da imunideficiência humana (HIV-1) como a principal causa de morte por doenças infecciosas no mundo. Em 2014, foram estimadas 1,2 milhões de mortes pelo HIV, incluindo 0,4 milhões com tuberculose. No mesmo ano, foram estimados 9,6 milhões de casos novos de TB, dentre entres 5,4 milhões de homens, 3,2 milhões de mulheres e 1,0 milhão de crianças. Globalmente, estima-se que 12% dos 9.6 milhões de casos novos de TB estejam co-infectados com o HIV, correspondendo a 1,2 milhões de casos [1]. Dos 9.6 milhões de casos novos estimados, 6 milhões foram notificados à Organização Mundial de Saúde (OMS), menos de dois terços (63%). Isto significa que em todo o mundo, 37% dos casos novos de tuberculose não foram diagnosticados ou relatados [1].

No Brasil, a cada ano, são notificados aproximadamente 70 mil casos novos de TB e ocorrem 4,6 mil mortes em decorrência da doença, o que deixa o Brasil no 17º lugar entre os 22 países responsáveis por 80% do total de casos de tuberculose no mundo [2]. Em 2014, foram diagnosticados 67.966 casos novos de tuberculose. Ao longo dos anos, observou-se redução do coeficiente de incidência, passando de 41,5/100 mil hab. em 2005 para 33,5 por 100 mil hab. em 2014, o que corresponde a uma redução média de 2,3% ao ano nesse período [3].

A testagem para HIV é uma recomendação do Ministério da Saúde voltada para todos os pacientes com tuberculose, priorizando o teste rápido. Isso se deve ao fato de as pessoas com HIV serem mais propensas a desenvolver a tuberculose ativa em comparação à população geral; frequentemente, o diagnóstico da infecção pelo HIV ocorre durante o curso da tuberculose. No Brasil, em 2014, 62,7% dos casos novos de tuberculose foram submetidos à testagem para HIV. No Estado da Bahia em 2014, foram notificados 30,7/100 mil hab. de casos novos de tuberculose com 6,3% de soropositivos para o HIV [3].

O HIV aumenta o risco de transição da infecção tuberculosa latente (LTBI) para a forma ativa da doença, e aumenta o risco de morte comparando-se com indivíduos sem infecção por HIV [4]. O HIV e o *Mycobacterium tuberculosis* (*Mtb*) sinergizam causando a diminuição da imunidade celular, de modo a favorecer a infecção por ambos os patógenos. O *Mtb* impacta o curso da infecção pelo HIV por facilitar a

replicação do vírus nos linfócitos e macrófagos alveolares, desregulando a resposta imune contra o bacilo [5]. A resposta efetora contra o *Mtb* envolve a coordenação das respostas imunes inata e adaptativa, ambas alteradas pelo HIV [6]. A morte celular por apoptose constitui um importante mecanismo da resposta imune inata para a proteção do hospedeiro que reduz a viabilidade do *Mtb* in vitro [7].

A apoptose dos macrófagos alveolares é um mecanismo importante para promover a eliminação do *Mtb* [8]. Contudo, macrófagos infectados pelo HIV não sofrem morte celular como ocorre com os linfócitos T CD4+, persistindo a infecção do vírus no interior dessas células [9]. Cepas clínicas do *Mtb* também inibem a apoptose através da inibição da via dependente de TNF [10], e macrófagos co-infectados com *Mtb* e HIV apresentam ainda maior redução da apoptose dependente de TNF [11].

A apoptose é regulada por diferentes vias e alguns estudos já demonstraram que polimorfismos genéticos que regulam a expressão de moléculas envolvidas com a apoptose podem influenciar o curso das doenças, incluindo a tuberculose [12-13]. Reconhecendo a importância da apoptose para a resistência do hospedeiro contra o *Mycobacterium tuberculosis*, o presente trabalho propôs investigar o papel de polimorfismos em genes de moléculas envolvidas na apoptose na imunopatogênese da co-infecção TB-HIV.

2. REVISÃO DE LITERATURA

2.1. Fisiopatologia da Tuberculose

A infecção tuberculosa é adquirida pela inalação de partículas suspensas contendo bacilos de *Mtb* liberadas por indivíduos com a doença ativa. Existem certos grupos de risco que são mais susceptíveis à infecção pelo *Mtb*: adultos jovens, profissionais de saúde que têm contato frequente com a doença e indivíduos imunodeficientes, como portadores do HIV [14-15]. Uma vez que o bacilo é inalado, ele reside nas células da linhagem mielóide, os macrófagos, onde persiste dentro dessas células e interfere nos eventos celulares que estimulam a maturação do fagossomo e apoptose para replicar ativamante no compartimento endossomal [16] .

As células apresentadoras de antígenos, incluindo os macrófagos, detectam o bacilo através dos receptores de reconhecimento padrão (PRRs). O *Mtb* interage com várias classes desses receptores nos fagócitos, incluindo receptores tipo Toll (TLRs), receptores tipo selectina (CLRs), porção Fc das imunoglobulinas (FcRs), receptores scavengers (SRs), receptores tipo NOD (NLRs) e receptor de células dendrítica DC-SIGN [17]. Alguns desses receptores ativam os macrógafos para síntese de moléculas antimicrobianas, quimiocinas e citocinas. Outros permitem a entrada silenciosa do *Mtb* e replicação no interior dos macrófagos. O DC-SIGN está envolvido na fagocitose do bacilo pelas células dendríticas, mas não torna as células permissíveis à replicação do bacilo [18].

Adicionalmente, se o bacilo não for eliminado logo após a fagocitose, ele pode proliferar-se no interior de células dendríticas e macrófagos alveolares rapidamente, estimulando a secreção de citocinas inflamatórias, como Interleucina-1 alfa (IL-1α), interleucina-1 beta (IL-1β), Fator de Necrose Tumoral (TNF), Interleucina-6 (IL-6) e Interleucina-8 (IL-8), que atuam como quimioatraentes recrutando novos macrófagos e granulócitos para o local da infecção e formação do granuloma [19].

Dentro de duas a quatro semanas após a infecção, as células dendríticas transportam os antígenos do *Mtb* para os linfonodos mediastinais, para a ativação da resposta imune adaptativa, mediada pelas células T. O bacilo que reside no interior do endossomo e seus antígenos são apresentados via complexo principal de histocompatibilidade (MHC) de classe I e II. A apresentação via MHC II facilita a

ativação das células T helper (Th) naive no subtipo Th1 secretoras de Interferon-gamma (IFN-γ), as quais rapidamente migram para o pulmão. Não somente as células T CD4+ são ativadas, há uma forte ativação das células T CD8+, onde essas células coletivamente geram uma resposta imune adaptativa polarizada do tipo 1 [20].

Os produtos das células Th1 estimulam a síntese de radicais livres e óxido nítrico (NO) pelos macrófagos. O efeito do IFN-γ não está associado apenas aos produtos do estresse oxidativo, mas também induz a autofagia, mecanismo antimicobacteriano essencial para a defesa do hospedeiro [21-22]. O TNF controla a morte celular via apoptose das células infectadas. Muitos estudos já demonstraram que as cepas virulentas do *Mtb* inibem a apoptose e preferencialmente induzem a necrose [23-24]. Na tuberculose, a apoptose é um mecanismo benéfico para o hospedeiro [25-26]. Já a necrose ajuda o escape do *Mtb* e provoca inflamação [24].

IFN-γ e do TNF também aceleram a infiltração de células com perfil inflamatório no sítio de infecção, iniciando a formação do granuloma. As células T que migram para o pulmão interagem com as células apresentadoras de antígenos, incluindo macrófagos infectados e não infectados em vários estágios de ativação. Na presença das células T ativadas o granuloma torna-se totalmente organizado com a micobactéria no interior do macrófago circundado pelos linfócitos T (Figura1). A formação do granuloma é benéfica para o hospedeiro e o patógeno, pois controla a infecção e fornece um nicho para o *Mtb* replicar e disseminar [27].

No interior do fagossomo, o *Mtb* fica privado de nutrientes e fontes de carbono. Para facilitar a sua adaptação, a bacilo modifica seu metabolismo dando preferência para os lípideos. Estudos já revelaram 250 genes do *Mtb* envolvidos no metabolismo dos lípideos, mostrando o potencial do bacilo para se adaptar às fontes de energia disponíveis do hospedeiro [28]. O bacilo também induz o acúmulo de lípideos no interior dos macrófagos, que se diferenciam em macrófagos espumosos (FMs), garantindo maior disponibilidade de substrato lípidico [29-30]. Os FMs ricos em lípideos no seu interior asseguram a sobrevivência dos bacilos pela disponibilidade da fonte de energia, e conferem proteção contra as atividades bactericidas, como autofagia e acidificação lisossomal [30-31]. A morte dos MFs encontrados no centro do granuloma provoca o acúmulo de lípideos como debris caseosos que induzem cavitação e liberação da bactéria para disseminação (Figura 1) [29]. Estudos

demonstraram que os macrófagos espumosos contribuem para a persistência do bacilo, pois perdem suas principais funções como fagocitose e produção de agentes oxidantes, tornando-se reservatórios do *Mtb* [37].

Outro mecanismo de escape do *Mtb* é a alteração da resposta imune no interior do granuloma, criando um ambiente inibitório através da indução de interleucina (IL)-10 [32-35]. A IL-10 inibe as células T e a ativação dos macrófagos no interior do granuloma. A inibição da resposta imune mediada pela IL-10 não é apenas um mecanismo de escape do patógeno, mas também pode ser um mecanismo mediado pelo hospedeiro para limitar a imunopatologia [36].

Com a progressão da infecção, o granuloma formará um foco necrótico devido à caseação no centro onde encontram-se os macrófagos. Acredita-se que a necrose de células no interior do granuloma facilite a transmissão do *Mtb* por alterar a estrutura pulmonar e permitir a liberação do bacilo para as vias aéreas superiores. Está claro que a evolução do granuloma é a dinâmica entre a persistência do bacilo e a resposta imune do hospedeiro, evoluindo de forma continuada durante o curso da infecção [38]. Esta visão está em conformidade com o processo de disseminação do bacilo em indivíduos co-infectados com HIV. Nesses indivíduos, há diminuição da liberação da bactérias para as vias aéreas superiores, pois o bacilo falha na indução da necrose e cavitação do granuloma. Estas observações sugerem que o *Mtb* utiliza o granuloma necrótico como uma porta para a transmissão pessoa a pessoa [39].

Outra característica que acompanha a evolução da infecção é o estresse oxidativo sistêmico e o aumento da peroxidação [40-41]. Estudos em pacientes com tuberculose ativa demonstraram diminuição sistêmica de antioxidantes e aumento de radicais livres comparados com indivíduos sem tuberculose, refletindo o estresse oxidativo associado com TB [42]. A Heme oxigenase-1 (HO-1) é o principal agente anti-oxidante expresso no tecido pulmonar, uma enzima de resposta ao estresse que degrada moléculas heme para a liberação de íons ferro, monóxido de carbono (CO) e biliverdina (BV) [43]. A expressão da HO-1 está associada à citoproteção em modelos de doenças vasculares e pulmonares [44-46].

Alguns estudos em modelos animais mostraram que a infecção pelo *M. tuberculosis* induz a expressão de HO-1 em macrófagos in vitro e in situ, promovendo o desenvolvimento do granuloma e a resistência do hospedeiro [42-44]. A proteção do tecido envolve a inibição das vias de morte incluindo a apoptose, através das

propriedades anti-inflamatórias e antioxidantes mediadas por CO e BV [47]. Já foi descrito que o CO exógeno inibe a apoptose dependente de TNF em fibroblastos e células endoteliais [48-49]. Em culturas de células do músculo liso vascular, a apoptose induzida por TNF, IL-1β e IFN-γ foi inibida pela liberação de CO [50-51]. Elevados níveis de HO-1 foram encontrados no plasma de pacientes com TB pulmonar e extrapulmonar, assim como níveis diminuídos de TNF [52].

A elevada expressão da HO-1 está associada à proteção em diversas doenças inflamatórias, como isquemia cardíaca [53] e hipóxia pulmonar [54]. Na infecção pelo HIV há diminuição da expressão da HO-1 no cérebro dos pacientes com neurodegeneração acompanhada de disfunção cognitiva e replicação do HIV [55]. O uso do antiretroviral aumenta a expressão do RNAm para o HO-1 e da proteína em células de carcinoma do cólon em pacientes com HIV [56].

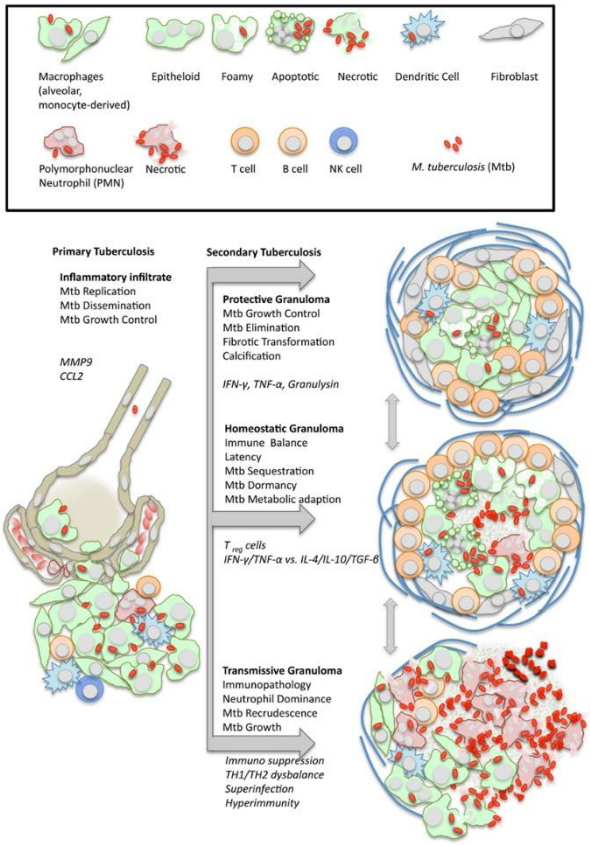

Figura 1: Dinâmica da evolução do granuloma com formação de necrose central e escape do bacilo
Fonte: [Front. Immunol., 07 January 2013 http://dx.doi.org/10.3389/fimmu.2012.0041

2.2. Fisiopatologia da infecção pelo HIV

A infecção com o HIV resulta numa destruição progressiva dos linfócitos T CD4+, geralmente associada com a progressão da doença. O primeiro receptor identificado para o HIV foi a molécula CD4. O receptor CXCR4 foi identificado como um co-receptor responsável pela entrada eficiente nas células-alvo de cepas HIV-1 com tropismo para células T, e o receptor de quimiocina CCR5 foi identificado como um co-receptor para cepas HIV-1 com tropismo para macrófagos [57].

A transmissão do HIV ocorre através de fluído genital, sangue e mucosa. A rota para a transmissão sexual do vírus inclui a vagina, ânus, pênis e o prepúcio. Essas superfícies têm barreiras anatômicas (barreiras epitelial e secreção de muco) e biológicas (sistema imune) para resistir à infecção viral [58]. O trato genital feminino é protegido por ter várias camadas de epitélio colunar e escamoso na ectoderme e endoderme vaginal que dificulta a penetração do vírus. O reto é composto por uma única camada de epitélio colunar, o que contribui para a maior rota de transmissão [59]. A transmissão do HIV ocorre quando virions infecciosos ou células infectadas de um doador atravessam essas barreiras e encontram uma célula susceptível do hospedeiro. Nas mucosas o vírus preferencialmente infecta células T CD4+ co-expressando o receptor CCR5 [60].

Uma vez que a infecção ocorre, o vírus replica-se localmente, e esta fase antes do RNA viral ser detectado no plasma é chamada fase de eclipse [61]. O vírus ou células infectadas pelo vírus trafegam para os linfonodos onde se encontram as células T CD4+CCR5+ ativadas que representam os alvos para a infecção. As principais células que trafegam com os virions são as células dendríticas. Essas células ligam-se ao vírus na mucosa através do receptor de lectina tipo-C (DC-SIGN) e migram para o tecidos linfóides secundários, onde o HIV será transmitido para os linfócitos TCD4+ contribuindo para a disseminação da infecção [62]. Por possuirem receptor CD4 e co-receptores para entrada do vírus, esperava-se que as células dendríticas fossem susceptíveis à infecção pelo HIV como as células T CD4+ e macrófagos. Contudo, estudos já demonstraram que as células dendríticas são resistentes à infecção, sendo necessária grande carga viral para que a infecção ocorra [63].

As células dendríticas retém o vírus em um compartimento endossomal não clássico que protege o vírus da degradação citosólica [64-65]. Atingindo os tecidos linfóides secundários, o vírus é transmitido para as células T ativadas onde se replicam e se disseminam [66]. O HIV tem maior predileção para o tecido linfóide associado ao trato gastrointestinal (GALT), onde as células T de memória efetora CD4+CCR5+ estão presentes em altos níveis [67].

A viremia no plasma atinge o pico máximo 21-28 dias após a infecção junto com a queda no número das células T CD4+, principalmente no GALT [67]. Estudos demonstram que no período agudo da infecção há maior expansão clonal de células T CD8+ com controle da viremia. Esses dados demonstram a importante função das células T CD8+ na inibição do HIV [68-69]. No período da viremia os pacientes podem desenvolver sintomas da síndrome viral aguda, como gripe, febre, dor de garganta, linfadenopatia e exantema. Seguindo esta fase, uma forte resposta imune resulta na diminuição da carga viral e aumento do número de células T CD4+ vírus-específicas circulantes, mas nesta fase o hospedeiro não é capaz de eliminar a infecção [70]. Finalmente, após 12-20 semanas a carga viral diminui e permanece num nível estável, iniciando a fase crônica da infecção, também conhecida como fase de latência [71].

A latência é definida como um período da infecção onde as células infectadas sintetizam partículas virais não infecciosas e persistem no interior do hospedeiro infectado [72-73]. Geralmente a infecção do linfócito T CD4+ resulta em morte, contudo, alguns estudos já demonstraram que algumas células infectadas tornam-se células de memória e permanecem num estado de repouso que contribui para a persistência do vírus [74-75]. Nesta fase, o número das células T CD4+ retorna próximo ao nível normal e os pacientes tornam-se assintomáticos por um período prolongado (Figura 2) [76-77].

Nessa fase ocorre a ativação da resposta imune pelos constituintes virais, como a glicoproteína (gp) 120, nef (uma proteína viral localizada no citoplasma) e produtos dos ácidos nucleicos liberados durante a replicação viral. A ativação do sistema imune resulta na secreção de citocinas proinflamatórias e de Interferons tipo I, incluindo o IFN-α e IFN-β [78-79]. A última consequência da ativação imune é a depleção das células TCD4+ por diferentes mecanismos (citotoxidade direta e apoptose), incluindo a diminuição da meia-vida das células T CD4+ e CD8+, tráfego

anormal das células T e exaustão das células T. Em pacientes não tratados, a perda gradual de linfócitos T ativados contribui para as infecções oportunistas e desenvolvimento das características da Síndrome da Imunodeficência Adquirida (SIDA) [79-80].

As células T CD4+ depletadas são substituídas por células T de vida curta com potencial regenerativo mais limitado [80]. Outro fator importante é a evolução viral acelerada neste estágio, observada pela alta mutação viral e alteração do tropismo celular. Há uma mudança no perfil do tropismo das cepas para o CCR5, para o CXCR4 ou para ambos os receptores, resultando no aumento da virulência e tropismo para as células-alvo [80]. A infecção pelo HIV também afeta o sangue e os tecidos linfóides com apoptose massiva das células B e perda dos centros germinativos dos tecidos linfóides [81-82].

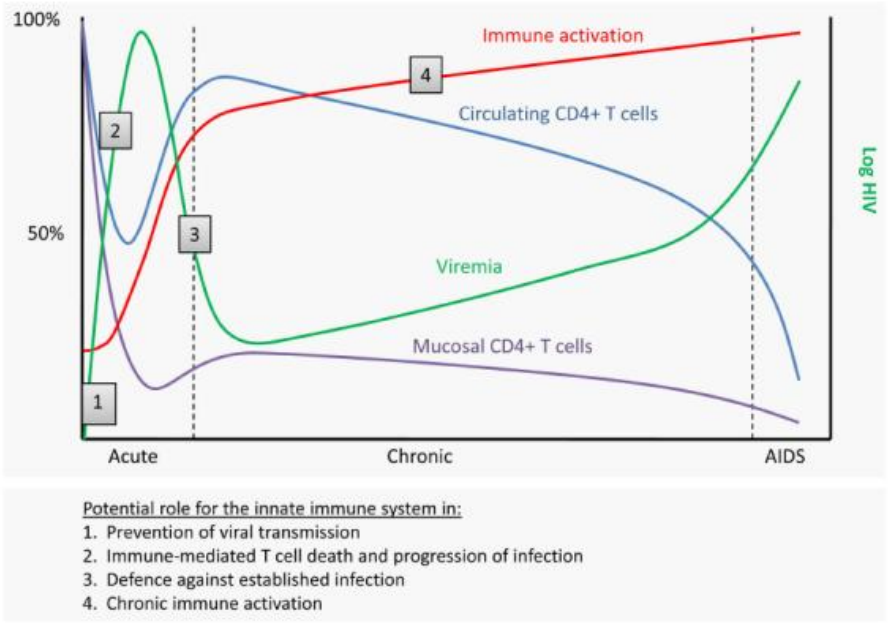

Figura 2: Curso da infecção pelo HIV

Fonte: [Int J Epidemiol. 20 de Abril de 2010 doi: 10.1093/ije/dyq057]

2.3. Cooperação entre o *Mycobacterium tuberculosis* e HIV

Os linfócitos T CD4+ mediam a resposta imune celular contra o *Mtb*. A função central dessas células no granuloma tem sido demonstrada na infecção pelo HIV, onde a depleção dos linfócitos T CD4+ afeta a resposta granulomatosa contra o *Mtb* [83].

