Review: Reactivation of tuberculosis by tumor necrosis factor neutralization

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ABSTRACT. Tumor necrosis factor (TNF) is required in the control of infection with *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis. TNF is essential and non-redundant for forming microbiocidal granulomas, and cannot be replaced by other members of the TNF family. We established a model of latent Mtb infection in mice, allowing investigation of the reactivation of latent Mtb as observed in patients receiving TNF-neutralizing therapy used in rheumatoid arthritis and Crohn's disease. Antibody neutralization of TNF is able to reactivate clinically silent Mtb infection. Using mutant mice expressing solely membrane, but not soluble TNF, we demonstrated that membrane TNF is sufficient to control acute Mtb infection. Therefore, we hypothesize that TNF-neutralizing therapy, sparing membrane TNF, may have an advantage as compared to complete neutralization. In conclusion, endogenous TNF is critical for the control of tuberculosis infection. Genetic absence or pharmacological neutralization of TNF results in uncontrolled infection, while selective neutralization might retain the desired anti-inflammatory effect but reduce the infectious risk.

Keywords: tuberculosis, tumor necrosis factor

Tuberculosis (TB) infection is major health problem caused by several strains of *Mycobacterium tuberculosis* (Mtb). While the introduction of chemotherapy after the second world war had almost eradicated active disease, the emergence of HIV/AIDS infection and increasing poverty and malnutrition has allowed a massive re-emergence of active tuberculosis infection in endemic areas, especially in Sub-Saharan Africa and Asia [1]. The present estimate is that one third of the world population harbors Mtb in a latent form (http://www.who.int), which may be reactivated by suppressing the host immune response. Unraveling the host immune response during the primary infection, and identifying the factors controlling latent and reactivated stages of TB, are therefore major challenges, which are facilitated by *in vivo* animal models. Key immune factors that have been implicated in the control of tuberculosis infection and reactivation of disease, include T cells, macrophages, interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-12, nitric oxide (NO), reactive oxygen and reactive nitrogen intermediates (RNI), as recently reviewed [2, 3], as T cell depletion, and inhibition or neutralization of several mediators at different stages of tuberculosis leads to rapid disease progression and eventually death. Apart from its protective effects in the immune response to *M. tuberculosis*, excessive TNF may also cause pathology *in vivo*, including hyperinflammation, caseous necrosis and cachexia, which are correlated with elevated TNF levels [4, 5]. Here we review the role of TNF in the development of immunity using our own and published data in genetically modified mice. There are substantial differences in the susceptibility of different inbred strains pointing to other important, but so far unidentified genes, controlling TB infection [6].

TNF FAMILY AND GENETIC MOUSE MODELS

TNF is the founding member of the cytokine TNF-like superfamily [7]. The TNF gene is closely linked, along with lymphotoxin α and β genes, to the MHC locus on murine chromosome 17 (human 6) [8-10]. TNF and lymphotoxins bind different receptors such as TNF-R1 and LTβ receptor, and signals through a complex cascade to activate NFκB resulting in gene activation [11]. The TNF family comprises several additional members such as LIGHT, binding to LTβR, CD40, FAS-L and many more, for review see [12, 13] and for detailed nomenclature refer to http://www.gene.ucl.ac.uk/nomenclature/genefamily/tnftop.

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To investigate the role of TNF, several tools are available, which include neutralizing antibodies, soluble receptors, and genetic mouse models. Gene-deficient mice (KO) have proved to be useful for understanding the biological role of TNF in host resistance and pathology of TB infection. These investigations have revealed that soluble TNF, lymphotoxin (LT) α and membrane-bound LTαβ, as well the TNF-R1 and TNF-R2, and LTβR are critical and non-redundant for host resistance, while LIGHT and TRAIL are not required [14-20]. Tissue-specific TNF-KO mice [21] and LTβ–KO mice [22] were generated to investigate *in vivo* functions of TNF produced by different cell types of the immune system such as macrophages/neutrophils and lymphocytes. Recently two new models with functional, normally regulated and expressed membrane-bound TNF, which was obtained by knocking-in an uncleavable Δ1-9,K11E TNF allele were developed. This represents a major advance, as no soluble TNF is expressed, thereby allowing the analysis of the role of membrane TNF in lymphoid structure development and inflammation [23, 24].