A infecção pelo HIV diminui a capacidade do granuloma em conter o *Mtb* induzindo o aumento do crescimento bacteriano e patologia grave. A exacerbação da patologia pode ocorrer pelo aumento da carga viral no local da infecção pelo *Mtb*, rompimento dos macrófagos e depleção das células T CD4+ específicas para o *Mtb*, fatores que induzem mudanças funcionais e prejudiciais dentro do granuloma [84].

O HIV replica-se preferencialmente no interior das células T CD4+ ativadas e macrófagos, células encontradas no granuloma, local considerado ideal para a replicação do vírus. Estudos demonstram que existe um aumento da carga viral no fluído pleural comparado com o plasma entre pacientes com TB pleural e maior replicação do HIV em macrófagos co-infectados com o *Mtb* e o HIV quando comparados com macrófagos não infectados com o vírus, enfatizando que a replicação do HIV é aumentada nos locais da infecção pelo bacilo [85-86]. O aumento da replicação viral pode estar associado ao tipo de cepa do bacilo. O *Mtb* pode induzir a replicação do HIV em diferentes níveis. Por exemplo, em células mononucleares do sangue periférico (PBMCs) co-infectadas, a cepa CDC1551, uma cepa clínica menos virulenta [87], induz mais replicação viral do que cepas clínicas mais virulentas, como a HN878 [88].

O ambiente imune que se desenvolve com a infecção pelo *Mtb* promove a replicação viral ex vivo. O bacilo promove o aumento de citocinas inflamatórias in vivo, as quais podem induzir a ativação de células T e macrófagos aumentando a replicação do HIV. A incubação de PBMCs infectadas com HIV com fluído pleural de pacientes com TB induz maior replicação viral do que a observada em células incubadas com

fluído pleural de pacientes sem TB. Este aumento na replicação é dependente de citocinas inflamatórias como IL-6 e TNF [89].

A funcionalidade de macrófagos também está alterada em indivíduos co-infectados [90-91]. Após a entrada do *Mtb* no parênquima alveolar, monócitos migram o pulmão e se diferenciam em diferentes tipos de macrófagos no interior do granuloma. Estudos demonstram que o HIV infecta macrófagos in vivo, e provavelmente inibe a função dessas células infectadas com *Mtb*. Todos os tipos de macrófagos são susceptíveis à infecção pelo HIV [90-93]. Macrófagos co-infectados diminuem a liberação de TNF e diminuem a apoptose dependente de TNF quando comparados com macrófagos infectados apenas pelo *Mtb* [91]. Essas células são importantes para a patogênese da tuberculose e infecção pelo HIV e servem como reservatório para ambos os patógenos.

A apoptose dos macrófagos é um mecanismo importante durante a co-infecção. A adição da proteína nef do HIV à cultura de macrófagos infectados com o *Mtb* diminui a secreção de TNF e a apoptose dependente de TNF, sugerindo que não apenas o vírus, mas também seus constituintes podem alterar a funcionalidade dos macrófagos [92]. Estudos sugerem que o HIV pode manipular tanto a apoptose, quanto a acidificação do fagossomo em macrófagos infectados com o *Mtb* [93-94]. Macrófagos humanos infectados com HIV e o Mycobacterium tuberculosis mostram diminuição da síntese de TNF, degradação de I□B e translocação de NF-□B, sugerindo interação dos patógenos para a falha da resposta imune através da diminuição sinalização celular e liberação de citocinas pro-inflamatórias [95].

3. HIPÓTESE

Fatores genéticos (*TNF*-308G>A e *DDX39B* -22G>C e -348C>T) e imunológicos (HO-1 e MMP-1) estão associados com a susceptibilidade e gravidade da doença em pacientes com tuberculose ativa e co-infectados com o vírus da imunodeficiência humana.

4. OBJETIVOS

4.1 GERAL

Avaliar Fatores genéticos (*TNF*-308G>A e *DDX39B* -22G>C e -348C>T) e imunológicos (HO-1 e MMP-1) associados com a susceptibilidade e gravidade da doença em pacientes com tuberculose ativa e co-infectados com o vírus da imunodeficiência humana.

4.2 ESPECÍFICOS

1. Realizar o levantamento bibliográfico sobre os polimorfismos genéticos envolvidos com a apoptose e associados com tuberculose.
2. Comparar a frequência genotípica e alélica dos polimorfismos dos genes *TNF*-308G>A e *DDX39B* -22G>C e -348C>T em voluntários com infecção tuberculosa latente, monoinfectados com tuberculose ativa ou co-infectados com TB-HIV.
3. Comparar a produção de citocinas em culturas estimuladas com antígenos do *Mtb* associadas com a apoptose e patogênese da tuberculose (TNF, IL-6, IL-1α e lL-1β) em voluntários com infecção tuberculosa latente, monoinfectados com tuberculose ativa ou co-infectados com TB-HIV.
4. Correlacionar o perfil genotípico com a produção de citocinas em voluntários com infecção tuberculosa latente, monoinfectados com tuberculose ativa ou co-infectados com TB-HIV.
5. Correlacionar a produção de citocinas com a gravidade da doença tuberculosa.
6. Comparar a produção de mediadores do estresse oxidativo (HO-1 e MMP-1) do plasma de voluntários com infecção tuberculosa latente, monoinfectados com tuberculose ativa ou co-infectados com TB-HIV.

5. RESULTADOS ALCANÇADOS

5.1 CAPÍTULO 1: Systematic review of the contribution of polymorphisms in genes involved in cell death pathways to the severity and progression of tuberculosis

Elisabete L. Conceição, Theolis Barbosa*

Universidade Federal da Bahia - UFBA, Av. Reitor Miguel Calmon, s/n, Vale do Canela, 40110-902, Salvador, Bahia, Brazil and Centro de Pesquisas Gonçalo Moniz - CPqGM, FIOCRUZ, Rua Waldemar Falcão 121, Candeal, 40296-710, Salvador, Bahia, Brazil.

E-mail adresses: ELC, betejc@hotmail.com; TB, theolis@bahia.fiocruz.br.

Running title: Cell death polymorphisms in tuberculosis

Word count:

*Correspondence to: Dr. Theolis Barbosa

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ). Rua Waldemar Falcão 121, Candeal, 40296-710 Salvador, Bahia, Brazil. Phone: +55 71 3176-2215; Fax: +55 71 3176-2279, e-mail: theolis@bahia.fiocruz.br.

Abstract

Cell death is a primordial event in the innate immune response against invading pathogens. The triggering of programmed cell death in infected cells is part of the innate mechanisms of the host to fight infections. Various pathways are involved in this response. This mechanism is important for the control of mycobacterial replication, thus it is not surprising that virulent *Mycobacterium tuberculosis* interferes with the induction of programmed cell death deviating cell fate to necrosis. Necrosis allows the bacteria to evade host defense mechanisms by inducing cellular lysis and the spreading of viable bacilli that become available to infect the surrounding macrophages. Many genes for which polymorphisms have been described regulate infection-driven cell death. In this review, we focus on the significance of these polymorphisms to cell death induction and to the pathogenesis of mycobacterial infections, with emphasis in the host response to *M. tuberculosis* infection.

Keywords: Apoptosis, genetic variation, tuberculosis, cellular immunity

Introduction

The development of a protective immune response against *Mycobacterium tuberculosis* (*Mtb*) is regarded as crucial for the containment of tuberculosis infection, as only a small percentage (approximately 10%) of infected individuals will develop active disease during lifetime [1], and vaccination with Bacille of Calmette-Guérin (BCG) can provide at least partial protection against infection and disease [2]. Exciting new information on the importance of innate immune mechanisms for the development of a protective immune response has emerged in the past decade [3]. Among innate resistance mechanisms, the importance of programmed cell death has been highlighted by studies that have demonstrated that infected cell fate can influence bacilli survival and the mounting of a protective adaptive response [4]. *Mtb* infects macrophages and subverts its mechanisms of killing [5], as well as inhibits apoptosis and triggers necrosis of macrophages to evade innate immunity and delay the initiation of the adaptive immune response [6-7]. On the other hand, programmed cell death is regulated by different pathways and genetic variability in genes that regulate the expression of molecules involved in cell death can influence the successful mounting of a protective response against *Mtb* [7].

There are different forms of cell death, which are reviewed elsewhere [8–10]. They are broadly categorized as accidental (ACD) or regulated (RCD) cell death [8]. The former category comprises cell death involving sudden disassembly of the cell structures due to exposure to extreme physical, chemical or mechanical stress, in a way that no specific molecular machinery is involved. The latter, in contrast, involves a molecular machinery that is genetically encoded and allows its modulation and

pharmacological inhibition. Table 1 summarizes the most relevant molecules involved in regulated cell death.

Here we systematically review the current state-of-the-art regarding the role of cell death in the immunopathogenesis of tuberculosis, the single nucleotide polymorphisms (SNPs) in genes that regulate programmed cell death and their observed and potential role in the development and progression of tuberculous disease.

Methods

Data sources and search strategy

This systematic review followed the PRISMA guidelines and checklists [11]. A Medline/PubMed and Google Scholars search was conducted including the studies available online on January 27, 2016. Data collection from published articles was performed without time restriction. The search criteria used are listed in Supplementary Table 1.

Eligibility criteria

Eligibility for inclusion focused on studies reporting polymorphisms in molecules involved in cell death pathways among TB patients. We included all original articles in English published in academic journals until January, 2016. 7.064 non-duplicated records were identified through database searching and records were screened to check whether they were related with the topic. 6.884 studies were not included as they described cytokine polymorphisms not involved in cell death pathways. The reference lists of included articles were also screened to identify relevant studies not previously detected in the electronic search (Figure 1). The information obtained from

these studies was organized in topics regarding the role of death pathways in *M. tuberculosis* infection, the genetic polymorphisms reported in genes involved in cell death pathways, and the data available on the association between these polymorphisms and susceptibility to tuberculosis.

Genetic polymorphisms in genes involved in cell death pathways

Single nucleotide polymorphisms (SNPs) of genes coding molecules involved in cell death pathways play a crucial function in genetics host that contribute to microorganisms killing. There are variants of several genes involved in the process of cell death such as TNF, TNFR, TRAIL, Fas/FasL, Caspase, P2X7 and IL-1β, that are crucial to active the apoptosis.

TNF is a cytokine involved in apoptosis extrinsic pathway. The gene is located within the major histocompatibility complex (MHC) region in the short arm of chromosome 6, between the human leukocyte antigen-B (HLA-B) and the HLA class III genes. TNF production is both transcriptionally and post-transcriptionally regulated. Many allelic polymorphisms of the TNF gene have been described, including eleven single nucleotide polymorphisms (SNPs) in the promoter region at the 1031 (T/C), -863 (C/A), -857 (C/T), -308 (G/A), -238 (G/A), -1196 (C/T), -1125 (G/C), -572 (A/C), -316 (G/A), -163 (G/A), and -70 (G/A) loci [12]. The polymorphism at the −238 position lies within a putative regulator sequence where a G to A substitution defines two variants (TNF-G and TNF-A). The second polymorphism at position −308 the presence of G defines the common variant TNF1 and the presence of A defines the less common variant TNF2. The presence of the TNF -308A allele is considered to be associated with higher TNF gene transcription and TNF over production [13].

TNF cytokine binding to two receptors, TNFR-1 (encoded by the TNFRSF1A gene) and TNFR-2 (encoded by the TNFRSF1B gene). TNFR-1 is dominant receptor to TNF binding and can activate NF-kB, mediate apoptosis, and function as a regulator of inflammation [14]. TNFR-1 is located in the short arm of chromosome 12 (12p13) and TNFR-2 at 1p36 [15]. The SNPs to *TNFR-1* were found in promoter region, exon 1 and introns 2, 4, 6, 7, and 8 [16-18]. Four polymorphisms were described in promoter region (-383 A/C, -609 G/T, -580 A/G, and -383 A/C) and exon 1 (+36 A/G) [47-48]. TNFRSF1B gene contains two sequence alleles in exon 6 at 196 position (T/G) that encode differing amino acid sequences of TNFR-2 (M196R). This amino acid difference occurs close to the cleavage site that leads to the shedding of TNFR-2 receptors. Some studies observe decreased sTNFR-2 levels with the R196 receptor form [19-20]. The M196 and R196 forms differ in their ability to mediate TNF signaling and activate downstream pathways and pathologic processes (such as apoptosis), such that the R196 form allows less activation of NFκB and recruitment of TRAF2, and greater induction of apoptosis after activation of the TNFR-1 pathway [21]. The frequencies of the R196 TNFRSF1B allele is associated with various inflammatory and autoimmune diseases (including SLE, Crohn's disease, and familial rheumatoid arthritis), suggesting a role for this allele in inflammatory diseases [22-24].

The TNF-related apoptosis-inducing ligand (TRAIL), is a endogenous a homotrimeric cytokine that belongs of the TNF cytokine family that inducing apoptosis [25]. TRAIL gene is located on chromosome 8p21 with five exons. Five SNPs have been identified in TRAIL exons. Three of them are in the 3' untranslated region (3'UTR) at 1525(G/A), 1588 (G/A) and 1595 (C/T) positions. Some studies have shown that the 3'UTR of TRAIL has important role in TRAIL gene regulation and it is a promising

anticancer agent due to its critical regulatory role in apoptosis. There are other two in exon 1 at site of 1192 and 5912, which do not alter the encoded amino acid sequence [26-28]. TRAIL binds to the TRAIL receptor 1 (*TRAIL-R1*), a gene also known as *DR4* and *TNFRSF10A.* The TRAIL-R1 triggers apoptotic proteases to regulate apoptosis. There are multiple well-characterized polymorphisms in the *TRAIL-R1* gene, but the most extensively studied polymorphism has been the C/G substitution resulting in a threonine to arginine amino acid change in exon 4 (Thr209Arg, rs20575). Thr209Arg is of special interest in recent decade most likely due to the involvement in receptor ligand binding activity and stimulation of apoptotic pathways [29].

The Fas/FasL signaling system activates a major extrinsic apoptosis pathway that is responsible for maintaining immuno-privileged sites, which is of particular importance for the regulation of the acquired immune response. It also fulfils other regulatory functions [30-31]. Fas (TNFRSF6/CD95/APO-1) is a cell surface receptor, expressed in a variety of tissues [32-33]. The gene is located chromosome 10q 24.1 and consists of nine exons and eight introns. The promoter region is responsible for allelic variations in Fas, which can also modify the transcriptional rate [34]. Two SNPs have been identified in the *FAS* promoter region: one in the silencer region, G to A substitution at the nucleotide position -1377 (rs2234767), and the other in the enhancer region, A to G substitution at the nucleotide position -670 (rs1800682). These two polymorphisms are located within the sites that bind to the transcription factors stimulatory protein-1 (Sp1) and the signal transducers and activators of transcription 1 (STAT1), respectively. The *FAS* –1377A allele and the *FAS* –670G allele disrupt the Sp1 and STAT1 binding sites, respectively, thereby diminishing the promoter activity and decreasing Fas expression [35-37]. The FasL also known as

TNFSF6 or CD95L, is a member of the tumor necrosis factor superfamily and the natural ligand to Fas. The human FasL gene is located on chromosome 1q23. It consists of four exons spanning approximately 8 kb, and encode a protein of 281 amino acids [38]. To *FasL* gene there are two reported polymorphisms: a C to T change at the nucleotide position -844 (FASL-844 C/T, rs763110) of the promoter region and an A to G change at nucleotide position -124 of intron 2 (FASL INV2nt -124 A/G, rs5030772). *FASL -844 C/T* is located in a putative binding motif for a transcription factor, CAAT/enhancer-binding protein h, and the -844 C allele may increase basal expression of FASL compared with the -844 T allele [39], suggesting that the *FASL -844 C/T* polymorphism may influence FasL expression and mediated signaling. The *Fas/FasL* polymorphism has been associated with cancer, lupus erythematosus and rheumatoid arthritis [40-41].

Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. There are multiple polymorphisms in genes to caspase that are involved in multiple disease [42-45]. The human *CASP8* gene contains 11 exons located in chromosome 2q33–34 [46]. The common variants of the *CASP8* gene disrupt apoptosis and enhance the risk of developing various types of cancer, including breast cancer [47] and prostate cancer [48]. There were at least 474 single nucleotide polymorphisms in the CASP8 gene according to the SNP database (http://www.ncbi.nlm.nih.gov/SNP), including the most commonly occurring CASP8 −652 6N ins/del polymorphism (rs3834129). It was reported that the CASP8 −652 6N ins/del promoter variant destroy the binding element for stimulatory protein 1 and reduce the expression of CASP8, thus resulting in a reduction in the apoptosis reactivity of T lymphocytes upon stimulation by cancer cells [49].

The $P2X_7$ gene, located at position 12q24, encodes for a 595-aa long polypeptide with two transmembrane stretches [50]. The human $P2X_7$ gene is highly polymorphic with five previously described loss-of-function (LOF) single-nucleotide polymorphisms (SNP; c.151+1G/T, c.946G/A, c.1096C/G, c.1513A/C and c.1729T/A) and one gain-of-function SNP (c.489C/T). However, the majority of these SNPs are intronic, with approximately 150 nonsynonymous (or missense) [51]. The A common SNP in exon 13 (1513A/C) results in the substitution of glutamic acid at position 946 by alanine (G496A) (GU). This polymorphism occurs in the region of the gene that encodes the carboxyterminal tail of the protein, and different studies have demonstrated that homozygosity for the C allele (C/C) leads to almost complete loss of the $P2X_7$ function with lack of ATP-induced mycobacterial killing in these individuals [52-53] . This amino acid change impairs cation fluxes in a variety of cells, the release of IL-1β, IL-18, and matrix metalloproteinase (MMP)-9 from macrophages, and shedding of CD23 and CD62L from lymphocytes [54-55]. The other polymorphisms identified include the 1729T/A SNP that abolishes trafficking of the receptor to the cell surface [56], the 946G/A SNP that abolishes the binding of ATP to the extracellular domain of $P2X_7R$ [57], and the 151+1 G/T SNP, leading to a null allele [58]. IL-1β gene is involved in Pyroptosis pathway. In the IL-1 processing is involved interleukin (IL)-1-converting enzyme called caspase-1 that cleaves the pro-inflammatory cytokine pro-interleukin (IL)-1β to an active secreted molecule in monocytes and macrophages. Additional consequence of caspase-1 activation in macrophages following infection by intracellular pathogens is a rapid, and caspase-1- dependent cell death called pyroptosis Figure 2 [59] . The loci for human *IL1B* gene is found as a cluster on chromosome 2q12. Several common polymorphisms within the *IL-1* gene complex have been described, including single nucleotide polymorphisms (SNPs) in *IL1B* at

position −511 (T/C), −31 (C/T), -3737 (T/C), -889 (C/T) and -1464 (C/G) in the promoter region and at position +3954 (C/T) in exon 5 [60].

Role of death pathways in *M. tuberculosis* infection

The apoptosis of infected macrophages provides an important link to adaptive immunity, as apoptotic vesicles containing bacterial antigens are taken up by Dendritic cells (DC). The DCs can efficiently present these antigens to naive T cells, leading to their activation [61-62]. Avirulent *M. tuberculosis* strains induce apoptosis in antigen presenting cells (APCs) through TNF/TNFR, toll like receptors (TLRs), Fas/FasL or by altering the expression of Bax/Bcl-xL via an oxygen dependent pathway [63-65].

Necrotic cell death may be an important factor in inflammatory tissue damage and transmission of the bacilli [66]. Several studies have suggested that pathogenic *M. tuberculosis* strains use inhibition of apoptosis as a virulence mechanism and induces necrosis, which allows the bacteria to evade host defense mechanisms by inducing cellular lysis and spreading of the infection to the surrounding macrophages [67-70].

Virulent strains such as *Mtb* H37Rv induce less macrophage apoptosis than avirulent or attenuated strains such as *Mtb* H37Ra by activating the release of membrane-bound TNFR2 as the soluble form to evade TNF-dependent apoptosis [71-72], and by increasing the expression of Mcl-1 protein that is a member of anti-apoptotic B-cell lymphoma/leukemia 2 (Bcl-2) family. The Bcl-2 family proteins, which are located in the outer membrane of mitochondria, can block the release of cytochrome c from mitochondria preventing caspase activation [73-74].

Mtb cell wall components cause stimulation of TLR2, which leads to translocation of NF-κB to nucleus and induces expression of TNF. During certain stages of infection, virulent *Mtb* strains might cause higher expression of TNF than avirulent strains to induce more necroptosis. Alternatively, virulent strains could, while avirulent strains could not, secrete a biological factor which can block caspase-8 activity to inhibit apoptosis [75-76].

M. tuberculosis activates the NLRP3 inflammasome [77] and macrophages infected with virulent *Mtb* (H37Rv) release high levels of IL-1β [78]. Caspase-1 activity and the pyroptosis of infected cells are inhibited by the Mtb Rv3364c protein, that can bind to the host cell protease cathepsin G [79]. To IL-1β function in tuberculosis studies has been shown that *M. tuberculosis* prevents inflammasome activation and IL-1β processing, which normally lead to improved mycobacterial clearance and a lower bacterial burden in the lungs of aerosol-infected mice. However, it should be noted that macrophages infected with *M. tuberculosis* produce IL-1β in a manner that is dependent on bacterial virulence [80].

The role of P2X7 receptors in the pathogenesis of tuberculosis is controversial. Macrophages treated with ATP reduces of M. tuberculosis-colony-forming units and presenting high P2X7 receptor expression in the lung, while P2X7R−/− mice increase of *M. tuberculosis* burden in lung tissue [81]. However, others evidences show that P2X7R-/- mice have attenuated disease with substantially reduced bacillus dissemination and lung inflammation without evidence of necrosis [82]. In humans, P2X(7)R and extracellular ATP mediate apoptosis of human monocytes/macrophages infected with Mycobacterium tuberculosis reducing the intracellular bacterial viability [83].

Genetic polymorphisms in cell death pathways associated with susceptibility to tuberculosis disease

Susceptibility to TB is partly genetically determined [84] and variations in genes involved in the cell death pathway may affect the host response to *M. tuberculosis* infection. Indeed, human mononuclear cells with certain polymorphisms in death related genes displayed an impaired ability to control *M. tuberculosis* growth, thus suggesting that polymorphisms in death genes may be associated with TB [85].

Two biallelic polymorphisms in the promoter region of the TNF gene may be relevant for the production of TNF in the tuberculosis. The *TNFRSF1B* polymorphism has been associated with tuberculosis in different populations [86-87]. The *Fas/FasL* polymorphism has not been investigated in tuberculosis. However, Fas and FasL soluble are elevated in the serum levels of pleural tuberculosis patients suggesting that Fas-mediated apoptosis may be at least a part of protective immunity to tuberculosis [88-89].

A SNP in the promoter region of $P2X_7R$ gene at nucleotide position −762 (C/T) has been described that has a protective association with tuberculosis [90]. The functional polymorphism in the *IL1B* gene influences the susceptibility to TB. *IL1B* +3954 C to T (rs1143634) has been associated with tuberculosis [91].

In clinical studies, the association among TNF polymorphism and tuberculosis remain unclear. The frequency of *-308A TNF* polymorphism has been known to be linked to a high TNF production. In a study with Sicilian patients affected by chronic tuberculosis (TBC), the frequency *-308A* allele was significantly increased in TBC compared to healthy subjects. However, the effect of an allele is largely dependent by homo- or heterozygous state and the percentage of subjects carrying the "TNF

low producer" -308GG genotype was reduced in TBC group [92]. In a study systematic of the published studies was performed to clearer understanding of the association between TNF-308 (rs1800629 G/A) polymorphisms and the susceptibility tuberculosis. This study indicated that the TNF-308 polymorphism was not associated with the risk of TB in the total population, however the significant risk for TNF-308 A allele was found among Asians and not Caucasians [93]. In Pakistan, TB patients with different lung tissue involvement the *TNF-308G/A* polymorphism in combination with *IFNG+874T/A* show weak associations with severe form of the disease [94]. In a Indian population the *TNF -238* and *-308* polymorphisms not were associated with the disease severity in patients sub-groups and were found to don't affect the TNF levels in both the patients and controls populations and in the patients sub-groups [95]. A meta-analysis study including nine case-control studies revaluated that there was association between TNF -238G/A polymorphism and tuberculosis susceptibility [96]. In Colombia TNF -308G was associated with TB. However, the TNF -238A allele represented a susceptibility factor for TB [97]. A study evaluating genes cytokine associated with tuberculin test response in a Brazilian indigenous, showed that TNFR1 not were associated to immune response and anergy [98]. Other study in Brazil evaluated serum levels of the cytokines and polymorphisms in the genes in patients with pulmonary tuberculosis. The study showed no association among IFN-γ and TNF-α gene polymorphisms at positions +874 and -238, respectively with the corresponding cytokine serum levels [99].

Haplotype analysis in South Africans revealed an association in the 3′untranslated region of *TNFRSF1B* with tuberculosis [86]. In Asian population the *TNFRSF1B* polymorphism also found to be associated with TB [87-100].

The SNPs at ATP of $P2X_7$ gene are associated with TB in different population. A cohort study in Southeast Asians showed that +1513A/C polymorphism was strongly associated with extrapulmonary, but not pulmonary TB. The +1513C allele increases susceptibility to tuberculosis and this defect is associated with the reduction in the capacity of macrophages to kill *M. tuberculosis*. It can worsen the clinical situation in TB [60,101]. The analyze of two single-nucleotide polymorphisms (SNPs) in the P2RX7 gene (-762 C/T and +1513A/C) in the Slavic population revealed no significant association to -762 C/T P2RX7 promoter between pulmonary TB patients and control subjects. In contrast, the frequency of the C allele at position +1513 in exon 13, resulting in a loss of P2X(7) function, was significantly higher among pulmonary TB patients [102]. In a Mexican population *$P2X_7R$-762* gene polymorphism not was associated with tuberculosis. In contrast, *the $P2X_7R$ A1513C* polymorphism was associated significantly with tuberculosis [103]. In Punjab, North India, the distribution of +1513 A/C genotypes in the TB patient and the control groups revealed a significant association of +1513C allele such risk factor for the development of TB [104]. However, in a Gambian population five SNPs at promoter region of *$P2RX_7R$* were screened for associations with clinical tuberculosis. A significant protective association against tuberculosis was found for 1 promoter SNP, at nucleotide position -762 for variant C allele [105].

The functional polymorphism in the *IL1B* gene influences the susceptibility to TB in some populations. In Iranian patients with pulmonary tuberculosis a positive, significant difference was found in *IL1B* at position -889, , where the T/T genotype was over represented in PTB patients [106]. In Gambian TB patients, decreased risk of pulmonary TB was associated with both heterozygosity and homozygosity for the *IL1B* -511/C allele and no association there was between the IL1B+3953-T/C

polymorphism with TB in this population [107]. The association of IL-1β and IL-10 cytokine gene polymorphisms with risk of developing tuberculosis in TB patients, their HHC and healthy controls (HC) showed that the genotype and allele frequencies of IL-1β +3954C/T polymorphism was similar between TB patients and HC [108]. In extrapulmonary tuberculosis the *IL1B* +3953 C/T polymorphism was associated was associated with the disease compared to PPD+ controls [109]. In a Northwestern Colombian population, an endemic area of M. tuberculosis infection, the *IL1B* +3953 C/T polymorphism was significant different between patients and controls [110]. A meta-analysis about the association of the *IL1B* polymorphism with TB indicated there is a lack of association between the IL1B (-511 and +3954) polymorphisms and TB risk [111].

Conclusion

Cell death is a protective mechanism of the innate immune system to eliminate intracellular pathogens. Macrophage apoptosis during *Mycobacterium tuberculosis* infection has focused much attention on its possible role in disease pathogenesis. This is a mechanism that contribute to amplification of the immune response by activating the adaptive immune response. Genetic polymorphisms that are associated with the death pathways may be beneficial or not to the host. Polymorphisms in *TNF, TNFRSF1B, $P2X_7R$* and *IL-1β* may contribute to protection against *Mtb* infection, facilitating their elimination (Figure 2). Functional studies are necessary to elucidate whether these polymorphisms are associated with protection.

Acknowledgements

Cleidiane Daltro in contributing for search strategy. Financial support was provided by Fiocruz and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-(CAPES)

Figure legends

Figure 1: Flow of information through eligible criteria to systematic review [40].

Figure 2: Genetic variation in regulated cell death that has been associated with susceptibility to tuberculosis

Supplementary Table 1. Search strategies employed in this systematic review.

Search criteria
(Cytokine [All Fields] AND polymorphism[All Fields]) NOT review [PT]
(tuberculosis [All Fields] AND "cell death" [All Fields]) NOT review [PT]
(tuberculosis [All Fields] AND apoptosis [All Fields]) NOT review [PT]
(tuberculosis [All Fields] AND polymorphism [All Fields]) NOT review [PT]
(polymorphism [All Fields] AND apoptosis [All Fields] AND tuberculosis [All Fields]) NOT review [PT]
(Cytokine [All Fields] AND polymorphism [All Fields] tuberculosis [All Fields]) NOT review [PT]
(CD95 [All fields] AND polymorphism [All fields]) NOT review [PT]
(TNF [All fields] AND polymorphism [All fields]) NOT review [PT]
(TNFR [All fields] AND polymorphism [All fields]) NOT review [PT]
("TRAIL" [All fields] AND polymorphism [All fields]) NOT review [PT]
("FASL" [All fields] AND polymorphism [All fields]) NOT review [PT]
("TRAF" [All fields] AND polymorphism [All fields]) NOT review [PT]
("nemo" [All fields] AND polymorphism [All fields]) NOT review [PT]
("cIAP" [All fields] AND polymorphism [All fields]) NOT review [PT]
("TAK" [All fields] AND polymorphism [All fields]) NOT review [PT]
("IKK" [All fields] AND polymorphism [All fields]) NOT review [PT]
("caspase" [All fields] AND polymorphism [All fields]) NOT review [PT]
("Bid" [All fields] AND polymorphism [All fields]) NOT review [PT]
("cFLIP" [All fields] AND polymorphism [All fields]) NOT review [PT]
("CUL3" [All fields] AND polymorphism [All fields]) NOT review [PT]
("Bcl" [All fields] AND polymorphism [All fields]) NOT review [PT]
("BH3" [All fields] AND polymorphism [All fields]) NOT review [PT]
("Bax" [All fields] AND polymorphism [All fields]) NOT review [PT]
("Bak" [All fields] AND polymorphism [All fields]) NOT review [PT]
("Smac" [All fields] AND polymorphism [All fields]) NOT review [PT]
("cytochrome c" [All fields] AND polymorphism [All fields]) NOT review [PT]

("apaf" [All fields] AND polymorphism [All fields]) NOT review [PT]
("xiap" [All fields] AND polymorphism [All fields]) NOT review [PT]
("DR4" [All fields] AND polymorphism [All fields]) NOT review [PT]
("DR5" [All fields] AND polymorphism [All fields]) NOT review [PT]
("DIABLO" [All fields] AND polymorphism [All fields]) NOT review [PT]

Table 1: Molecules critically involved in regulated cell death.

RCD type Apoptosis (caspase-dependent)	Molecules involved	Role in RCD	Refs.
Extrinsic:	TNF receptor 1 (TNFR1/TNFRSF1A), FAS (CD95/APO1/TNFRSF6), TRAIL receptor 1 (TRAILR1/DR4/TNFRSF10A), TRAIL receptor 2 (TRAILR2/DR5/TNFRSF10B), DR3 (APO3/TNFRSF25), DR6 (TNFRSF21)	Death receptors	[9,10]
	lymphotoxin-α (LT-α/TNFSF1), tumor necrosis factor (TNF/TNFSF2), FAS/CD95 ligand (FASL/CD95L/TNFSF6), TNF ligand superfamily member 10 (TRAIL/Apo2l/TNFSF10), TL1A (TNFSF15)	Death receptor ligands; FASL, TNF and TRAIL also play a role in necroptosis	[9,10]
	Deleted in colorectal carcinoma (DCC), UNC5B	Dependence receptors	[9]
	Netrin-1	Dependence receptors ligand	[9]
	Fas-associated protein with a death domain (FADD)	DISC formation	[8,9]
	TNFR-associated death	Recruits	[9]

	domain (TRADD)	FADD/caspase-8 upon TNF signaling	
	Receptor-interacting protein kinase 1 (RIPK1/RIP1)	DISC component; also plays a role in necroptosis	[9,10]
	FADD-like apoptosis regulator (CFLAR/c-FLIP)	DISC component, regulates caspase-8	[8-10]
	Cellular inhibitor of apoptosis proteins (cIAPs)	DISC component	[9,10]
	TNFR-associated factor 2 (TRAF2), TRAF5	DISC component recruited upon TNF signaling	[9]
	Protein phosphatase 2A (PP2A), death-associated protein kinase 1 (DAPK1)	Activation of executioner caspases in the absence of dependence receptors ligation to netrin-1	[9]
	Procaspase- 8	DISC component, activates caspase-3, cleaves BID generating the mitochondrion-permeabilizing fragment tBID	[9]
	Procaspase 10	DISC component	[9]
Intrinsic:	Holocytochrome c (CYT*C*)	Apoptosome component, induces caspase 3/9 activation	[8,9]

	Deoxy-ATP- and apoptotic peptidase-activating fator 1 (APAF)	Apoptosome component, induces caspase 3/9 activation	[8,9]
	Multi-BH motif proteins: B-cell CLL/lymphoma 2(BCL2)-associated X protein (BAX), BCL2-antagonist/killer 1 (BAK1)	Death agonists, pore formation	[8, 10]
	Multi-BH motif proteins: BCL2, BCL2-like 1 (BCL2L1/BCL-X_L), BCL-$_W$, A1, Myeloid cell leukemia 1 (MCL-1)	Death antagonists, BCL2/BCL2L1, MCL-1: BAX/BAK1 inhibitor	[8,10]
	BH3-only proteins: BCL2binding component 3 (BBC3/PUMA), BCL2-like 11 (BCL2L11/BIM), BH3-interacting domain death agonist (BID), BAD, BIK, BMF, NOXA	Death agonists, regulation of BCL2-like proteins	[8, 10]
	Procaspase-9	Activates caspases-3, 7	[10]
Both:	Procaspases-3, 6, 7	Apoptosis executioners	[8]
	IAP-binding mitochondrial protein (DIABLO/SMAC)	IAP family inhibitor, sequesters XIAP	[8-10]
	X-linked inhibitor of apoptosis (XIAP)	Regulation of mitochondrial outer membrane	[8, 10]

		permeabilization, inhibition of caspases-3, 7 and 9	
Necroptosis (RIPK1/RIPK3/MLKL-dependent)	RIPK1	Induces RCD upon death receptors ligation in the presence of caspase inhibitors; regulates caspase activation When catalytically inactive (necrostatin(Nec)-1 bound), inhibits necroptosis triggered by TLR ligation/type I interferons	[8]
	Receptor-interacting protein kinase 3 (RIPK3/RIP3)	Induces necroptosis in response to TLR ligation/type I interferons, regulates caspase activation	[8]
	TLR adaptor molecule 1 (TICAM1/TRIF)	Induces necroptosis in response to TLR ligation/type I interferons	[8]
	Z-DNA binding protein 1 (ZBP1)	Triggers necroptosis in response to viral infection	[8]
	MLKL	Binds to membrane phospholipids	[8]

		resulting in loss of barrier function	
Pyroptosis (caspase-1 dependent)	Adaptor protein ASC	Inflammasome component, pyroptosome component	[9]
	NOD-like receptors, absent in melanoma 2 (AIM2)	Inflammasome component	[9]
	Caspase-1	Proteolysis and release of interleukin-1β (IL-1 β) and IL-18, activation of caspase-7	[9]
Netosis (NADPH oxidase dependent death restricted to granulocytes)	NADPH oxidase	Superoxide generation	[9]
Mitochondrial permeability transition-driven cell death	CYPD	Inducer, component of the permeability transition pore complex (PTCP)	[8]
	ATP synthasome	Putative pore-forming unit of PTCP	[8]
Parthanatos	PARP1		[8]
	Apoptosis-inducing factor, mitochondrion-associated 1 (AIFM1)		[8]
Ferroptosis	Gluthatione peroxidase 4 (GPX4)		[8]

Autosis (“autophagic cell death”, can be modulated by targeting at least two components of the autophagic machinery)	Microtubule-associated protein 1 light chain 3 (MAP1LC3/LC3/Atg8)		[8,9]

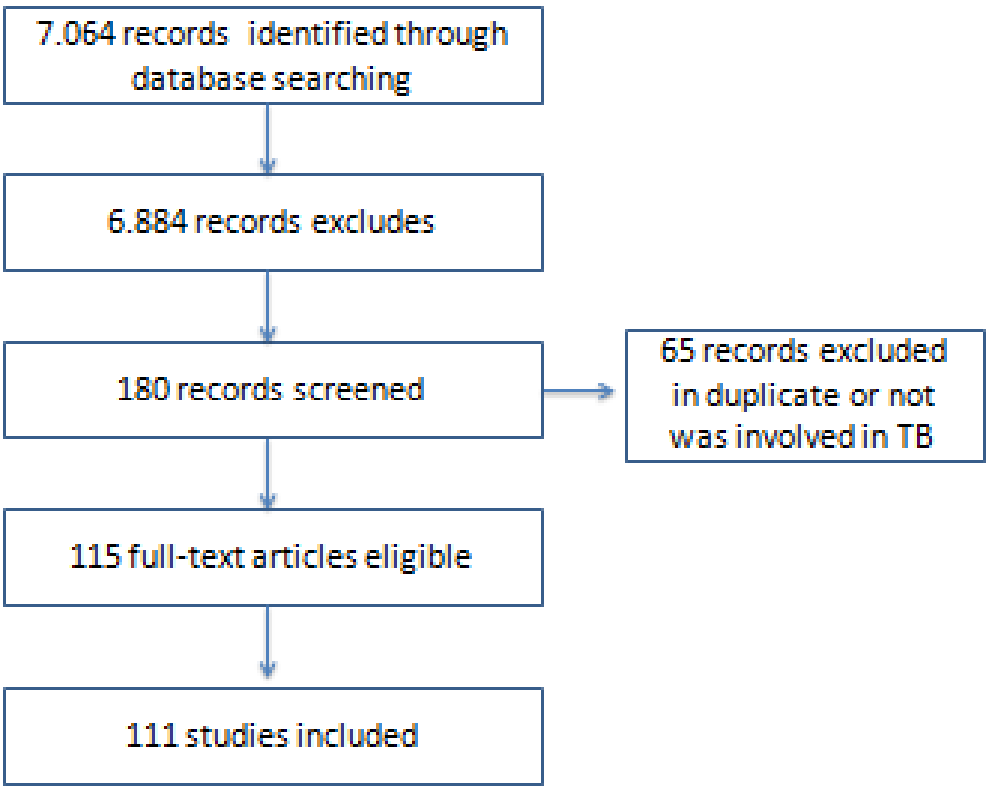

Figure 1: Flow of information through eligible criteria to systematic review [40]

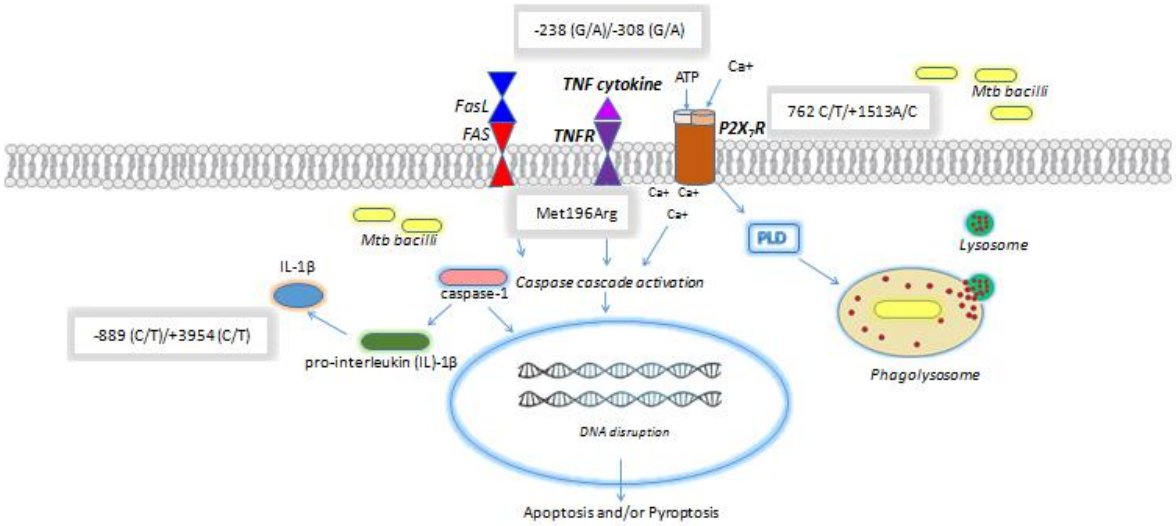

Figure 2: Genetic variation in regulated cell death that has been associated with susceptibility to tuberculosis. Susceptibility to tuberculosis was found to be associated to the extrinsic apoptotic pathways related with TNF and ATP signaling and with pyroptosis (bold characters). The TNF receptor is activated by TNF, promoting caspase activation and DNA disruption. Apoptosis induction through the purinergic receptor $P2X_7$ is gated by ATP. Activation of the $P2X_7$ receptor opens a cation-specific channel that alters the ionic environment of the cell, activating several pathways, as the caspase cascade and phospholipase D, stimulating phagosome-lysosome fusion to mycobacterial killing.

References

[1] Vynnycky E, Fine PE. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. Epidemiol Infect. 1997;119: 183–201.

[2] Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PEM, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis Off Publ Infect Dis Soc Am. 2014;58: 470–480.

[3] Aaby P, Benn CS. Saving lives by training innate immunity with bacille Calmette-Guerin vaccine. Proc Natl Acad Sci U S A. 2012;109: 17317–17318.

[4] Behar SM, Martin CJ, Nunes-Alves C, Divangahi M, Remold HG. Lipids, apoptosis, and cross-presentation: links in the chain of host defense against Mycobacterium tuberculosis. Microbes Infect Inst Pasteur. 2011;13: 749–756.

[5] Sia JK, Georgieva M, Rengarajan J. Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between Mycobacterium tuberculosis and Innate Immune Cells. J Immunol Res. 2015;2015: 747543.

[6] Divangahi M, Desjardins D, Nunes-Alves C, Remold HG, Behar SM. Eicosanoid pathways regulate adaptive immunity to Mycobacterium tuberculosis. Nat Immunol. 2010;11: 751–758.

[7] Amaral EP, Lasunskaia EB, D'Império-Lima MR. Innate immunity in tuberculosis: how the sensing of mycobacteria and tissue damage modulates macrophage death. Microbes Infect Inst Pasteur. 2016;18: 11–20.

[8] Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ. 2015;22: 58–73. doi:10.1038/cdd.2014.137.

[9] Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, et al. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ. 2012;19: 107

[10] Ashkenazi A, Salvesen G. Regulated cell death: signaling and mechanisms. Annu Rev Cell Dev Biol. 2014;30: 337–356. doi:10.1146/annurev-cellbio-100913-013226

[11] Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349:g7647.

[12] WANG Dejun, YANG Yuelin, XIA Qingjie, et al. (2005). On the Association of Tumor Necrosis Factor-α Gene Polymorphisms with the Susceptibility to Silicosis. J Sichuan Univ (Med Sci Ed) 36 (5), 679-682 (In Chinese).

[13] Yamamoto-Furusho JK, Uscanga LF, Vargas-Alarcón G, Rodríguez-Pérez JM, Zuñiga J, Granados J. Polymorphisms in the promoter region of tumor necrosis factor alpha (TNF-alpha) and the HLA-DRB1 locus in Mexican mestizo patients with ulcerative colitis. Immunol Lett. 2004 Aug 15;95(1):31-5.