CELL ACTIVATION, RECRUITMENT, EFFECTOR CELLS AND LONG-TERM CONTROL OF INFECTION IN MYCOBACTERIAL GRANULOMA

Macrophages and DC are probably the first cells encountering Mtb bacilli in the airways. Phagocytosis induces transcriptional machinery, resulting in the secretion of several proinflammatory cytokines and chemokines, expression of costimulatory molecules and effector molecules including nitrite, which has mycobactericidal activity *(figure 1)*. Mycobacterial proteins are degraded and presented by class II proteins to the T cell receptor inducing clonal activation of CD4 T cells. Interferon-γ (IFNγ) derived from T cells and NK or NKT cells are potent activator of APCs, enhancing the killing of Mtb and presentation of mycobacterial peptide to T cells. The concerted action of cytokines and chemokines leads to accumulation of activated macrophages containing a few surviving bacilli, surrounded by activated T cells. This constitutes the typical mycobacterial granuloma *(figure 1)*.

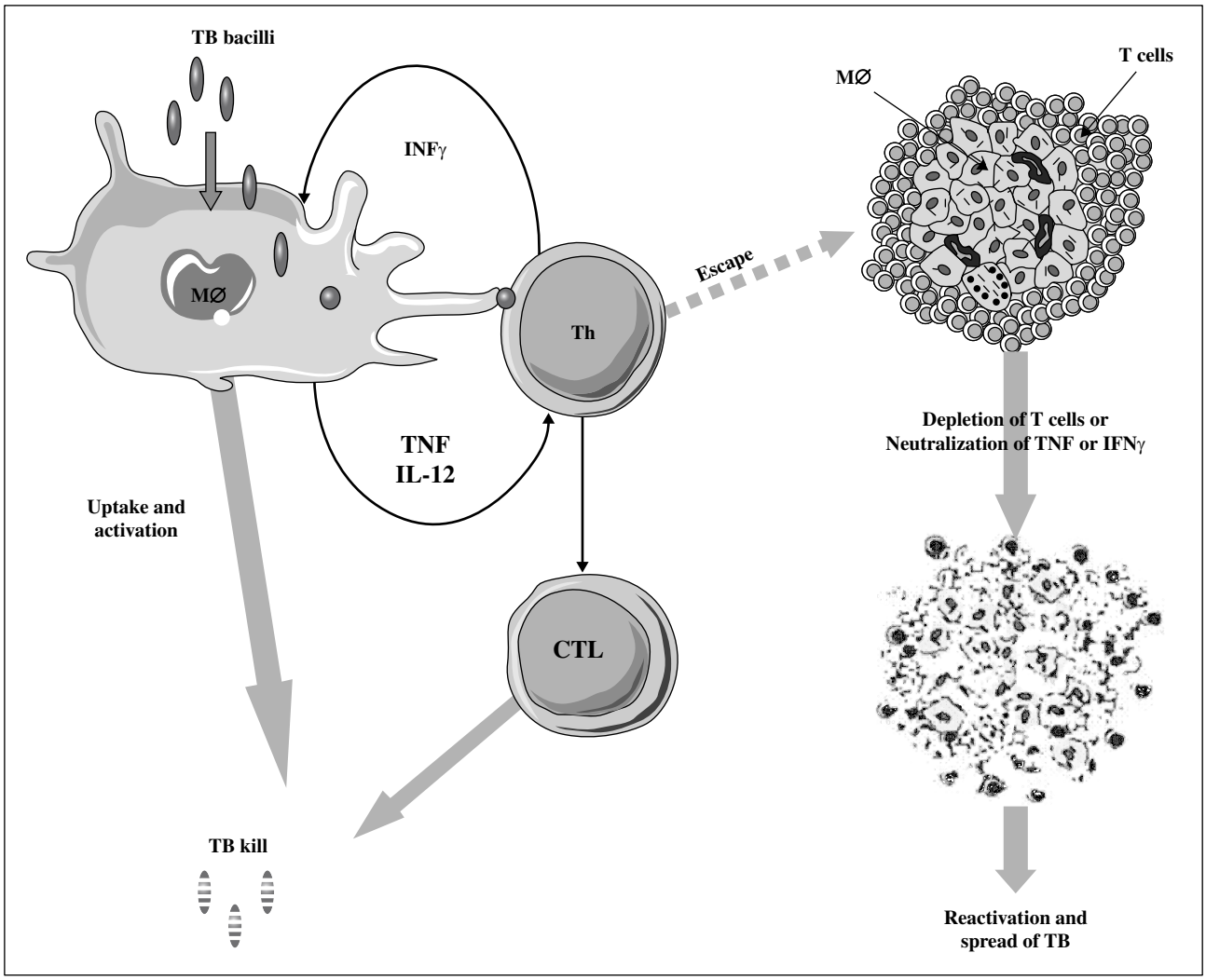

Figure 1

Macrophage and T cell activation, killing of TB bacilli and granuloma formation.
Macrophages are activated by TB bacilli to produce cytokines and T cell activation. Activated macrophages are mycobactericidal, but a few bacilli escape. The cell activation induces lymphocyte recruitment orchestrated by chemokines, leading to the formation of granulomas that contain bacilli. Antibody neutralization of TNF or IFNγ, or T cell depletion results in dissolution of the granuloma structure, rescue of surviving bacilli with dissemination of infection.