[14] Secher T, Vasseur V, Poisson DM, Mitchell JA, Cunha FQ, Alves-Filho JC, Ryffel B. Crucial role of TNF receptors 1 and 2 in the control of polymicrobial sepsis. J Immunol. 2009 Jun 15; 182(12):7855-64.

[15] Barton A, John S, Ollier WER, Silman A, Worthington J. Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not tumor necrosis factor receptor I, in Caucasians. Arthritis Rheum, 2001.

[16] Aksentijevich I, Galon J, Soares M, Mansfield E, Hull K, Oh HH. The tumor-necrosis-factor receptor-associated periodic syndrome: new mutations in

TNFRSF1A, ancestral origins, genotype-phenotype studies, and evidence for further genetic heterogeneity of periodic fevers. Am J Hum Genet, 2001; 31

[17] Weinshenker BG, Hebrink D, Wingerchuk DM, Klein CJ, Atkinson E, O'Brien PC. Genetic variants in the tumor necrosis factor receptor 1 gene in patients with MS Neurology, 1999; 32

[18] Bazzoni F, Gatto L, Lenzi L, Vinante F, Pizzolo G, Zanolin E. Identification of novel polymorphisms in the human TNFR1 gene: distribution in acute leukemia patients and healthy individuals. Immunogenetics, 2000.

[19] Stark GL, Dickinson AM, Jackson GH, Taylor PR, Proctor SJ, Middleton PG. Tumour necrosis factor receptor type II 196M/R genotype correlates with circulating soluble receptor levels in normal subjects and with graft-versus-host disease after sibling allogeneic bone marrow transplantation. Transplantation. 2003 Dec 27; 76(12):1742-9.

[20] Glossop JR, Dawes PT, Nixon NB, Mattey DL. Polymorphism in the tumour necrosis factor receptor II gene is associated with circulating levels of soluble tumour necrosis factor receptors in rheumatoid arthritis. Arthritis Res Ther. 2005; 7(6):R1227-34.

[21] Till A, Rosenstiel P, Krippner-Heidenreich A, Mascheretti-Croucher S, Croucher PJ, Schäfer H, Scheurich P, Seegert D, Schreiber S. The Met-196 -> Arg variation of human tumor necrosis factor receptor 2 (TNFR2) affects TNF-alpha-induced apoptosis by impaired NF-kappaB signaling and target gene expression. J Biol Chem. 2005 Feb 18; 280(7):5994-6004.

[22] Komata T, Tsuchiya N, Matsushita M, Hagiwara K, Tokunaga K. Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus.Tissue Antigens. 1999 Jun; 53(6):527-33.

[23] Dieudé P, Petit E, Cailleau-Moindrault S, Osorio J, Pierlot C, Martinez M, Fauré S, Alibert O, Lasbleiz S, De Toma C, Bardin T, Prum B, Cornélis F. Association between tumor necrosis factor receptor II and familial, but not sporadic, rheumatoid arthritis: evidence for genetic heterogeneity. Arthritis Rheum. 2002 Aug; 46(8):2039-44.

[24] Waschke KA, Villani AC, Vermeire S, Dufresne L, Chen TC, Bitton A, Cohen A, Thomson AB, Wild GE. Tumor necrosis factor receptor gene polymorphisms in Crohn's disease: association with clinical phenotypes. Am J Gastroenterol. 2005 May; 100(5):1126-33.

[25] Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity. 1995 Dec; 3(6):673-82.

[26] Gray HL, Sorensen EL, Hunt JS, Ober C. Three polymorphisms in the 3' UTR of the TRAIL (TNF-related apoptosis-inducing ligand) gene. Genes Immun. 2001 Dec; 2(8):469-70.

[27] Yan X, Xu L, Qi J, Liang X, Ma C, Guo C, Zhang L, Sun W, Zhang J, Wei X, Gao L. sTRAIL levels and TRAIL gene polymorphisms in Chinese patients with fatty liver disease. Immunogenetics. 2009 Aug; 61(8):551-6.

[28] Jun Luo, Jinmeng Xiong, Jianghua Wu, and Xujun Ye. Genetic polymorphisms and haplotypes of TRAIL gene correlate with NSCLC susceptibility in a group of Chinese patients. Int J Clin Exp Med. 2015; 8(9): 16223–16230.

[29] Fisher MJ, Virmani AK, Wu L, Aplenc R, Harper JC, Powell SM, Rebbeck TR, Sidransky D, Gazdar AF, El-Deiry WS. Nucleotide substitution in the ectodomain of trail receptor DR4 is associated with lung cancer and head and neck cancer. Clin Cancer Res. 2001 Jun; 7(6):1688-97.

[30] Los M, Wesselborg S, Schulze-Osthoff K (1999) The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity 10: 629–639.

[31] Rashedi I, Panigrahi S, Ezzati P, Ghavami S, Los M (2007) Autoimmunity and Apoptosis – Therapeutic Implications. Curr Med Chem 14: 3139–3159.

[32] Los M, van de Craen M, Penning CL, Schenk H, Westendorp M, et al. (1995) Requirement of an ICE/Ced-3 protease for Fas/Apo-1-1mediated apoptosis. Nature 371: 81–83.

[33] Villa-Morales M, Fernandez-Piqueras J (2012) Targeting the Fas/FasL signalingnpathway in cancer therapy. Expert Opin Ther Targets.

[34] Huang QR, Morris D, Manolios N (1997) Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. Molecular immunology 34: 577–582.

[35] Huang QR, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. Mol Immunol 1997;34:577–82.

[36] Sibley K, Rollinson S, Allan JM, Smith AG, Law GR, Roddam PL, et al. Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. Cancer Res 2003;63:4327–30.

[37] Kanemitsu S, Ihara K, Saifddin A, Otsuka T, Takeuchi T, Nagayama J, et al. A functional polymorphism in Fas (CD95/APO-1) gene promoter associated with systemic lupus erythematosus. J Rheumatol 2002;29:1183–8.

[38] Takahashi T, Tanaka M, Inazawa J, Abe T, Suda T, et al. (1994) Human Fas ligand: gene structure, chromosomal location and species specificity. International immunology 6: 1567–1574.

[39] Wu J, Metz C, Xu X, Abe R, Gibson AW, et al. (2003) A novel polymorphic CAAT/enhancer-binding protein beta element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. J Immunol 170: 132–138.

[40] Xu Y, He B, Li R, Pan Y, Gao T, Deng Q, Sun H, Song G, Wang S. Association of the polymorphisms in the Fas/FasL promoter regions with cancer susceptibility: a systematic review and meta-analysis of 52 studies. PLoS One. 2014 Mar 5;9(3):e90090. doi: 10.1371/journal.pone.0090090.

[41] Yıldır S, Sezgin M, Barlas IÖ, Türköz G, Ankaralı HÇ, Şahin G, Erdal ME. Relation of the Fas and FasL gene polymorphisms with susceptibility to and severity of rheumatoid arthritis. Rheumatol Int. 2013 Oct;33(10):2637-45. doi: 10.1007/s00296-013-2793-1.

[42] Bergheanu SC, Pons D, van der Hoeven BL, Liem SS, Siegerink B, Schalij MJ, van der Bom JG, Jukema JW. The 5352 A allele of the pro-inflammatory caspase-1 gene predicts late-acquired stent malapposition in STEMI patients treated with sirolimus stents. Heart Vessels. 2011 May;26(3):235-41.

[43] Yan S, Li YZ, Zhu XW, Liu CL, Wang P, Liu YL. HuGE systematic review and meta-analysis demonstrate association of CASP-3 and CASP-7 genetic polymorphisms with cancer risk. Genet Mol Res. 2013 May 13;12(2):1561-73.

[44] Zhang ZY, Xuan Y, Jin XY, Tian X, Wu R. CASP-9 gene functional polymorphisms and cancer risk: a large-scale association study plus meta-analysis. Genet Mol Res. 2013 Feb 28;12(3):3070-8

[45] Yan S, Li YZ, Zhu JW, Liu CL, Wang P, Liu YL. Role of CASP-10 gene polymorphisms in cancer susceptibility: a HuGE review and meta-analysis. Genet Mol Res. 2012 Nov 26;11(4):3998-4007.

[46] Grenet J, Teitz T, Wei T, Valentine V, Kidd VJ. Structure and chromosome localization of the human CASP8 gene. Gene. 1999 Jan 21; 226(2):225-32.

[47] Palanca Suela S, Esteban Cardeñosa E, Barragán González E, de Juan Jiménez I, Chirivella González I, Segura Huerta A, Guillén Ponce C, Martínez de Dueñas E, Montalar Salcedo J, Castel Sánchez V, Bolufer Gilabert P, CASP8 D302H polymorphism delays the age of onset of breast cancer in BRCA1 and BRCA2 carriers.Breast Cancer Res Treat. 2010 Jan; 119(1):87-93.

[48] Lubahn J, Berndt SI, Jin CH, Klim A, Luly J, Wu WS, Isaacs S, Wiley K, Isaacs WB, Suarez BK, Hayes RB, Kibel AS. Association of CASP8 D302H polymorphism with reduced risk of aggressive prostate carcinoma. Prostate. 2010 May 1; 70(6):646-53.

[49] Sun T, Gao Y, Tan W, Ma S, Shi Y, Yao J, Guo Y, Yang M, Zhang X, Zhang Q, Zeng C, Lin D. A six-nucleotide insertion-deletion polymorphism in the CASP8 promoter is associated with susceptibility to multiple cancers. Nat Genet. 2007 May; 39(5):605-13.

[50] Buell GN, Talabot F, Gos A et al. Gene structure and chromosomal localization of the human P2X7 receptor. Receptor Channels 1998; 5:347–54.

[51] Bartlett R, Yerbury JJ, and Sluyter R (2013) P2X7 receptor activation induces reactive oxygen species formation and cell death in murine EOC13 microglia. Mediators Inflamm 2013:271813.

[52] Sluyter R, Shemon AN, Wiley JS. Glu^{496} to Ala polymorphism in the $P2X_7$ receptor impairs ATP-induced IL-1β release from human monocytes. J Immunol 2004; 172:3399–405.

[53] Cabrini G, Falzoni S, Forchap SL et al. A His-155 to Tyr polymorphism confers gain-of-function to the human $P2X_7$ receptor of human leukemic lymphocytes. J Immunol 2005; 175:82-89.

[54] Saunders BM, Fernando SL, Sluyter R, Britton WJ, Wiley JS. A lossof- function polymorphism in the human P2X7 receptor abolishes ATPmediated killing of mycobacteria. J Immunol 2003;171:5442–5446

[55] Gu BJ, Wiley JS. Rapid ATP-induced release of matrix metalloproteinase 9 is mediated by the P2X7 receptor. Blood 2006;107:4946–4953.

[56] Wiley JS, Dao-Ung LP, Li C, Shemon AN, Gu BJ, Smart ML, Fuller SJ, Barden JA, Petrou S, Sluyter R. An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human P2X7 receptor. J Biol Chem 2003;278:17108–17113.

[57] Gu BJ, Sluyter R, Skarratt KK, Shemon AN, Dao-Ung LP, Fuller SJ, Barden JA, Clarke AL, Petrou S, Wiley JS. An Arg307 to Gln polymorphism within the ATP-binding site causes loss of function of the human P2X7 receptor. J Biol Chem 2004;279:31287–31295.

[58] Skarratt KK, Fuller SJ, Sluyter R, Dao-Ung LP, Gu BJ, Wiley JS. A 5_intronic splice site polymorphism leads to a null allele of the P2X7 gene in 1–2% of the Caucasian population. FEBS Lett 2005;579:2675–2678.

[59] Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 2009; 7: 99–109.

[60] Shete, A. R., Joseph, R., Vijayan, N. N., Srinivas, L. & Banerjee, M. Association of single nucleotide gene polymorphism at interleukin-1beta +3954, -511, and -31 in chronic periodontitis and aggressive periodontitis in Dravidian ethnicity. J Periodontol. 81, 62–69 (2010).

[61] Divangahi M, et al. Mycobacterium tuberculosis evades macrophage defenses by inhibiting plasma membrane repair. Nat Immunol. 2009; 10:899–906.

[62] Winau F, et al. Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. Immunity. 2006; 24:105–117.

[63] Chen M, Gan H, Remold HG A mechanism of virulence: virulent Mycobacterium tuberculosis strain H37Rv, but not attenuated H37Ra, causes significant mitochondrial inner membrane disruption in macrophages leading to necrosis. J Immunol 2006; 176:3707–3716.

[64] Velmurugan, K. et al. Mycobacterium tuberculosis nuoG is a virulence gene that inhibits apoptosis of infected host cells. PLoS Pathog. 3, e110 (2007).

[65] Park,J.S., Tamayo,M.H., Gonzalez-Juarrero,M., Orme,I.M., and Ordway,D. J. (2006).Virulent clinical isolates of Mycobacterium tuberculosis grow rapidly and induce cellular necrosis but minimal apoptosis in murine macrophages. J. Leukoc.Biol. 79, 80–86.

[66] Butler RE, Brodin P, Jang J, Jang MS, Robertson BD, Gicquel B, Stewart GR. The balance of apoptotic and necrotic cell death in Mycobacterium tuberculosis infected macrophages is not dependent on bacterial virulence. PLoS One. 2012;7(10):e47573.

[67] Sohn H, Lee KS, Kim SY, Shin DM, Shin SJ, Jo EK, Park JK, Kim HJ. Induction of cell death in human macrophages by a highly virulent Korean Isolate of Mycobacterium tuberculosis and the virulent strain H37Rv. Scand J Immunol. 2009 Jan;69(1):43-50.

[68] Sly LM, Hingley-Wilson SM, Reiner NE, McMaster WR. Survival of Mycobacterium tuberculosis in host macrophages involves resistance to apoptosis

dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. J Immunol. 2003 Jan 1; 170(1):430-7.

[69] Zhang J, Jiang R, Takayama H, Tanaka Y. Survival of virulent Mycobacterium tuberculosis involves preventing apoptosis induced by Bcl-2 upregulation and release resulting from necrosis in J774 macrophages. Microbiol Immunol. 2005; 49(9):845-52.

[70] Sohn H, Lee KS, Kim SY, Shin DM, Shin SJ, Jo EK, Park JK, Kim HJ. Induction of cell death in human macrophages by a highly virulent Korean Isolate of Mycobacterium tuberculosis and the virulent strain H37Rv. Scand J Immunol. 2009 Jan; 69(1):43-50.

[71] Keane J, Remold HG, Kornfeld H. Virulent Mycobacterium tuberculosis strains evade apoptosis of infected alveolar macrophages. J Immunol. 2000 164: 2016–2020.

[72] Rakotosamimanana N, Doherty TM, Andriamihantasoa LH, Richard V, Gicquel B, Soares JL, Zumla A, Razanamparany VR. Expression of TNF-alpha-dependent apoptosis-related genes in the peripheral blood of malagasy subjects with tuberculosis. Plos One (2013) 8:61154

[73] [44] Sly LM, Hingley-Wilson SM, Reiner NE, McMaster WR. Survival of Mycobacterium tuberculosis in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. J Immunol. 2003 170:430–437.

[74] Mogga, S., T. Mustafa, L. Sviland, R. Nilsen. Increased Bcl-2 and reduced Bax expression in infected macrophages in slowly progressive primary murine Mycobacterium tuberculosis infection. Scand. J. Immunol. 2002 56: 383.

[75] Kundu M, Pathak SK, Kumawat K, Basu S, Chatterjee G, Pathak S, Noguchi T, Takeda K, Ichijo H, Thien CB, Langdon WY, Basu J. A TNF- and c-Cbl-dependent FLIP(S)-degradation pathway and its function in Mycobacterium tuberculosis-induced macrophage apoptosis.Nat Immunol. 2009 Aug; 10(8):918-26.

[76] Remijsen Q, Goossens V, Grootjans S, Van den Haute C, Vanlangenakker N, Dondelinger Y, Roelandt R, Bruggeman I, Goncalves A, Bertrand MJ, Baekelandt V, Takahashi N, Berghe TV, Vandenabeele P. Depletion of RIPK3 or MLKL blocks TNF-driven necroptosis and switches towards a delayed RIPK1 kinase-dependent apoptosis. Cell Death Dis. 2014 Jan 16; 5:e1004.

[77] Mishra BB, Moura-Alves P, Sonawane A, Hacohen N, Griffiths G, et al. Mycobacterium tuberculosis protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. Cell Microbiol. 2010 Aug;12(8):1046-63

[78] Welin A, Eklund D, Stendahl O, Lerm M. Human macrophages infected with a high burden of ESAT-6-expressing M. tuberculosis undergo caspase-1- and cathepsin B-independent necrosis. PLoS One. 2011;6(5):e20302.

[79] Danelishvili,L, Everman, J.L, Mcnamara, M.J, and Bermudez, L. E. Inhibition of the Plasma- Membrane-Associated Serine Protease Cathepsin G by Mycobacterium tuberculosis Rv3364c Suppresses Caspase-1 and Pyroptosis in Macrophages. Front.Microbiol. 2011; 2:281. doi: 10.3389/fmicb.2011.00281.

[80] Master SS, Rampini SK, Davis AS, Keller C, Ehlers S, Springer B, Timmins GS, Sander P, Deretic V. Mycobacterium tuberculosis prevents inflammasome activation. Cell Host Microbe. 2008 Apr 17;3(4):224-32.

[81] Santos AA Jr, Rodrigues-Junior V, Zanin RF, Borges TJ, Bonorino C, Coutinho-Silva R, Takyia CM, Santos DS, Campos MM, Morrone FB. Implication of purinergic

P2X7 receptor in M. tuberculosis infection and host interaction mechanisms: a mouse model study. Immunobiology. 2013 Aug;218(8):1104-12.

[82] Amaral EP, Ribeiro SC, Lanes VR, Almeida FM, de Andrade MR, Bomfim CC, Salles EM, Bortoluci KR, Coutinho-Silva R, Hirata MH, Alvarez JM, Lasunskaia EB, D'Império-Lima MR. Pulmonary infection with hypervirulent Mycobacteria reveals a crucial role for the P2X7 receptor in aggressive forms of tuberculosis. PLoS Pathog. 2014 Jul 3;10(7):e1004188.

[83] Placido R, Auricchio G, Falzoni S, Battistini L, Colizzi V, Brunetti E, Di Virgilio F, Mancino G: P2X(7) purinergic receptors and extracellular ATP mediate apoptosis of human monocytes/macrophages infected with Mycobacterium tuberculosis reducing the intracellular bacterial viability. Cell Immunol 2006;244:10-18.

[84] Yim JJ, Selvaraj P. Genetic susceptibility in tuberculosis. Respirology. 2010 Feb;15(2):241-56.

[85] Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Wiley JS, et al. (2005) Gene Dosage Determines the Negative Effects of Polymorphic Alleles of the P2X7 Receptor on Adenosine Triphosphate Mediated Killing of Mycobacteria by Human Macrophages. J Infect Dis 192: 149–155. doi: 10.1086/430622.

[86] Möller M, Flachsbart F, Till A, Thye T, Horstmann RD, Meyer CG, Osei I, van Helden PD, Hoal EG, Schreiber S, Nebel A, Franke A. A functional haplotype in the 3'untranslated region of TNFRSF1B is associated with tuberculosis in two African populations. Am J Respir Crit Care Med. 2010 Feb 15;181(4):388-93.

[87] Kusuhara K, Yamamoto K, Okada K, Mizuno Y, Hara T. Association of IL12RB1 polymorphisms with susceptibility to and severity of tuberculosis in Japanese: a gene-based association analysis of 21 candidate genes. Int J Immunogenet. 2007 Feb; 34(1):35-44.

[88] Budak F, Uzaslan EK, Cangür S, Göral G, Oral HB. Increased pleural soluble fas ligand (sFasL) levels in tuberculosis pleurisy and its relation with T-helper type 1 cytokines. Lung. 2008 Sep Oct;186(5):337-43. doi: 10.1007/s00408-008-9107-5. Epub 2008 Jul 29..

[89] El-Masry S, Lotfy M, Nasif WA, El-Kady IM, Al-Badrawy M. 18. Elevated serum level of interleukin (IL)-18, interferon (IFN)-gamma and soluble Fas in patients with pulmonary complications in tuberculosis. Acta Microbiol Immunol Hung. 2007 Mar;54(1):65-77.

[90] Singla N, Gupta D, Joshi A, Batra N, Singh J. Genetic polymorphisms in the P2X7 gene and its association with susceptibility to tuberculosis. Int J Tuberc Lung Dis. 2012 Feb;16(2):224-9.

[91] Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Assessment of the interleukin 1 gene cluster and other candidate gene polymorphisms in host susceptibility to tuberculosis. Tuberc Lung Dis 1998;79:83–9.

[92] Scola L, Crivello A, Marino V, Gioia V, Serauto A, Candore G, Colonna-Romano G, Caruso C, Lio D. IL-10 and TNF-alpha polymorphisms in a sample of Sicilian patients affected by tuberculosis: implication for ageing and life span expectancy. Mech Ageing Dev. 2003 Apr;124(4):569-72.

[93] Wang Q, Zhan P, Qiu LX, Qian Q, Yu LK. TNF-308 gene polymorphism and tuberculosis susceptibility: a meta-analysis involving 18 studies. Mol Biol Rep. 2012 Apr;39(4):3393-400. doi: 10.1007/s11033-011-1110-x.

[94] Ansari A, Hasan Z, Dawood G, Hussain R. Differential combination of cytokine and interferon- γ +874 T/A polymorphisms determines disease severity in pulmonary tuberculosis. PLoS One. 2011;6(11):e27848. doi: 10.1371/journal.pone.0027848.

[95] Sharma S, Rathored J, Ghosh B, Sharma SK. Genetic polymorphisms in TNF genes and tuberculosis in North Indians. BMC Infect Dis. 2010 Jun 10;10:165. doi: 10.1186/1471-2334-10-165.

[96] Zhang ZJ, Zhu H, Pu XD, Zhang F, Lei X, Zhou WJ, Wang Y. [Tumor necrosis factor alpha-238G/A polymorphism and tuberculosis susceptibility: a meta-analysis]. Zhonghua Jie He He Hu Xi Za Zhi. 2013 Jan;36(1):33-7.

[97] Correa PA, Gomez LM, Cadena J, Anaya JM: Autoimmunity and tuberculosis. Opposite association with TNF polymorphism. J Rheumatol 2005, 32:219-224.

[98] Correa PA, Gomez LM, Cadena J, Anaya JM: Autoimmunity and tuberculosis. Opposite association with TNF polymorphism. J Rheumatol 2005, 32:219-224.

[99] Muller BL, Ramalho DM, Santos PF, Mesquita ED, Kritski AL, Oliveira MM. Inflammatory and immunogenetic markers in correlation with pulmonary tuberculosis. J Bras Pneumol. 2013 Nov-Dec;39(6):719-27. doi: 10.1590/S1806-37132013000600011.

[100] Mokrousov I, Wu XR, Vyazovaya A, Feng WX, Sun L, Xiao J, Miao Q, Jiao WW, Shen A. Polymorphism of 3'UTR region of TNFR2 coding gene and its role in clinical tuberculosis in Han Chinese pediatric population. Infect Genet Evol. 2011 Aug;11(6):1312-8.

[101] Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Goldberg H, Marks GB, Wiley JS, Britton WJ: A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. Am J Respir Crit Care Med 2007;175:360-366.

[102] Mokrousov I, Sapozhnikova N, Narvskaya O. Mycobacterium tuberculosis co-existence with humans: making an imprint on the macrophage P2X(7) receptor gene? J Med Microbiol. 2008 May;57(Pt 5):581-4. doi: 10.1099/jmm.0.47455-0.

[103] Niño-Moreno P, Portales-Pérez D, Hernández-Castro B, Portales-Cervantes L, Flores-Meraz V, Baranda L, Gómez-Gómez A, Acuña-Alonzo V, Granados J, González-Amaro R. P2X7 and NRAMP1/SLC11 A1 gene polymorphisms in Mexican mestizo patients with pulmonary tuberculosis. Clin Exp Immunol. 2007 Jun;148(3):469-77.

[104] Sharma S, Kumar V, Khosla R, Kajal N, Sarin B, Sehajpal P. Association of P2X7 receptor +1513 (A>C) polymorphism with tuberculosis in a Punjabi population. Int J Tuberc Lung Dis. 2010 Sep;14(9):1159-63.

[105] Li CM, Campbell SJ, Kumararatne S et al. Association of a polymorphism in the $P2X_7$ gene with tuberculosis in a Gambian population. J Infect Dis 2002; 186:1458–62.

[106] Amirzargar AA, Rezaei N, Jabbari H, Danesh AA, Khosravi F, Hajabdolbaghi M, Yalda A, Nikbin B. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. Eur Cytokine Netw. 2006 Jun;17(2):84-9.

[107] Awomoyi AA, Charurat M, Marchant A, Miller EN, Blackwell JM, McAdam KP, Newport MJ. Polymorphism in IL1B: IL1B-511 association with tuberculosis and decreased lipopolysaccharide-induced IL-1beta in IFN-gamma primed ex-vivo whole blood assay. J Endotoxin Res. 2005;11(5):281-6.

[108] Meenakshi P, Ramya S, Shruthi T, Lavanya J, Mohammed HH, Mohammed SA, Vijayalakshmi V, Sumanlatha G. Association of IL-1β +3954 C/T and IL-10-1082 G/A cytokine gene polymorphisms with susceptibility to tuberculosis. Scand J Immunol. 2013 Jul;78(1):92-7. doi: 10.1111/sji.12055.

[109] Motsinger-Reif AA, Antas PR, Oki NO, Levy S, Holland SM, Sterling TR. Polymorphisms in IL-1beta, vitamin D receptor Fok1, and Toll-like receptor 2 are

associated with extrapulmonary tuberculosis. BMC Med Genet. 2010 Mar 2;11:37. doi: 10.1186/1471-2350-11-37.

[110] Gomez LM, Camargo JF, Castiblanco J, Ruiz-Narváez EA, Cadena J, Anaya JM. Analysis of IL1B, TAP1, TAP2 and IKBL polymorphisms on susceptibility to tuberculosis. Tissue Antigens. 2006 Apr;67(4):290-6.

[111] Huang QP, Liao N, Zhao H, Chen ML, Xie ZF. Lack of Association Between the IL1B (-511 and +3954), IL1RN VNTR Polymorphisms and Tuberculosis Risk: A Meta-analysis. Lung. 2015 Sep 2.

5.2 CAPÍTULO 2: Association of TNF-308 and DDX39B -22/-348 polymorphisms with disease extension and human immunodeficiency virus coinfection among patients with tuberculosis in a Brazilian population.

Elisabete L. Conceição[a,b], Michael Santos Rocha[b], Silas Gabriel Souza de Oliveira, Iza Cristina Araujo Pina[b], Ana Paula Torres[b], Carla Cunha de Souza[b], Carolina Neves Amoedo, Luiz Eduardo Lago de Castro[d], Marcio de Oliveira Silva[b,e], Vitor Ramos Mendonça[b], Jamocyr Moura Marinho[c,d], Theolis Barbosa[a,b]*

[a]Universidade Federal da Bahia - UFBA, Av. Reitor Miguel Calmon s/n , Vale do Canela , 40110-902, Salvador, Bahia, Brazil. [b]Centro de Pesquisas Gonçalo Moniz - CPqGM, FIOCRUZ, Rua Waldemar Falcão 121, Candeal, 40296-710, Salvador, Bahia, Brazil. [c]Escola Bahiana de Medicina e Saúde Pública (EBMSP), Rua Frei Henrique 08, Nazaré, 40050-420, Salvador, Bahia, Brazil. [d]Hospital Especializado Octávio Mangabeira, Praça Conselheiro João Alfredo, s/n, Pau Miúdo, 40320-350, Salvador, Bahia, Brazil. [e]Centro Especializado em Diagnóstico, Assistência e Pesquisa, R. Comendador José Alves Ferreira, 240, Garcia, 40100-160, Salvador, Bahia, Brazil.

*Correspondence to : Dr. Theolis Barbosa

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ). Rua Waldemar Falcão 121, Candeal, 40296-710 Salvador, Bahia, Brasil. Phone: +55 71 3176-2215; Fax +55 71 3176-2279, e-mail: theolis@bahia.fiocruz.br.

Introduction

Mycobacterium tuberculosis (*Mtb*) and human immunodeficiency virus (HIV)-1 infections are the leader killers worldwide [1]. Their wide geographic distribution facilitates the occurrence of co-infection, which is associated with up to 40-fold greater risk of active tuberculosis (TB) disease and death [2]. These pathogens synergize to dowmodulate the immune response by influencing several aspects of the host's innate and acquired immunity (reviewed in [3]).

The risk of progression in TB and HIV has been partly associated with the genetic background of the host [4-5]. Genetic variation in this context involves alterations in key immune mediators that regulate the immune system and influence outcomes in TB and HIV disease progression [6-7]. *Mtb*-infected alveolar macrophages produce increased levels of TNF, IL-1 and IL-6, leading to enhanced HIV replication and persistence within macrophages [8]. TNF has a critical role in the induction of apoptosis of infected cells. Some studies have shown that *Mtb*-mediated apoptosis of human alveolar macrophages (AM) is reduced in HIV+ subjects compared with healthy subjects in a TNF-dependent manner [9]. On the other hand, in vitro HIV infection of AM from healthy persons reduces both TNF release and AM apoptosis in response to irradiated *Mtb* [10] suggesting that the inhibition of the macrophage response to *Mtb* may contribute to disease pathogenesis in HIV+ individuals. The TNF gene is located within the major histocompatibility complex (MHC) region in the short arm of chromosome 6, between the human leukocyte antigen-B (HLA-B) and the HLA class III genes. Many allelic variants of the TNF gene have been described, of which two biallelic single nucleotide polymorphisms (SNPs) have been shown to influence TNF production in TB: -238G<A and -308G<A [11]. The *TNF*-308G>A polymorphism leads to increased TNF production due to augmented gene

transcription [11]. In TB patients with chronic disease the frequency of the -308A allele was significantly increased when compared with healthy subjects [12].

In the vicinity of the *TNF* gene, the *DDX39B* gene encodes a nuclear protein called HLA-B-associated transcript 1 (BAT1). The human *DDX39B* gene (D6S81E, UAP56) is a member of the DEAD ([Asp-Glu-Ala-Asp])-box family of ATP-dependent RNA helicases. DDX39B gene is situated in the central region of the major histocompatibilty complex (MHC) on the short arm of human chromosome 6 and is 40 kb telomeric to *TNF* [13]. Two polymorphisms in the promoter region of *DDX39B,* -22C/G and -348C/T, have been shown to affect the transcriptional activity and the binding of nuclear transcription factors at the start site of the *DDX39B* gene. The nuclear transcription factors are two proteins identified as YY1 (binding directly at -348) and Oct1 oligonucleotide (binding directly at -22) [14]. BAT1 downregulates the production of inflammatory cytokines such as TNF, IL-1 and IL-6, which suggests that it plays a protective role in several immune mediated disorders [15], but its role in TB and HIV infection has not been appreciated. IL-6 is produced in response to *Mtb* in the early phase of infection. Its levels are increased in plasma, sputum, and cell culture supernatant in active pulmonary tuberculosis patients and in macrophages treated with *M. bovis* Bacillus of Calmette-Guérin (BCG) [16-17]. IL-1 is critical for the host control of *Mtb* infection. It is an important mediator of innate immunity but can it also promote inflammatory tissue damage. *iNos*$^{-/-}$ animals infected with *Mtb* produce high amounts of mature IL-1β and show extensive tissue necrosis, neutrophil infiltration, and relatively high bacterial burdens [18]. Mice deficient in IL-1R1 infected with *Mtb* die shortly after infection with 2-log higher bacterial load in the lung and necrotic pneumonia as compared with wild type animals [19].

The present study describes the frequency of polymorphisms in *TNF*-308 and *DDX39B* -22/-348 in a TB endemic setting, and its association with cytokine production and clinical outcome in TB infected individuals with or without HIV co-infection.

Material & Methods

Study settings

The present study was carried out in Salvador, Bahia, Brazil from 2011 to 2014 in three reference centers: one hospital that is a reference for TB diagnosis and complicated TB treatment and two outpatient clinics that are reference for HIV infection diagnosis and clinical management.

Inclusion and exclusion criteria

Three groups of individuals were invited to participate of this study: 1) newly diagnosed pulmonary TB (**PTB**) patients with bacteriological confirmation by positive sputum smear and/or culture; 2) **TB-HIV** co-infected PTB patients (individuals newly diagnosed in the routine HIV infection sceening upon TB confirmation, or inpatients from the TB reference hospital, or outpatients under follow-up in the two reference clinics); 3) undergraduate students screened for tuberculin skin test positivity prior to engagement in voluntary or academic stage at the reference TB hospital, which presented a positive test (induration equal or above 10mm, **LTBI**). For all groups individuals from both sexes were eligible if aged 18-60 years. Exclusion criteria were HIV test refusal, indeterminate HIV test result, nonadherence to the present treatment for tuberculosis disease and / or acquired immunodeficience syndrome,

absence of amplified PCR product after 3 attempts of DNA extraction from 2 different aliquots.

Ethics statement

All the study protocols were reviewed and approved by the institutional review boards of the Centro de Pesquisas Gonçalo Moniz (FIOCRUZ), Hospital Especializado Octavio Mangebeira (reference TB hospital), Centro Estadual Especializado em Diagnóstico, Assistência e Pesquisa (outpatient reference clinic), and Secretaria Municipal de Saúde (for the Servico Municipal de Assistencia Especializada outpatient reference clinic of Salvador). Written informed consent was obtained from all the participants enrolled in the study. This study complies with the Brazilian current legislation (CNS Resolution 466/2012) and is registered with the CAAE 21823713.8.0000.0040.

Culture and cytokine production

Whole blood cultures were performed by diluting vacuum-collected heparinized blood 1:10 with RPMI 1640 (Gibco) supplemented with antibiotics streptomycin, with or without mycobacterial antigen (10 µg/ml of *Mycobacterium tuberculosis* H37Rv culture lysate, kindly provided by the Colorado State University, USA as part of NIH, NIAID Contract No. HHSN266200400091C, entitled "Tuberculosis Vaccine Testing and Research Materials", which was awarded to Colorado State University). The cultures were incubated for 72h at 37°C, 5% CO_2 in a humidified atmosphere. Culture supernatants were kept at -20°C until analysis. The cytokines (TNF, IL-6 and IL-1α/β) were measured in culture supernatants using multiplexed bead-based

immunoassays (BD Cytometer Bead Array™, Becton-Dickinson Bioscience, San Jose, CA).

Single nucleotide polymorphism genotyping

DNA was extracted from blood collected in EDTA vaccum tubes using a commercial kit Qiagen QIAamp DNA Blood Mini Kit (Valencia, CA, USA) according to the manufacturer's protocol. The DNA was quantified by spectrophotometry Nanodrop 2000 UV-Vis Spectophotomer and was stored at -20°C until further processing. Polymorphisms in the promoter region of *DDX39B* at the positions -22 (G>C; rs2239527; G ancestral allele) and -348 (C>T; rs2239528; C ancestral allele) were typed using PCR restriction fragment-length polymorphism analysis with the restriction enzyme *Nla*III (New England Biolabs) [22]. The polymorphism of *TNF*-308 (G>A; rs1800629; G ancestral allele) was evaluated using PCR restriction fragment-length polymorphism analysis with the restriction enzyme *Nco*I (New England Biolabs) [23].

Evaluation of disease extension

The extension of PTB was evaluated based on radiographic examination, smear grade, nadir HIV viral load and CD4+ cell counts, and HIV viral load / CD4+ cell counts in the last three months. Two experienced medical evaluators (one pneumologist and one radiologist) blindly reviewed the x-ray photographs independently, regarding the presence of cavitation in one or both lungs, in agreement with the Brazilian Ministry of Health guidelines for tuberculosis control [24]. The discordant cases were resolved by a third experienced evaluator (a pneumologist). The smear results obtained with the first sputum sample (or with the

second sputum sample if the first result was negative) were used to grade the smear positivity from 1 to 3, in agreement with the Brazilian Ministry of Health guidelines for tuberculosis control [24]. HIV viral load and CD4+ cell counts were obtained from medical records.

Statistical analyses

Categorical variables were described using absolute and relative frequency values. They were compared using the chi-square test (with Yates correction when applicable) and the Fisher's exact test (with the corresponding odds ratio, OR, and associated 95% confiance interval, 95% CI). Continuous variables were described using median and interquartile range (IQR), except for age that was described using mean and standard deviation. They were compared using the Wilcoxon test for unpaired data, the Mann-Whitney test for paired data and the Kruskal-Wallis ANOVA for three or more groups. Spearman regression was used to relate the cytokine production with the radiographic classification, the sputum smear grade, the HIV viral load and the CD4+ cell counts. The Hardy-Weinberg equilibrium (HWE) was assessed by comparing the observed genotypic frequencies with the expected frequencies under HWE estimated from the allelic frequencies.

The databanks were mounted in EpiData Entry (EpiData Association, Denmark), and the analyses were performed using GraphPad prism software version 6 (GraphPad Software, San Diego, CA, USA). The linkage disequilibrium was analyzed using Plink 1.9 beta program (available in: https://www.cog-genomics.org/plink2).

Results

Study sample

The study included 109 PTB, 60 TB-HIV and 74 LTBI volunteers (Table 1). The LTBI group had a significantly lower proportion of males, and was composed of younger individuals. The highest frequency of Bacille of Calmette-Guérin vaccination scar was found in the LTBI group, and BMI was above 18.5 for 96% of the individuals, in contrast with 68% in the PTB group and 61% in the TB-HIV group (P<0.0001).

Frequency of TNF *-308 (G>A) and* DDX39B *-22 (G>C), -348 (C>T) polymorphisms*

The genotypic and alellic frequencies of the *TNF -308* (G>A), *DDX39B* -22 (G>C) and *-348* (C>T) polymorphisms were compared among the studied groups (Table 2). LTBI volunteers exhibited increased frequency of the *TNF -308* GG genotype (post-hoc estimated power of 99.8% and 68.6 for the LTBI x PTB and LTBI x TB-HIV, respectively). The G allele frequency was also augmented among LTBI volunteers (post-hoc estimated power of 44.0% and 97.7% for the LTBI x PTB and LTBI x TB-HIV groups, respectively). *TNF -308* GG was the most frequent genotype found in LTBI and TB-HIV individuals, while the GA genotype was the most frequent in the PTB group. PTB volunteers exhibited increased frequency of *DDX39B*-22 GG genotype (post-hoc estimated power of 77.5% and 60.0% for the PTB x TB-HIV and PTB x LTBI groups, respectively). No differences in the allelic frequencies were observed. *DDX39B*-22CG genotype was the most frequent in all groups. The G allele was the most frequently observed in PTB, while the C allele was the most frequently observed in both TB-HIV and LTBI. PTB volunteers presented the highest frequencies of *DDX39B*-348 CC, but differences were only observed between PTB and TB-HIV volunteers (post-hoc estimated power of 68.3%). No differences in the

allelic frequencies were observed. The C allele was the most frequently observed in all groups.

Correlation between alleles at two or more genetic loci is referred to as linkage disequilibrium (LD). LD is generated as a consequence of a number of factors and results in the shared ancestry of a population of chromosomes at nearby loci [33]. The variants studied are not in linkage disequilibrium (Figure 1).

Dominant model and haplotype analyses

Considering a common dominant model, the odds of PTB against TB-HIV co-infection were increased among individuals bearing the *DDX39B*-348 recessive genotype (Table 3). The odds of active versus latent TB infection were decreased among individuals with the *TNF*-308 GG recessive genotype for both mono- and co-infected subjects. No association was found between genotype and odds of co-infection versus PTB disease or latent versus active TB for the *DDX39B* -22 locus.

As *DDX39B* and *TNF* are located in the same major histocompatibility complex (MHC) region on chromosome 6, we assessed the association of these polymorphisms with TB outcomes using haplotype analysis. The frequencies of haplotypes representing all possible combinations of *TNF-308* G>A, *DDX39B-22* G>C and *DDX39B*-348 C>T SNPs are shown in Table 4. The combinations *TNF*-308G/*DDX39B*-22C/*DDX39B*-348T and TNF-308A/*DDX39B*-22G/*DDX39B*-348C were more frequently found in TB-HIV and TB volunteers, respectively.

Cytokine production and its association with TNF *-308 (G>A) and* DDX39B *-22 (C>G), -348 (C>T) polymorphisms*

The levels of TNF, IL-6, IL-1α and IL-1β were measured in the culture supernatants of 42 PTB, 47 TB-HIV and 49 LTBI volunteers. PTB subjects produced higher levels of TNF (Figure 2A), and this association was evidenced among individuals bearing the *TNF*-308 GG genotype (Figure 3A). The genetic variants of *DDX39B* -22 and -348 were not associated with differential TNF production (Figures 3B and C, respectively). PTB volunteers also produced higher levels of IL-1β (Figure 2D), which was observed among individuals bearing the *DDX39B* -22 GG+GC genotype (Figure 3 H). When comparing PTB and LTBI volunteers, IL-1β was increased in PTB among the individuals bearing the *DDX39B*-348 CC genotype, while when comparing PTB and TB-HIV individuals this cytokine was elevated in PTB patients of the *DDX39B*-348 CT+TT genotype (Figure 3I). Among volunteers with the *DDX39B*-348 CC genotype it was also possible to observe higher production of IL-1β when comparing TB-HIV with LTBI (Figure 3I), which was not observed for the whole group (Figure 2D). The levels of IL-1α were increased in the cultures from PTB volunteers when compared with the TB-HIV group (Figure 2C), and this difference was associated with the *DDX39B*-22CC/-348CC genotype (Figures 3F and G).

Cytokine production and disease severity in PTB and TB-HIV patients

The levels of the cytokines TNF, IL-6, IL1-α and IL-1β were compared among PTB and TB-HIV patients with different degrees of disease severity, regarding the presence of cavitation and the number of bacilli in sputum smears, nadir HIV viral load and CD4+ cell counts, and HIV viral load and CD4+ cell counts in the last evaluation (within three months of blood collection). In the PTB group, no association was found between cytokine production and disease severity based on the presence of cavitation or smear grade (Figures 4 and 5 respectively). Regarding the TB-HIV

group, no association was found between cytokine levels and disease extension based on HIV viral load and CD4+ cells (data not shown).

Discussion

Several studies have examined the potential contribution of *TNF* promoter polymorphisms to TB and HIV susceptibility, but the findings of these studies are inconsistent [25-28]. TNF is a strong pro-inflammatory mediator and contributes both to the protection and immunopathology in tuberculosis. Increased levels of TNF were reported in cultures from TB patients with moderate and advanced disease [28] and in the serum [30-31] of TB patients compared with healthy controls, and TNF deficiency affects phagocyte activation, chemokine expression, cellular influx to the granuloma and apoptosis of infected cells [9,32-33]. The presence of the *TNF* -308A allele was associated with higher TNF gene transcription and increased production of TNF and may be associated with tissue destruction [11]. TNF -308A has been associated with increased risk of TB in patients affected by chronic lung tuberculosis and of HIV in long-term non-progressive patients [34-36]. The*TNF* -308G allele was associated with protection against HIV infection among children, suggesting that the G allele might be a marker of differential susceptibility to HIV transmission [7]. Our study indicated a significant association between *TNF* -308G with protection and TNF -308A with risk of active TB disease in our population. The odds of having a GA/AA genotype were increased 4.82 fold among PTB volunteers, and 2.71 among TB-HIV co-infected subjects, compared with the LTBI group. We found increased TNF production in the PTB group compared to TB-HIV and LTBI individuals, but the levels

of this cytokine were not related with disease severity parameters neither among PTB volunteers nor among TB-HIV co-infected subjects.

This is the first study undertaken to examine the potential influence of the DDX39B gene promoter polymorphisms -22C>G and -348C>T in the outcomes of PTB and TB-HIV. The *DDX39B* polymorphism has been associated with inflammatory disease and downregulation of proinflammatory cytokines [15, 51-52]. The *DDX39B* promoter polymorphisms alter the binding of transcription factors (Oct1 and YY1), and the sequences with -22G and -348 T alleles were expressed more efficiently than sequences containing -22C and-348C alleles [14]. In Chronic Chagas cardiomyopathy both *DDX39B* -22C and -348C were associated with disease development [22]. In malaria, the *DDX39B*-22G allele was associated with resistance against the disease, and conversely the the C allele was associated with increased risk of disease complications [23]. A strong association of TT+CT with Leprosy was observed in three cohort studies in India [53].

The *DDX39B* -22 GG genotype and the *DDX39B*-22 G allele were associated with PTB, while the -348 CC genotype was more frequent among PTB when compared with TB-HIV. The haplotype analysis revealed that the allelic combinations *TNF*-308G/*DDX39B*-22C/*DDX39B*-348T and *TNF*-308A/*DDX39B*-22G/*DDX39B*-348C are associated with PTB. The latter haplotype is related with higher production of proinflammatory cytokines?. *DDX39B* polymorphisms did not relate with TNF production, but the increased production of IL-1α and IL-1β among PTB individuals was dependent of the *DDX39B* genotype. The *DDX39B* -22 GG+GC genotype was associated with higher IL-1β production and the *DDX39B*-22CC/-348CC genotype was associated with higher IL-1 α production in this group. The relationship between the *DDX39B*-348 polymorphisms and the production of IL-1 β was less clear for the

PTB group, but the *DDX39B*-348 CC genotype was associated with higher IL-1β production among TB-HIV individuals.

IL-1 is critical for the host control of *Mtb* infection. Mice deficient in IL-1R or MyD88 adaptor succumb rapidly to low dose aerosol infection with *Mtb* [37-38]. In humans, a role for IL-1 signaling in host resistance to *Mtb* is supported by a number of genetic studies demonstrating an association of polymorphisms in the IL-1 or IL-1R genes with altered disease progression and susceptibility [39-40]. Some studies showed decreased IL-β levels in patients with AIDS compared to patients with AIDS and dementia [41-42]. High levels of TNF and IL-1β are associated with dementia in HIV patients causing neuronal apoptosis [43-44]. In our study IL-1 levels were not related with disease severity parameters.

None of the studied polymorphisms affected the IL-6 response, and its production was not different among the groups studied. This is a potent cytokine that is induced by *Mtb* but it is dependent of the nature of the challenge. High serum levels of IL-6 were observed in the serum from pulmonary tuberculosis patients compared with healthy controls [45-47]. In vitro, cells cotransfected with HIV-1 long terminal repeat sequence (LTR) and infected with *Mycobacterium smegmatis*, *Mycobacterium avium*, *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* showed silimar levels of IL-1β and IL-6 [48].

Our study was limited by the reduced sample size. We also experienced technical difficulties especially in the assessment of *DDX39B* polymorphisms.

Conclusion

The results of the present study indicate that polymorphisms in the *TNF* and *DDX39B* genes may be associated with susceptibility with PTB in our population. However, the genotypes studied were not related with HIV co-infection. Although the variants studied have influenced the TNF and IL-1 response, the disease extension was independent from cytokine production, suggesting that other factors may play a role in disease severity.

Acknowledgements

To Dr. José Rocha and Dra. Eliana Matos for contributing with the analysis of radiographies and to Ryan Santos Costa for collaborating with data analysis. To the HEOM, CEDAP and SEMAE team for logistics support for the recruitment of volunteers. Financial support was provided by Fundação de Amparo à Pesquisa do Estado da Bahia - FAPESB, Fiocruz and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

References

[1] World Health Organization. Global Tuberculosis Control 2011: WHO Report 2011.

[2] Toossi Z, Mayanja-Kizza H, Hirsch CS, Edmonds KL, Spahlinger T, Hom DL, Aung H, Mugyenyi P, Ellner JJ, Whalen CW. Impact of tuberculosis (TB) on HIV-1 activity in dually infected patients.Clin Exp Immunol. 2001 Feb; 123(2):233-8.

[3] Shankar EM, Vignesh R, Ellegård R, Barathan M, Chong YK, Bador MK, Rukumani DV, Sabet NS, Kamarulzaman A, Velu V, Larsson M. HIV-Mycobacterium tuberculosis co-infection: a 'danger-couple model' of disease pathogenesis. Pathog Dis. 2014 Mar;70(2):110-8.

[4] Lindenau JD, Guimarães LS, Friedrich DC, Hurtado AM, Hill KR, Salzano FM, Hutz MH. Cytokine gene polymorphisms are associated with susceptibility to tuberculosis in an Amerindian population. Int J Tuberc Lung Dis. 2014 Aug;18(8):952-7.

[5] S. Raghavan a, K. Alagarasu b, P. Selvaraj. Immunogenetics of HIV and HIV associated tuberculosis. Tuberculosis 92 (2012) 18e30.

[6] Mabunda N, Alvarado-Arnez LE, Vubil A, Mariamo A, Pacheco AG, Jani IV, Moraes MO. Gene polymorphisms in patients with pulmonary tuberculosis from Mozambique. Mol Biol Rep. 2015 Jan;42(1):71-6. doi: 10.1007/s11033-014-3741-1.

[7] Ahir S, Mania-Pramanik J, Chavan V, Kerkar S, Samant-Mavani P, Nanavati R, Mehta P. Genetic variation in the promoter region of pro-inflammatory cytokine TNF-α in perinatal HIV transmission from Mumbai, India. Cytokine. 2015 Mar;72(1):25-30.

[8] Kedzierska K, Crowe SM, Turville S, Cunningham AL. The influence of cytokines, chemokines and their receptors on HIV-1 replication in monocytes and macrophages. Rev Med Virol. 2003 Jan-Feb;13(1):39-56.

[9] Patel, N. R., K. Swan, X. Li, S. D. Tachado, and H. Koziel. 2009. Impaired M. tuberculosis-mediated apoptosis in alveolar macrophages from HIV^{+} persons: potential role of IL-10 and BCL-3. J. Leukoc. Biol. 86:53-60.

[10] Patel, N. R., et al. 2007. HIV impairs TNF-alpha mediated macrophage apoptotic response to Mycobacterium tuberculosis. J. Immunol. 179:6973-6980.

[11] Yamamoto-Furusho JK, Uscanga LF, Vargas-Alarcón G, Rodríguez-Pérez JM, Zuñiga J, Granados J. Polymorphisms in the promoter region of tumor necrosis factor alpha (TNF-alpha) and the HLA-DRB1 locus in Mexican mestizo patients with ulcerative colitis. Immunol Lett. 2004 Aug 15;95(1):31-5.

[12] Scola L, Crivello A, Marino V, Gioia V, Serauto A, Candore G, Colonna-Romano G, Caruso C, Lio D. IL-10 and TNF-alpha polymorphisms in a sample of Sicilian patients affected by tuberculosis: implication for ageing and life span expectancy. Mech Ageing Dev. 2003 Apr;124(4):569-72.

[13] Shafat Ali, Rupali Chopra, Shweta Aggarwal, Amit Kumar Srivastava, Ponnusamy Kalaiarasan, Dheeraj Malhotra, Sailesh Gochhait, Vijay K. Garg, S. N. Bhattacharya, Rameshwar N. K. Bamezai. Association of variants in BAT1-LTA-TNF-BTNL2 genes within 6p21.3 region show graded risk to leprosy in unrelated cohorts of Indian population. Hum Genet (2012) 131:703–716.

[14] Price P, Wong AM-L, Williamson D, Voon D, Baltic S, Allcock RJN, Boodhoo A,Christiansen FT: Polymorphisms at positions −22 and −348 in the promoter of the BAT1 gene affect transcription and the binding of nuclear factors. Hum Mol Genet 2004, 13:967–974.

[15] Allcock RJ, Williams JH, Price P. The central MHC gene, BAT1, may encode a protein that down-regulates cytokine production. Genes Cells. 2001 May;6(5):487-94.

[16] Zeng JC, Xiang WY, Lin DZ, Zhang JA, Liu GB, Kong B, Gao YC, Lu YB, Wu XJ, Yi LL, Zhong JX, Xu JF. Elevated HMGB1-related interleukin-6 is associated with dynamic responses of monocytes in patients with active pulmonary tuberculosis. Int J Clin Exp Pathol. 2015 Feb 1;8(2):1341-53.

[17] Sousa-Vasconcelos PD, Seguins WD, Luz ES, Pinho/ RT. Pattern of cytokine and chemokine production by THP-1 derived macrophages in response to live or heat-killed Mycobacterium bovis bacillus Calmette-Guérin Moreau strain. Mem Inst Oswaldo Cruz. 2015 Sep;110(6):809-813.

[18] Mishra BB, Rathinam VA, Martens GW, Martinot AJ, Kornfeld H, Fitzgerald KA, Sassetti CM. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. Nat Immunol. 2013 Jan;14(1):52-60. doi: 10.1038/ni.2474. Epub 2012 Nov 18

[19] Fremond CM, Togbe D, Doz E, Rose S, Vasseur V, Maillet I, Jacobs M, Ryffel B, Quesniaux VF. IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to Mycobacterium tuberculosis infection. J Immunol. 2007 Jul 15;179(2):1178-89.

[20] World Health Organization. Global Tuberculosis Report 2015. Available in http://www.who.int/tb/publications/global_report/en/. Accessed in November, 15, 2015.

[21] World Health Organization. Mortality and burden of disease. Available in: https://extranet.who.int/sree/Reports?op=Replet&name=/WHO_HQ_Reports/G2/PROD/EXT/TBCountryProfile&ISO2=BR&outtype=html. Accessed in November, 15, 2015.

[22] Ramasawmy R, Cunha-Neto E, Faé KC, Müller NG, Cavalcanti VL, Drigo SA, Ianni B, Mady C, Kalil J, Goldberg AC. BAT1, a putative anti-inflammatory gene, is associated with chronic Chagas cardiomyopathy. J Infect Dis 2006, 193:1394–1399.

[23] Vitor RR Mendonça, Ligia CL Souza, Gabriela C Garcia, Belisa ML Magalhães, Marcus VG Lacerda,Bruno B Andrade, Marilda S Gonçalves and Manoel Barral-Netto. DDX39B (BAT1), TNF and IL6 gene polymorphisms and association with clinical outcomes of patients with Plasmodium vivax malaria. Malaria Journal 2014, 13:278.

[24] Ministry of Health. Recommendations manual for TB control in Brazil. 2011. Availablehttp://www.cve.saude.sp.gov.br/htm/TB/mat_tec/manuais/MS11_Manual_Recom.pdfAccessed in January, 10, 2016.

[25] Veloso S, Olona M, García F, Domingo P, Alonso-Villaverde C, Broch M, Peraire J, Viladés C, Plana M, Pedrol E, López-Dupla M, Aguilar C, Gutiérrez M, Leon A, Tasias M, Gatell JM, Richart C, Vidal F. Effect of TNF-alpha genetic variants and CCR5 Delta 32 on the vulnerability to HIV-1 infection and disease progression in Caucasian Spaniards. BMC Med Genet. 2010 Apr 26;11:63.

[26] Deghaide NH, Rodrigues Mde L, Castelli EC, Mendes-Junior CT, Figueiredo JF, Donadi EA. Tumor necrosis factor region polymorphisms are associated with AIDS and with cytomegalovirus retinitis. AIDS. 2009 Aug 24;23(13):1641-7.

[27] Oh JH, Yang CS, Noh YK, Kweon YM, et al.. Polymorphisms of interleukin-10 and tumour necrosis factor-alpha genes are associated with newly diagnosed and recurrent pulmonary tuberculosis. Respirology 2007, 12: 594-598.

[28] Vejbaesya S, Chierakul N, Luangtrakool P and Sermduangprateep C. NRAMP1 and TNF-alpha polymorphisms and susceptibility to tuberculosis in Thais. Respirology. 2007; 12: 202-206.

[28] Dlugovitzky D, Bay ML, Rateni L, Fiorenza G, Vietti L, Farroni MA, Bottasso OA. Influence of disease severity on nitrite and cytokine production by peripheral blood mononuclear cells (PBMC) from patients with pulmonary tuberculosis (TB). Clin Exp Immunol. 2000 Dec;122(3):343-9.

[29] Kart L, Buyukoglan H, Tekin IO, Altin R, Senturk Z, Gulmez I, et al. Correlation of serum tumor necrosis factor-alpha, interleukin-4 and soluble interleukin-2 receptor levels with radiologic and clinical manifestations in active pulmonary tuberculosis. Mediators Inflamm. (2003) 12(1):9–14.

[30] Nakaya M, Yoneda T, Yoshikawa M, Tsukaguchi K, Tokuyama T, Fu A, et al. The evaluation of interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-alpha) level in peripheral blood of patients with active pulmonary tuberculosis. Kekkaku. (1995) 70(8):461–466.

[31] Kawaguchi H, Ina Y, Ito S, Sato S, Sugiura Y, Tomita H, et al. Serum levels of soluble tumor necrosis factor (TNF) receptors in patients with pulmonary tuberculosis. Kekkaku. (1996) 71(3):259–265.

[32] Roach DR, Bean AG, Demangel C, France MP, Briscoe H, Britton WJ. TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. J Immunol. 2002 May 1; 168(9):4620-7.

[33] Keane, J., H. G. Remold, H. Kornfeld. Virulent Mycobacterium tuberculosis strains evade apoptosis of infected alveolar macrophages. J. Immunol. 2000, 164: 2016-2020.

[34] Scola L, Crivello A, Marino V, Gioia V, et al. IL-10 and TNF-alpha polymorphisms in a sample of Sicilian patients affected by tuberculosis: implication for ageing and life span expectancy. Mech. Ageing Dev. 2003; 124: 569-572.

[35] Merza M, Farnia P, Anoosheh S, Varahram M, et al. (2009). The NRAMPI, VDR and TNF-alpha gene polymorphisms in Iranian tuberculosis patients: the study on host susceptibility. Braz. J. Infect. Dis.13: 252-256.

[36] Nasi M, Riva A, Borghi V, D'Amico R, Del Giovane C, Casoli C, Galli M, Vicenzi E, Gibellini L, De Biasi S, Clerici M, Mussini C, Cossarizza A, Pinti M. Novel genetic association of TNF-α-238 and PDCD1-7209 polymorphisms with long-term non-progressive HIV-1 infection. Int J Infect Dis. 2013 Oct;17(10):e845-50.

[37] Fremond CM, Togbe D, Doz E, Rose S, Vasseur V, Maillet I, Jacobs M, Ryffel B, Quesniaux VF. IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to Mycobacterium tuberculosis infection. J Immunol. 2007 Jul 15; 179(2):1178-89.

[38] Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A, Kugler D, Hieny S, Caspar P, Núñez G, Schlueter D, Flavell RA, Sutterwala FS, Sher A. Caspase-1 independent IL-1beta production is critical for host resistance to mycobacterium tuberculosis and does not require TLR signaling in vivo.J Immunol. 2010 Apr 1; 184(7):3326-30.

[39] Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Assessment of the interleukin 1 gene cluster and other candidate gene polymorphisms in host susceptibility to tuberculosis. Tuber Lung Dis. 1998; 79(2):83-9.

[40] Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. Wilkinson RJ, Patel P, Llewelyn M, Hirsch CS, Pasvol G, Snounou G, Davidson RN, Toossi Z J Exp Med. 1999 Jun 21; 189(12):1863-74.

[41] Zhao L, Pu SS, Gao WH, Chi YY, Wen HL, Wang ZY, Song YY, Yu XJ. Effects of HIV-1 tat on secretion of TNF-α and IL-1β by U87 cells in AIDS patients with or without AIDS dementia complex. Biomed Environ Sci. 2014 Feb;27(2):111-7.

[42] .Brabers NA and Nottet HS. Role of the pro-inflammatory cytokines TNF-α and IL-1β in HIVassociated dementia. Eur J Clin Invest, 2006;36,447-58.

[43] Yan YF, Wang ZY, Pu SS, Wen HL, Huang T, Song YY, Xu HZ, Zhao L. HIV-1B gp120 genes from one patient with AIDS dementia complex can affect the secretion of tumor necrosis factor and interleukin 1β in glial cells. Chin Med J (Engl). 2011 Dec;124(24):4217-22.

[44] Brabers NA, Nottet HS. Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia. Eur J Clin Invest. 2006 Jul;36(7):447-58.

[45] Tang S, Cui H, Yao L, Hao X, Shen Y, Fan L, et al. Increased cytokines response in patients with tuberculosis complicated with chronic obstructive pulmonary disease. PLoS One (2013) 8(4): 0062385.

[46] Ellertsen LK, Storla DG, Diep LM, Brokstad KA, Wiker HG, Hetland G. Allergic sensitisation in tuberculosis patients at the time of diagnosis and following chemotherapy. BMC Infect Dis. (2009) 9:100. doi:10.1186/1471-2334-9-100.

[47] Shu CC, Wu MF, Hsu CL, Huang CT, Wang JY, Hsieh SL, et al. Apoptosis-associated biomarkers in tuberculosis: promising for diagnosis and prognosis prediction. BMC Infect Dis. (2013) 13:45 2334 -13–45.

[48] Kitaura H, Ohara N, Kobayashi K, Yamada T. TNF-alpha-mediated activation of HIV-1 LTR in monocytoid cells by mycobacteria. FEMS Immunol Med Microbiol. 2001 Aug;31(2):97-103.

[49] Ben-Selma W, Harizi H and Boukadida J. Association of TNF-alpha and IL-10 polymorphisms with tuberculosis in Tunisian populations. Microbes Infect. 201113: 837-843.

[50] Bouma G, Crusius JB, Oudkerk Pool M, Kolkman JJ, von Blomberg BM, Kostense PJ, Giphart MJ, Schreuder GM, Meuwissen SG, Peña AS: Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. Scand J Immunol 1996, 43:456–463.

[51] Gnjec A, D'Costa KJ, Laws SM, Hedley R, Balakrishnan K, Taddei K, Martins G, Paton A, Verdile G, Gandy SE, Broe GA, Brooks WS, Bennett H, Piguet O, Price P, Miklossy J, Hallmayer J, McGeer PL, Martins RN: Association of alleles carried at TNFA −850 and BAT1–22 with Alzheimer's disease. J Neuroinflammation 2008, 5:36.

[52] Koch W, Hoppmann P, Michou E, Jung V, Pfeufer A, Mueller JC, Gieger C, Wichmann H-E, Meitinger T, Schömig A, Kastrati A: Association of variants in the BAT1-NFKBIL1-LTA genomic region with protection against myocardial infarction in Europeans. Hum Mol Genet 2007, 16:1821–1827.

[53] Quiñones-Lombraña A, Lopez-Soto A, Ballina-Garcia FJ, Alperi-López M, Queiro-Silva R, Lopez-Vazquez A, Lopez-Larrea C, Gonzalez S: BAT1 promoter polymorphism is associated with rheumatoid arthritis susceptibility. J Rheumatol 2008, 35:741–744.

[54] Fenhalls G, Wong A, Bezuidenhout J, van Helden P, Bardin P, Lukey PT. In situ production of gamma interferon, interleukin-4, and tumor necrosis factor alpha mRNA in human lung tuberculous granulomas. Infect Immun. 2000 May;68(5):2827-36.

[55] Fenhalls G, Stevens L, Bezuidenhout J, Amphlett GE, Duncan K, Bardin P, Lukey PT. Distribution of IFN-gamma, IL-4 and TNF-alpha protein and CD8 T cells

producing IL-12p40 mRNA in human lung tuberculous granulomas. Immunology. 2002 Mar;105(3):325-35.

[56] Nikitina IY, Kondratuk NA, Kosmiadi GA, Amansahedov RB, Vasilyeva IA, Ganusov VV, Lyadova IV. Mtb-specific CD27low CD4 T cells as markers of lung tissue destruction during pulmonary tuberculosis in humans. PLoS One. 2012;7(8):e43733. doi: 10.1371/journal.pone.0043733. Epub 2012 Aug 24.

[57] Soria A, Trabattoni D, Squillace N, Rainone V, Gnudi F, Clerici M, Gori A, Bandera A. Prospective immune dynamics during the first 24 weeks of efavirenz based-antiretroviral therapy in HIV-1-infected subjects, according to CD4+ T-cell counts at presentation: the

[58] González-Nicolás J, Resino S, Jiménez JL, Alvarez S, Fresno M, Muñoz-Fernández MA. Tumor necrosis factor-alpha and nitric oxide in vertically HIV-1-infected children: implications for pathogenesis. Eur Cytokine Netw. 2001 Jul-Sep;12(3):437-44.

[59] Day CL, Mkhwanazi N, Reddy S, Mncube Z, van der Stok M, Klenerman P, Walker BD. Detection of polyfunctional Mycobacterium tuberculosis-specific T cells and association with viral load in HIV-1-infected persons. J Infect Dis. 2008 Apr 1;197(7):990-9

Figure legends

Figure 1: Linkage disequilibrium between DDX39B -22 (rs2239527), DDX39B -348 (rs2239528) and TNF-308 (rs1800629)

Figure 2: Cytokine levels in culures from PTB, LTBI and TB-HIV groups

Figure 3: Cytokine levels in culures from PTB, LTBI and TB-HIV groups stratified by the *TNF*-308, *DDX39B* -22 and -348 genotypes.

Figure 4: Cytokine levels in PTB volunteers stratified by presence of cavitation.

Figure 5: Cytokine levels in PTB volunteers stratified by sputum smear grade.

Table 1 Clinical characteristics of the study groups.

Clinical characteristics	PTB (n=109)	TB-HIV (n=60)	LTBI (n=74)	p value
Age (mean±SD)	37.34±12.3	37.47±8.52	28.12±6.17	<0.0001*
Gender				
Male N (%)	76 (70)	44 (73)	19 (26)	<0.0001#
BCG				
Vaccinated N (%)	91 (83)	55 (92)	71 (96)	0.0221#
BMI (mean±SD)	19.93± 3.49	20.15±3.16	23.75±3.77	<0.0001*

*P value by chi-square analysis
P value by Kruskal-Wallis test

Table 2 Allelic and Genotypic frequencies in *TNF* and *DDX39B* among PTB, TB-HIV and LTBI

		PTB	TB-HIV	LTBI	PTB vs TB-HIV		PTB vs LTBI		TB-HIV vs LTBI	
		N (%)	N (%)	N (%)	χ^2	p value	χ^2	p value	χ^2	p value
TNF-308 (G > A)	**Genotypes**									
	GG	42 (42)	28 (56)	55 (77)	3.11	0.2104	24.08	<0.0001 ***	9.68	0.0079 **
	GA	54 (53)	21 (42)	12 (17)						
	AA	5 (5)	1 (2)	4 (6)						
	Alleles									
	G	138 (68)	77 (77)	122 (86)	2.45	0.1169	13.99	0.0002 ***	3.19	0.074
	A	64 (32)	23 (23)	20 (14)						
DDX39B -22 (C > G)	**Genotypes**									
	GG	18 (23)	1 (3)	1 (4)	6.29	0.043 *	6.06	0.0483 *	0.36	0.8347
	CG	46 (58)	21 (68)	15 (60)						
	CC	15 (19)	9 (29)	9 (36)						
	Alleles									
	G	82 (52)	17 (37)	16 (37)	3.18	0.0743	1.8	0.1788	0.08	0.7722
	C	76 (48)	29 (63)	24 (66)						
DDX39B -348 (C > T)	**Genotypes**									
	CC	57 (72)	14 (47)	11 (61)	9.54	0.0084 **	3.769, 2	0.1519	1.35	0.5082
	CT	16 (20)	15 (50)	7 (39)						
	TT	6 (8)	1 (3)	0						
	Alleles									
	C	130 (82)	35 (80)	18 (81)	0.17	0.6785	1.47	0.2247	0.5	0.4753
	T	28 (18)	9 (20)	7 (19)						

* P value <0,05

Table 3 Dominant model analysis in *TNF* and *DDX39B* among PTB, TB-HIV and LTBI

		PTB	TB-HIV	LTBI	PTB vs TB-HIV		PTB vs LTBI		TB-HIV vs LTBI	
		N	N	N	p value	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)
TNF-308 (G > A)	**Genotypes**									
	GG	42	28	55	0.1189	1.78 (0.9017-3.545)	P<0.0001*	4.82 (2.439 - 9.562)	0.0168*	2.701 (1.227 - 5.943)
	GA + AA	59	22	16						
DDX39B -22 (C > G)	**Genotypes**									
	CC	15	9	9	0.3061	1.745 (0.6696 - 4.550)	0.1026	2.400 (0.8902 - 6.470)	0.7741	1.375 (0.4457 - 4.242)
	CG +GG	64	22	16						
DDX39B -348 (C > T)	**Genotypes**									
	CC	57	14	11	0.0233*	0.3377 (0.1415 - 0.8061)	0.3979	0.6065 (0.2085 - 1.765)	0.3831	1.796 (0.5470 - 5.896)
	CT+ TT	22	16	7						

P value by Fisher's exact test
* P value <0,05

Table 4 Haplotype frequencies in *TNF* and *DDX39B* among PTB, TB-HIV and LTBI vs all groups

Haplotypes				
TNF -308 G>A	***DDX39B-22 G>C***	***DDX39B -348 C>T***	**n (%)**	**p value All groups**
G	G	C	87 (30)	0.2746
G	G	T	33 (9)	0.1710
G	C	C	92 (33)	0.2771
G	C	T	32 (9)	0.0443 *
A	G	C	44 (13)	0.0378 *
A	G	T	18 (5)	0.6500
A	C	C	49 (15)	0.2947
A	C	T	19 (18)	0.6141

P value by chi-square analysis
* P value <0,05

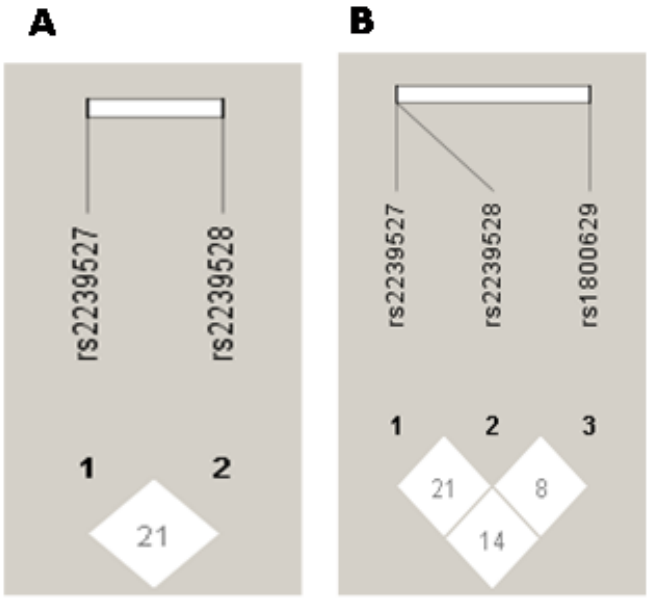

Figure 1: Linkage disequilibrium between DDX39B -22 (rs2239527), DDX39B -348 (rs2239528) and TNF-308 (rs1800629)

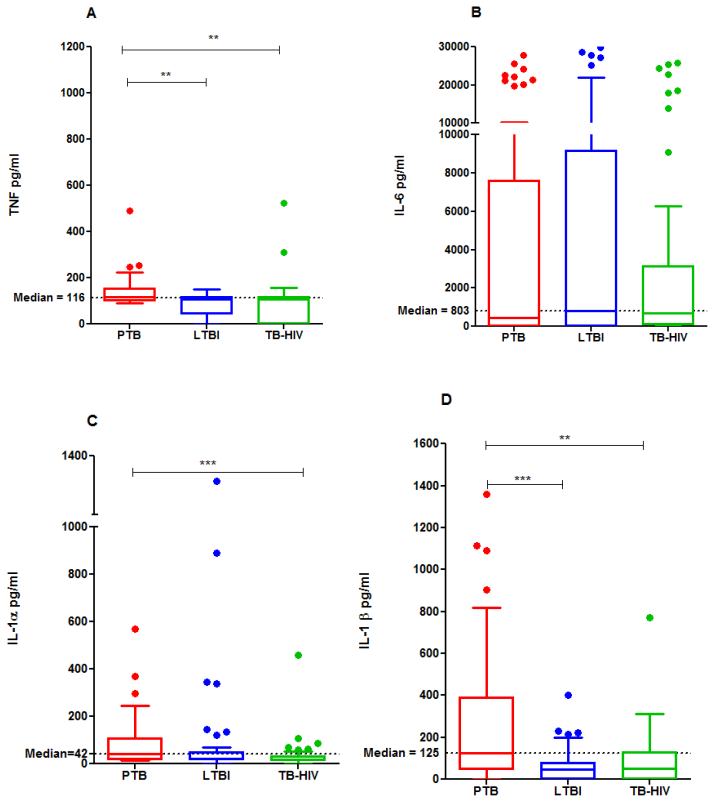

Figure 2: Cytokine levels among PTB, LTBI and TB-HIV groups. Levels of TNF, IL-6, IL-1α and IL-1β (2a, 2b, 2c,2d) The lines within the boxes represent the median, and the linoutside the boxes

represent the minimum and maximum values. PTB: Pulmonary Tuberculose; LTBI: Latente Tuberculsis Infection; TB-HIV: TB patients co-infected with HIV

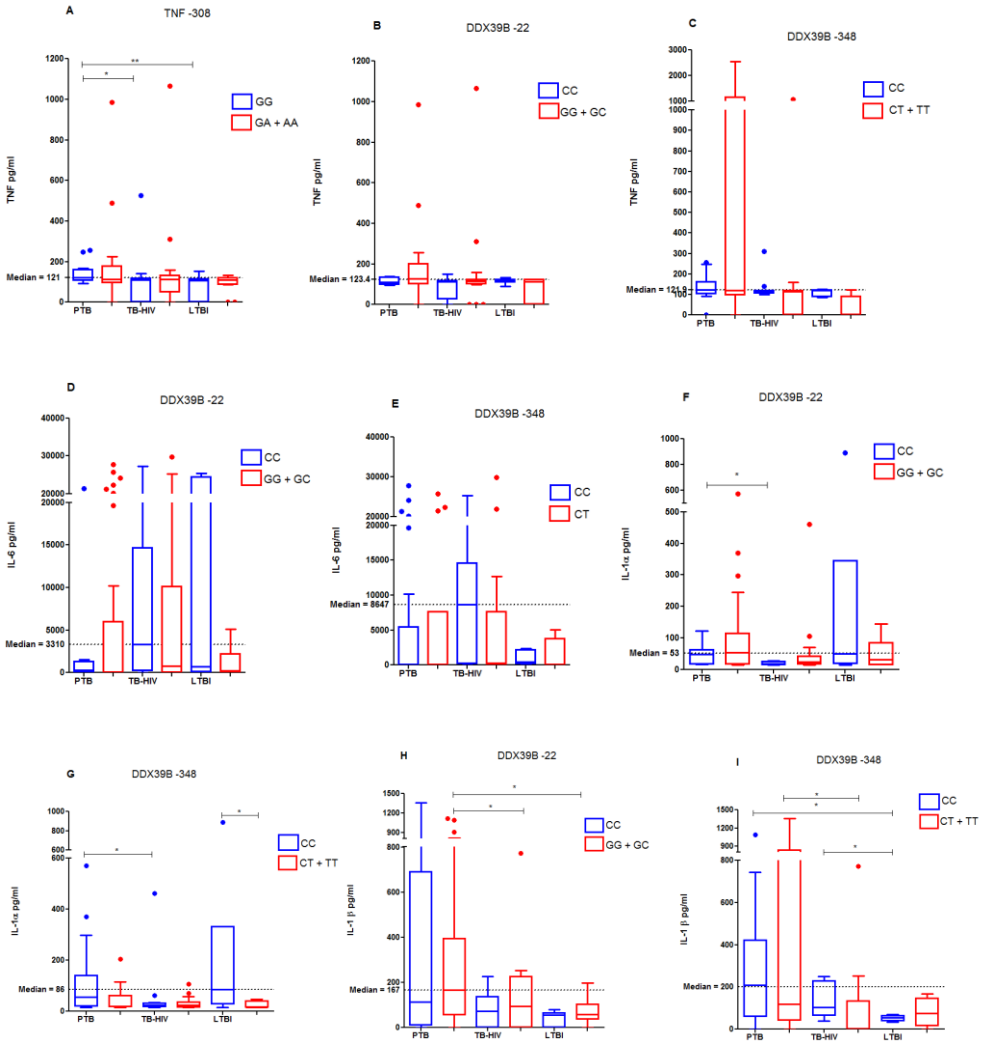

Figure 3: Cytokine levels stratified by genotype of *TNF*-308, *DDX39B* -22 and -348 polymorphisms among PTB, TB-HIV and LTBI groups. Levels of TNF, IL-6, IL-1α and IL-1β stratified by genotypes (3a-i) The lines within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values. PTB: Pulmonary Tuberculose; LTBI: Latente Tuberculsis Infection; TB-HIV: TB patients co-infected with HIV

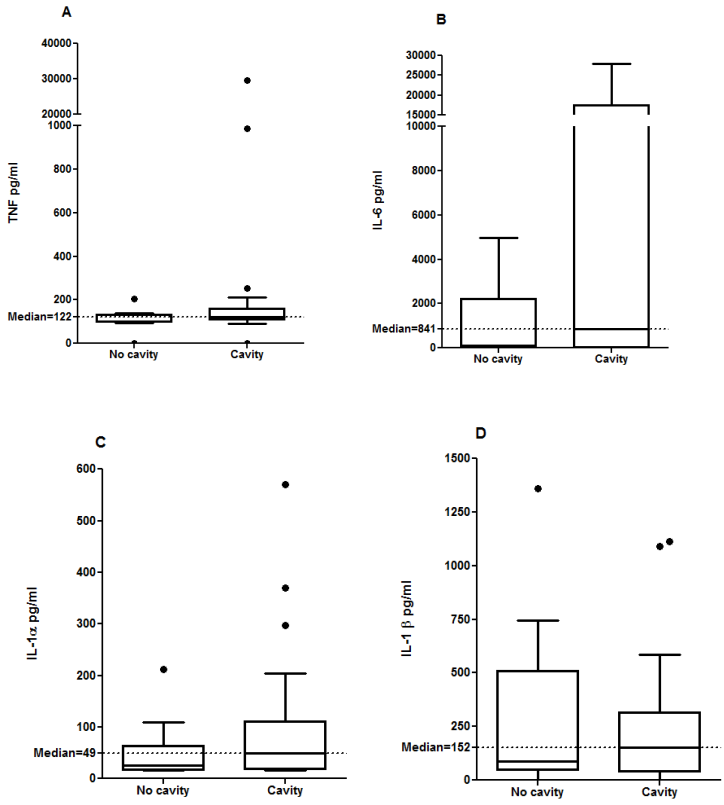

Figure 4: Cytokine levels and disease extension scored by radiographies classification among PTB patients. Levels of TNF, IL-6, IL-1α and IL-1β stratified by genotypes (4a,4b,4c,4d) The lines within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values.

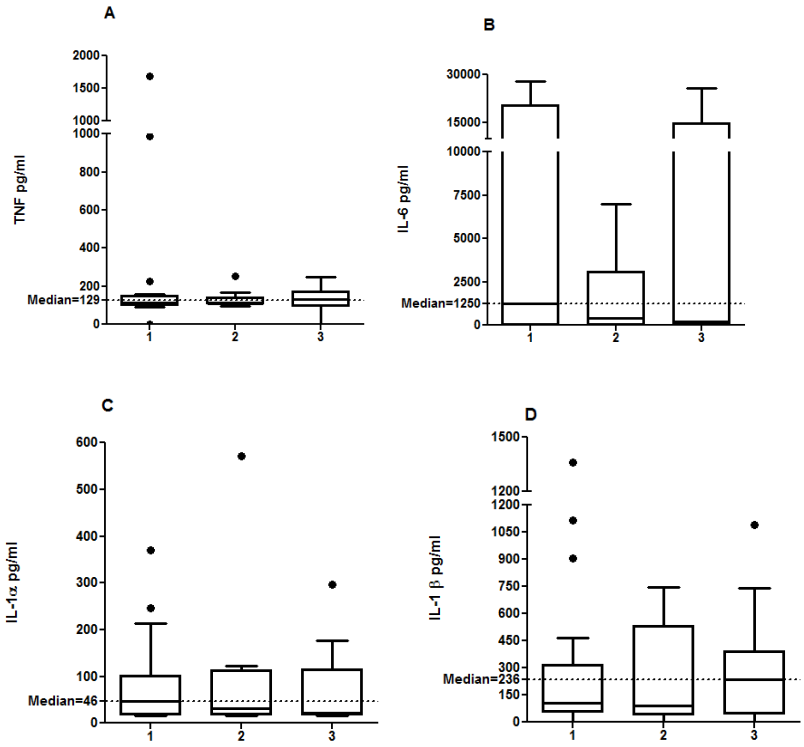

Figure 5: Cytokine levels and disease extension scored by classification smear among PTB patients. Levels of TNF, IL-6, IL-1βand IL-1α stratified by genotypes (5a,5b,5c,5d) The lines within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values. The numbers represent:1-one +, 2-two ++ and 3-three +++

5.3 CAPÍTULO 3: Heme oxygenase-1 regulation of MMP-1 underlies distinct disease profiles in tuberculosis

6. DISCUSSÃO

Dentre os mecanismos da resposta imune inata, a morte celular programada tem sido destaque nos estudos que demonstraram o papel deste mecanismo na sobrevivência do bacilo e no desenvolvimento da resposta imune adaptativa [96]. O *Mtb* infecta macrófagos e consegue evadir deste mecanismo de morte [97]. A apoptose pode ser regulada por diferentes vias e SNPs em genes que regulam a expressão de moléculas envolvidas na morte celular podem influenciar o desenvolvimento da resposta protetora contra o *Mtb* [98-99]. No estudo de revisão sistemática, conseguimos identificar quatro genes envolvidos nas vias de morte celular associados com o desfecho da tuberculose, nomeadamente TNF, TNFR, P2X7R e IL-1β. Entretanto, os estudos associando o polimorfismo no gene do TNF com TB em diferentes populações são controversos [100-101].

Na nossa população, encontramos associação entre o genótipo *TNF*-308 GG com proteção contra a TB, pois houve maior frequência do genótipo GG e do alelo G entre os indivíduos com infecção latente. Foi observada maior produção de TNF entre os pacientes com PTB portadores do genótipo *TNF*-308 GG. Um estudo anterior no Brasil investigou a posição -238 do gene do TNF e não encontrou associação do polimorfismo com a doença [102].

O nosso estudo foi o primeiro a investigar a associação de polimorfismos no gene *DDX39B* com a TB e coinfecção TB-HIV. Polimorfismos nas posições *DDX39B* -22 e -348 alteram a expressão de citocinas importantes na imunopatogênese da TB, como TNF, IL-1 e IL-6 [103]. Polimorfimos no gene DDX39B alteram a ligação de fatores de transcrição e consequentemente, alteram a expressão de genes. Os alelos *DDX39B* -22G e -348 T expressam maior eficiência na ligação aos fatores de transcrição, sugerindo maior expressão de citocinas inflamatórias [104]. Nosso

estudo revelou maior frequência dos genótipos ancestrais *DDX39B* -22GG e *DDX39B* -348CC entre os pacientes com PTB. Nos indivíduos coinfectados foi observado que o genótipo heterozigoto foi o mais frequente. A produção de IL-α e IL-1β foi influenciada pelos polimorfismos no gene *DDX39B*. Nosso estudo também revelou que a produção de citocinas inflamatórias in vitro não se correlacionou com a gravidade da doença.

Ao avaliar a possível interação entre os biomarcadores HO-1 e MMP-1 com TB em duas coortes diferentes, Brasil e India, observamos que pacientes com TB ativa possuem níveis elevados de HO-1 e MMP1- no plasma comparados com indivíduos com LTBI. O estudo também revelou que existe uma correlação negativa entre os biomarcadores avaliados no plasma destes pacientes, apresentando um perfil de HO-1 ($HO\text{-}1^{hi}MMP\text{-}1^{lo}$) ou de MMP-1 ($HO\text{-}1^{lo}MMP\text{-}1^{hi}$) com dicotomia. Estes mediadores também estão relacionados com a evolução da doença durante o tratamento. SNPs que impactam a expressão de HO-1 e MMP-1 já foram demonstrados em diferentes condições [105-112]. A heterogeneidade genética para essas enzimas pode explicar a dicotomia existente na produção destes marcadores nas duas diferentes populações de pacientes com TB. Por outro lado, será importante avaliar a relação entre a produção das citocinas estudadas e os níveis destas enzimas no soro dos pacientes para entender sua possível relação com a progressão da doença.

7. CONCLUSÕES

O presente trabalho revelou que polimorfismos nos genes *TNF, TNFR, P2X7R* e *IL-1β* (moléculas envolvidas em vias de morte celular programada) podem contribuir para o desfecho clínico da tuberculose. Observamos em nossa população que polimorfimos nos genes *TNF*-308G>A, *DDX39B* -22 G>C e -348 C>T podem estar associados com a tuberculose pulmonar ativa, porém não parecem contribuir para a resposta contra o HIV. Estudos futuros são necessários na investigação de genes de ancestralidade para confirmar se a ocorrência dos genótipos está associada com susceptibilidade à infecção pelo *Mtb.*

8. REFERÊNCIAS BIBLIOGRÁFICAS

[1] Reuters. Tuberculosis now rivals AIDS as leading cause of death: WHO Acessado em 04/10/2015.

[2] Ministério da Saúde. Portal da Saúde SUS. 2015 http://portalsaude.saude.gov.br/index.php?option=com_content&view=article&id=11045&Itemid=674. Acessado em 04/10/2015

[3] Ministério da Saúde. Boletim Epidemiológico da Secrataria de de Vigilância em Saúde. Volume 46 (2014). http://funed.mg.gov.br/wp content/uploads/2014/06/Boletim_Tuberculose_PNCT_2014.pdf. Acessado em 04/10/2015.

[4] Diedrich, C. R. Flynn, J. L. HIV-1/Mycobacterium tuberculosis Coinfection Immunology: How Does HIV-1 Exacerbate Tuberculosis? Infection and Immunity; 2011; 79 4 1407-1417.

[5] Hoshino Y, Nakata K, Hoshino S, Honda Y, Tse DB, Shioda T, Rom WN, Weiden M. Maximal HIV-1 replication in alveolar macrophages during tuberculosis requires both lymphocyte contact and cytokines. J Exp Med. 2002 Feb 18; 195(4):495-505.

[6] Patel NR, Koziel H. Lung defenses in the immunosuppressed patient. In: Agusti C, Torres A, editors. Pulmonary infection in the immunocompromised patient. Oxford: Wiley-Blackwell; 2009.

[7] Sly, L. M., S. M. Hingley-Wilson, N. E. Reiner, W. R. McMaster. Survival of Mycobacterium tuberculosis in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. J. Immunol. 2003.

[8] Kornfeld, H., G. Mancino, V. Colizzi. The role of macrophage cell death in tuberculosis. Cell Death Differ 1999.. 6: 71-78.

[9] Fauci, A. S. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science 1988 239: 617-622.

[10] Balcewicz-Sablinska, M. K., J. Keane, H. Kornfeld, H. G. Remold. Pathogenic Mycobacterium tuberculosis evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-α. J. Immunol. 1998 161: 2636-2641.

[11] Patel NR, Zhu J, Tachado SD, Zhang J, Wan Z, Saukkonen J, Koziel H. HIV impairs TNF-alpha mediated macrophage apoptotic response to Mycobacterium tuberculosis. J Immunol. 2007 Nov 15;179(10):6973-80.

[12] Yıldır S, Sezgin M, Barlas IÖ, Türköz G, Ankaralı HÇ, Şahin G, Erdal ME. Relation of the Fas and FasL gene polymorphisms with susceptibility to and severity of rheumatoid arthritis. Rheumatol Int. 2013 Oct;33(10):2637-45. doi: 10.1007/s00296-013-2793-1.

[13] Bahari G, Hashemi M, Taheri M, Naderi M, Moazeni-Roodi A, Kouhpayeh HR, Eskandari-Nasab E. Association of P2X7 gene polymorphisms with susceptibility to

pulmonary tuberculosis in Zahedan, Southeast Iran. Genet Mol Res. 2013 Jan 24;12(1):160-6. doi: 10.4238/2013.

[14] World Health Organization. Tuberculosis fact sheet. In: Geneva (Switzerland): WHO global TB programme; 2015 Oct [Internet] [cited 2015 Nov 2]; [about 5 screens]. Available from: http://www.who.int/mediacentre/ factsheets/fs104/en/.

[15] Goldman L, Schafer AI. Tuberculosis: disease overview. In: Goldman L, Schafer AI, editors. Goldman's cecil medicine: expert consult premium edition. 24th ed. St. Louis (MO): Saunders Elsevier; 2011.

[16] Russell DG. Who puts the tubercle in tuberculosis? Nat Rev Microbiol 2007;5:39–47.

[17] Mukhopadhyay S, Pluddemann A, Gordon S. Macrophage pattern recognition receptors in immunity, homeostasis and self
tolerance. Adv Exp Med Biol 2009;653:1–14.

[18] Pompei L, et al. Disparity in IL-12 release in dendritic cells and macrophages in response to Mycobacterium tuberculosis is due to use of distinct TLRs. J Immunol 2007;178:5192– 5199.

[19] Cruz-Knight W, Blake-Gumbs L. Tuberculosis: an overview. Prim Care 2013;40(3):743e56.

[20] Winau, F., Weber, S., Sad, S., De Diego, J., Hoops, S. L., Breiden, B., et al. (2006). Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. Immunity 24, 105–117.

[21] Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 2004;119:753–766.

[22] Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science 2006;313:1438–1441.

[23] Velmurugan K, et al. Mycobacterium tuberculosis nuo G is a virulence gene that inhibits apoptosis of infected host cells. PLoS Pathog 2007;3:e110.

[24] Divangahi M, et al. Mycobacterium tuberculosis evades macrophage defenses by inhibiting plasma membrane repair. Nat Immunol 2009;10:899–906.

[25] Barry DP, Beaman BL. Modulation of eukaryotic cell apoptosis by members of the bacterial order Actinomycetales. Apoptosis 2006;11:1695–1707.

[26] Schaible UE, et al. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. Nat Med 2003;9:1039–1046.

[27] Ulrichs T, Kaufmann SH. New insights into the function of granulomas in human tuberculosis. J Pathol 2006;208:261–269.

[28] Cole, S. T. et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 1998 393, 537–544.

[29] Russell, D. G., Cardona, P. J., Kim, M. J., Allain, S. & Altare, F. Foamy macrophages and the progression of the human tuberculosis granuloma. Nature immunology 2009 10, 943–948.

[30] Singh, V. et al. Mycobacterium tuberculosis-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. Cell host & microbe 2012 12, 669–68.

[31] Peyron, P. et al. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. PLoS pathogens 2008 4, e1000204.

[32] Hossain MM, Norazmi MN. Pattern recognition receptors and cytokines in Mycobacterium tuberculosis infection-the double-edged sword? Biomed Res Int 2013;2013:179174

[33] de Waal Malefyt, R., Yssel, H., and De Vries, J. E. (1993). Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. J. Immunol. 150, 4754–4765.

[34] Chiu, B. C., Stolberg, V. R., and Chensue, S. W. (2008). Mononuclear phagocyte-derived IL-10 suppresses the innate IL-12/IFN-gamma axis in lung-challenged aged mice. J. Immunol. 181, 3156–3166.

[35] Chiu, B. C., Stolberg, V. R., Freeman, C. M., and Chensue, S. W. (2007). Mononuclear phagocyte-derived interleukin-10 suppresses the innate pulmonary granuloma cytokine response in aged mice. Am. J. Pathol. 171, 829–837.

[36] Davis, J. M., and Ramakrishnan, L. (2009). The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136, 37–49.

[37] Peyron, P., Vaubourgeix, J., Poquet, Y., Levillain, F., Botanch, C., Bardou, F., et al. (2008). Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. PLoS Pathog. 4:e1000204. doi: 10.1371/journal.ppat.1000204.

[38] Cardona,P.J. Adynamic reinfection hypothesis of latent tuberculosis infection. Infection .2009.37, 80–86.

[39] Glynn, J.R.,Murray,J.,Bester,A., Nelson,G.,Shearer,S.,andSonnen- berg,P.(2008).Effectsofdurationof HIV infectionandsecondarytuber- culosis transmissionontuberculosis incidenceintheSouthAfricangold mines.
AIDS 22, 1859–1867.

[40] Madebo T, Lindtjorn B, Aukrust P, and Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. Am J Clin Nutr. 2003;78(1):117-22.

[41] Palanisamy GS, Kirk NM, Ackart DF, Shanley CA, Orme IM, and Basaraba RJ. Evidence for oxidative stress and defective antioxidant response in guinea pigs with tuberculosis. PLoS One. 2011;6(10):e26254.

[42] Madebo T, Lindtjørn B, Aukrust P, Berge RK Am J. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. Clin Nutr. 2003 Jul; 78(1):117-22.

[43] Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proc Natl Acad Sci U S A. 1968 Oct; 61(2):748-55.

[44] Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol. Rev. 2006; 86:583–650.

[45] Ryter S, Otterbein LE, Morse D, Choi AM. Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. Mol. Cell. Biochem. 2002; 234–235:249–263.

[46] Dennery PA, Visner G, Weng YH, Nguyen X, Lu F, Zander D, Yang G. Resistance to hyperoxia with heme oxygenase-1 disruption: role of iron. Free Radic. Biol. Med. 2003; 34:124–133.

[47] Gozzelino R, Jeney V, Soares MP (2010) Mechanisms of cell protection by heme oxygenase-1. Annu Rev Pharmacol Toxicol 50: 323–354.

[48] Petrache I, Otterbein LE, Alam J, Wiegand GW, Choi AM. Heme oxygenase-1 inhibits TNFalpha-induced apoptosis in cultured fibroblasts. Am. J. Physiol. Lung Cell Mol. Physiol. 2000; 278:L312–L319.

[49] Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares MP. Carbon monoxide generated by heme oxygenase-1 suppresses endothelial cell apoptosis. J. Exp. Med. 2000; 192:1015–1026.

[50] Liu XM, Chapman GB, Peyton KJ, Schafer AI, Durante W. Antiapoptotic action of carbon monoxide on cultured vascular smooth muscle cells. Exp. Biol. Med. (Maywood). 2003; 228:572–575.

[51] Liu XM, Chapman GB, Peyton KJ, Schafer AI, Durante W. Carbon monoxide inhibits apoptosis in vascular smooth muscle cells. Cardiovasc. Res. 2002; 55:396–405.

[52] Andrade BB, Pavan Kumar N, Amaral EP, Riteau N, Mayer-Barber KD, Tosh KW, Maier N, Conceição EL, Kubler A, Sridhar R, Banurekha VV, Jawahar MS, Barbosa T, Manganiello VC, Moss J, Fontana JR, Marciano BE, Sampaio EP, Olivier KN, Holland SM, Jackson SH, Moayeri M, Leppla S, Sereti I, Barber DL, Nutman TB, Babu S, Sher A. Heme Oxygenase-1 Regulation of Matrix Metalloproteinase-1 Expression Underlies Distinct Disease Profiles in Tuberculosis. J Immunol. 2015 Sep 15;195(6):2763-73.

[53] Yet SF, Tian R, Layne MD, Wang ZY, Maemura K, Solovyeva M, Ith B, Melo LG, Zhang L, Ingwall JS, Dzau VJ, Lee ME, Perrella MA. Cardiac-specific expression of heme oxygenase-1 protects against ischemia and reperfusion injury in transgenic mice. Circ Res. 2001 Jul 20; 89(2):168-73.

[54] Minamino T, Christou H, Hsieh CM, Liu Y, Dhawan V, Abraham NG, Perrella MA, Mitsialis SA, Kourembanas S. Targeted expression of heme oxygenase-1 prevents the pulmonary inflammatory and vascular responses to hypoxia. Proc Natl Acad Sci U S A. 2001 Jul 17; 98(15):8798-803.

[55] Gill AJ, Kovacsics CE, Cross SA, Vance PJ, Kolson LL, Jordan-Sciutto KL, Gelman BB, Kolson DL. Heme oxygenase-1 deficiency accompanies neuropathogenesis of HIV-associated neurocognitive disorders. J Clin Invest. 2014 Oct;124(10):4459-72.

[56] Mühl H, Paulukat J, Höfler S, Hellmuth M, Franzen R, Pfeilschifter J. The HIV protease inhibitor ritonavir synergizes with butyrate for induction of apoptotic cell death and mediates expression of heme oxygenase-1 in DLD-1 colon carcinoma cells. Br J Pharmacol. 2004 Dec;143(7):890-8. Epub 2004 Oct 25.

[57] Levy JA. Dispelling myths and focusing on notable concepts in HIV pathogenesis. Trends Mol Med. 2015 Jun;21(6):341-53.

[58] Baggaley RF, White RG, Boily MC: HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. Int J Epidemiol 2010, 39:1048-1063.

[59] Anderson DJ, Marathe J, Pudney J: The structure of the human vaginal stratum corneum and its role in immune defense. Am J Reprod Immunol 2014, 71:618-623.

[60] Haase AT: Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu Rev Med 2011, 62:127-139.

[61] Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, Heil ML, Kasolo F, Musonda R, Hahn BH, Shaw GM, Korber BT, Allen S, Hunter E: Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 2004, 303:2019-2022.

[62] Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen IL, Nottet HS, KewalRamani VN, Littman DR, Figdor CG, van Kooyk Y. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cellsCell. 2000 Mar 3; 100(5):587-97.

[63] Bakri Y, Schiffer C, Zennou V, Charneau P, Kahn E, Benjouad A, Gluckman JC, Canque B, The maturation of dendritic cells results in postintegration inhibition of HIV-1 replication. J Immunol. 2001 Mar 15; 166(6):3780-8.

[64] Garcia E, Pion M, Pelchen-Matthews A, Collinson L, Arrighi JF, Blot G, Leuba F, Escola JM, Demaurex N, Marsh M, Piguet V. HIV-1 trafficking to the dendritic cell-T-cell infectious synapse uses a pathway of tetraspanin sorting to the immunological synapse. Traffic. 2005 Jun; 6(6):488-501.

[65] Kwon DS, Gregorio G, Bitton N, Hendrickson WA, Littman DR, DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. Immunity. 2002 Jan; 16(1):135-44.

[66] Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC: CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004, 200:749-759.

[67] Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, Dandekar S. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol. 2003;77:11708–11717. doi: 10.1128/JVI.77.21.11708-11717.2003.

[68] Pantaleo G, Demarest JF, Soudeyns H, Graziosi C, Denis F, Adelsberger JW, Borrow P, Saag MS, Shaw GM, Sekaly RP. Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV. Nature. 1994;370:463–467. doi: 10.1038/370463a0.

[69] Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol. 1994;68:6103–6110.

[70] Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. N Engl J Med. 1998;339:33–39. doi: 10.1056/NEJM199807023390107.

[71] Schacker TW, Hughes JP, Shea T, Coombs RW, Corey L. Biological and virologic characteristics of primary HIV infection. Ann Intern Med. 1998;128:613–620.

[72] Redefining the viral reservoirs that prevent HIV-1 eradication. Eisele E, Siliciano RF Immunity. 2012 Sep 21; 37(3):377-88.

[73] Persaud D, Zhou Y, Siliciano JM, Siliciano RF, Latency in human immunodeficiency virus type 1 infection: no easy answers. J Virol. 2003 Feb; 77(3):1659-65.

[74] Han Y, Lassen K, Monie D, Sedaghat AR, Shimoji S, Liu X, Pierson TC, Margolick JB, Siliciano RF, Siliciano JD. Resting CD4+ T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. J Virol. 2004 Jun; 78(12):6122-33.

[75] Siliciano RF, Greene WC. HIV latency.Cold Spring Harb Perspect Med. 2011 Sep; 1(1):a007096.

[76] Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373:123–126. doi: 10.1038/373123a0.

[77] Ford ES, Puronen CE, Sereti I. Immunopathogenesis of asymptomatic chronic HIV Infection: the calm before the storm. Curr Opin HIV AIDS. 2009;4:206–214. doi: 10.1097/COH.0b013e328329c68c.

[78] McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. The immune response during acute HIV-1 infection: clues for vaccine development. Nat Rev Immunol. 2010;10:11–23. doi: 10.1038/nri2674

[79] Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med. 2006;12:289–295. doi: 10.1038/nm1380.

[80] Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM, Paul WE. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? Nat Med. 2002;8:319–323. doi: 10.1038/nm0402-319.

[81] Alam SM, Scearce RM, Parks RJ, Plonk K, Plonk SG, Sutherland LL, Gorny MK, Zolla-Pazner S, Vanleeuwen S, Moody MA, Xia SM, Montefiori DC, Tomaras GD, Weinhold KJ, Karim SA, Hicks CB, Liao HX, Robinson J, Shaw GM, Haynes BF. Human immunodeficiency virus type 1 gp41 antibodies that mask membrane proximal region epitopes: antibody binding kinetics, induction, and potential for regulation in acute infection. J Virol. 2008;82:115–125. doi: 10.1128/JVI.00927-07.

[82] He B, Qiao X, Klasse PJ, Chiu A, Chadburn A, Knowles DM, Moore JP, Cerutti A. HIV-1 envelope triggers polyclonal Ig class switch recombination through a CD40-independent mechanism involving BAFF and C-type lectin receptors. J Immunol. 2006;176:3931–3941.

[83] D.O. Adams, The granulomatous inflammatory response, Am.J. Pathol. 84 (1976) 164–191.

[84] Imperiali FG, Zaninoni A, La Maestra L, Tarsia P, Blasi F, Barcellini W(2001) Increased Mycobacterium tuberculosis growth in HIV-1-infected human macrophages: role of tumor necrosis factor-alpha. Clin Exp Immunol 123: 435–442

[85] Hoshino, Y., et al. 2002. Maximal HIV-1 replication in alveolar macrophages during tuberculosis requires both lymphocyte contact and cytokines. J. Exp. Med. 195:495-505.

[86] Lawn, S. D., S. T. Butera, and T. M. Shinnick. 2002. Tuberculosis unleashed: the impact of human immunodeficiency virus infection on the host granulomatous response to Mycobacterium tuberculosis. Microbes Infect. 4:635-646.

[87] Reed, M. B., et al. 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 431:84-87.

[88].Ranjbar, S., et al. 2009. HIV-1 replication is differentially regulated by distinct clinical strains of Mycobacterium tuberculosis. PLoS One 4:e6116.

[89] Garrait, V., et al. 1997. Tuberculosis generates a microenvironment enhancing the productive infection of local lymphocytes by HIV. J. Immunol. 159:2824-2830.

[90] Patel, N. R., K. Swan, X. Li, S. D. Tachado, and H. Koziel. 2009. Impaired M. tuberculosis-mediated apoptosis in alveolar macrophages from HIV^{+} persons: potential role of IL-10 and BCL-3. J. Leukoc. Biol. 86:53-60.

[91] Patel, N. R., et al. 2007. HIV impairs TNF-alpha mediated macrophage apoptotic response to Mycobacterium tuberculosis. J. Immunol. 179:6973-6980.

[92] Hoshino, Y., et al. 2002. Maximal HIV-1 replication in alveolar macrophages during tuberculosis requires both lymphocyte contact and cytokines. J. Exp. Med. 195:495–505.

[92] Hoshino, Y., et al. 2004. Mycobacterium tuberculosis-induced CXCR4 and chemokine expression leads to preferential X4 HIV-1 replication in human macrophages. J. Immunol. 172:6251–6258.

[93] Mattapallil, J. J., et al. 2005. Massive infection and loss of memory CD4_ T cells in multiple tissues during acute SIV infection. Nature 434:1093–

1097.

[92] Kumawat, K., S. K. Pathak, A. L. Spetz, M. Kundu, and J. Basu. 2010. Exogenous Nef is an inhibitor of Mycobacterium tuberculosis-induced tumor necrosis factor-alpha production and macrophage apoptosis. J. Biol. Chem. 285:12629–12637.

[93] Deretic, V., et al. 2004. Endosomal membrane traffic: convergence point targeted by Mycobacterium tuberculosis and HIV. Cell. Microbiol. 6:999–1009.

[94] Mwandumba, H. C., et al. 2004. Mycobacterium tuberculosis resides in nonacidified vacuoles in endocytically competent alveolar macrophages from patients with tuberculosis and HIV infection. J. Immunol. 172:4592–4598.

[95] Anandaiah A, Sinha S, Bole M, Sharma SK, Kumar N, Luthra K, Li X, Zhou X, Nelson B, Han X, Tachado SD, Patel NR, Koziel H. Vitamin D rescues impaired Mycobacterium tuberculosis-mediated tumor necrosis factor release in macrophages of HIV-seropositive individuals through an enhanced Toll-like receptor signaling pathway in vitro. Infect Immun. 2013 Jan;81(1):2-10. doi: 10.1128/IAI.00666-12. Epub 2012 Oct 15.

[96] Behar SM, Martin CJ, Nunes-Alves C, Divangahi M, Remold HG. Lipids, apoptosis, and cross-presentation: links in the chain of host defense against Mycobacterium tuberculosis. Microbes Infect Inst Pasteur. 2011;13: 749–756.

[97] Sia JK, Georgieva M, Rengarajan J. Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between Mycobacterium tuberculosis and Innate Immune Cells. J Immunol Res. 2015;2015: 747543.

[98] Divangahi M, Desjardins D, Nunes-Alves C, Remold HG, Behar SM. Eicosanoid pathways regulate adaptive immunity to Mycobacterium tuberculosis. Nat Immunol. 2010;11: 751–758.

[99] Amaral EP, Lasunskaia EB, D'Império-Lima MR. Innate immunity in tuberculosis: how the sensing of mycobacteria and tissue damage modulates macrophage death. Microbes Infect Inst Pasteur. 2016;18: 11–20.

[100] Scola L, Crivello A, Marino V, Gioia V, Serauto A, Candore G, Colonna-Romano G, Caruso C, Lio D. IL-10 and TNF-alpha polymorphisms in a sample of Sicilian patients affected by tuberculosis: implication for ageing and life span expectancy. Mech Ageing Dev. 2003 Apr;124(4):569-72.

[101] Wang Q, Zhan P, Qiu LX, Qian Q, Yu LK. TNF-308 gene polymorphism and tuberculosis susceptibility: a meta-analysis involving 18 studies. Mol Biol Rep. 2012 Apr;39(4):3393-400. doi: 10.1007/s11033-011-1110-x.

[102] Muller BL, Ramalho DM, Santos PF, Mesquita ED, Kritski AL, Oliveira MM. Inflammatory and immunogenetic markers in correlation with pulmonary tuberculosis. J Bras Pneumol. 2013 Nov-Dec;39(6):719-27. doi: 10.1590/S1806-37132013000600011.

[103] Allcock RJ, Williams JH, Price P. The central MHC gene, BAT1, may encode a protein that down-regulates cytokine production. Genes Cells. 2001 May;6(5):487-94.

[104] Vejbaesya S, Chierakul N, Luangtrakool P and Sermduangprateep C. NRAMP1 and TNF-alpha polymorphisms and susceptibility to tuberculosis in Thais. Respirology. 2007; 12: 202-206.

[105] Exner M, Minar E, Wagner O, and Schillinger M. The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med.* 2004;37(8):1097-104.

[106] Ganachari M, Ruiz-Morales JA, Gomez de la Torre Pretell JC, Dinh J, Granados J, and Flores-Villanueva PO. Joint effect of MCP-1 genotype GG and

MMP-1 genotype 2G/2G increases the likelihood of developing pulmonary tuberculosis in BCG-vaccinated individuals. *PLoS One.* 2010;5(1):e8881.

[107] Hsieh MH, Chou PC, Chou CL, Ho SC, Joa WC, Chen LF, Sheng TF, Lin HC, Wang TY, Chang PJ, et al. Matrix metalloproteinase-1 polymorphism (-1607G) and disease severity in non-cystic fibrosis bronchiectasis in Taiwan. *PLoS One.* 2013;8(6):e66265. 26

[108] Mendonca VR, Luz NF, Santos NJ, Borges VM, Goncalves MS, Andrade BB, and Barral-Netto. M. Association between the haptoglobin and heme oxygenase 1 genetic profiles and soluble CD163 in susceptibility to and severity of human malaria. *Infect Immun.* 2012;80(4):1445-54.

[109] Ninomiya S, Niimi T, Shimizu S, Sato S, Achiwa H, Ito H, Akita K, Maeda H, and Ueda R. Matrix metalloproteinase-1 polymorphism of promoter region in sarcoidosis and tuberculosis patients. *Sarcoidosis Vasc Diffuse Lung Dis.* 2004;21(1):19-24.

[110] Saukkonen K, Lakkisto P, Kaunisto MA, Varpula M, Voipio-Pulkki LM, Varpula T, Pettila V, and Pulkki K. Heme oxygenase 1 polymorphisms and plasma concentrations in critically ill patients. *Shock.* 2010;34(6):558-64.

[111] Sheu CC, Zhai R, Wang Z, Gong MN, Tejera P, Chen F, Su L, Thompson BT, and Christiani DC. Heme oxygenase-1 microsatellite polymorphism and haplotypes are associated with the development of acute respiratory distress syndrome. *Intensive Care Med.* 2009;35(8):1343-51.

[112] Sri Manjari K, Nallari P, Balakrishna N, Vidyasagar A, Prabhakar B, Jyothy A, and Venkateshwari A. Influence of matrix metalloproteinase-1 gene -1607 (1G/2G) (rs1799750) promoter polymorphism on circulating levels of MMP-1 in chronic pancreatitis. *Biochem Genet.* 2013;51(7-8):644-54.