Other cell types may participate in this process, including neutrophils, eosinophils, NK, NKT and mast cells, and possibly γδ-T cells [2, 3].

Infection with the vaccine strain M*ycobacterium bovis* BCG is well controlled in normal BL6 mice. However, BCG infection following neutralization of TNF or in TNF-R1-KO mice leads to fatal infection [20, 25]. We used TNF-LTα-deficient mice, which display high susceptibility and succumb to BCG infection between 8 and 10 weeks. The granuloma response was severely impaired, with reduced T cell recruitment and macrophage activation, expressing low levels of inducible nitric oxide synthase (NOS2) – a key mediator of antibacterial defense. We compared the susceptibility of TNF- and LTα-deficient mice, and showed that both types of single gene-deficient mice succumbed to BCG infection, suggesting that TNF and LTα are necessary and non-redundant in the control of BCG infection (Jacobs M, in preparation).

The T cell response is critical for controlling mycobacterial infection as antibody-mediated depletion of T cells or CD4 T cells in immunosuffcient BL6 mice leads to uncontrolled infection similar to that observed in T cell-deficient mice [26]. Interestingly, depletion of CD4 cells may also lead to reactivation of silent, chronic TB infection, despite almost normal levels of IFNγ. One explanation of this would be that TNF produced by CD4 cells is critical for host-resistance. Little information is available on the antigen specificity of the T cell response, especially of CD8 T cells [27]. It has been demonstrated that CD8 T cells were specific for culture filtrate protein-10 (CFP10) in purified protein derivative positive donors and in T cells from Mtb infected mice. CFP10-specific T cells were detected as early as week three post-infection and reached 30% of the CD8 T cells in the lung with long persistence [27].

In addition to the classical cells of the immune system, such APC and T lymphocytes, other cells are involved in the control of infection. NK cells have been associated with early resistance against intracellular pathogens and are known to be potent producers of IFNγ. Aerosol infection increased NK cell recruitment and activation, resulting in IFNγ production. However, *in vivo* depletion of NK cells using a lytic antibody had no influence on bacterial clearance. Therefore, NK cells do not appear to alter host resistance [28].

However, NKT cells may play a role in infection control. *In vivo* activation of NKT cells by α-galactosyl-ceramide augmented host-resistance in mice, which may also be mediated in part by the production of IFNγ [29]. Moreover, activation of CD1-restricted human T cells increased killing, probably via granulolysin [30]. It is important to note here that some of the mycobacterial antigens can be presented *in vivo* to NKT cells in a context of CD1b non-classical MHC, thereby mediating interaction of NKT cell with infected cells.

Mast cells (MC) are abundant in the lung and interact directly with a wide variety of infectious agents, including Mtb, triggering the release of histamine and β-hexosaminidase, TNF and IL-6, the latter being critically involved in antimycobacterial resistance. Mtb appears to interact with CD48 on MC inducing histamine release, which is inhibited by CD48 Abs. Therefore, Mtb and its antigens recognize and activate MC [31]. Further investigations in mast cell-deficient mice are necessary to define the role of MC in the host response to Mtb infection.

MOLECULAR MECHANISMS OF MYCOBACTERIAL KILLING/RESISTANCE

Activation of macrophages and dendritic cells by Mtb induces on one hand, several proinflammatory cytokines including TNF, LTα and IL-12, and on the other hand expression of costimulatory molecules that enhance antigen presentation and activation of T cells. Activated T cells produce TNF, IFNγ and LTα inducing further macrophage activation. Activated macrophages express NOS2, producing nitric oxide and reactive nitrogen intermediates (RNI), which are critical for killing and inhibiting growth of virulent Mtb and BCG [32-34].

Mycobacteria may inhibit phagosome maturation and fusion with lysosomes, and thereby escape killing [35-38]. Activated macrophages recruit T cells and form granulomas, in which bacteria can grow. The granuloma is a dynamic structure, which requires a permanent signal from activated T cells and macrophages [39]. Any perturbation of this signaling such as neutralization of IFNγ or TNF, causes dissolution of granulomas [25] and allows reactivation and spread of infection *(figure 1)*. Activated T cells not only provide help, but acquire cytotoxic functions, which eradicate bacilli, although the relative contribution of CD4 versus CD8 cells to control TB infection is not fully established.

In order to better understand the effect of TNF on intracellular replication of mycobacteria, we investigated the growth of the vaccine strain BCG in TNF-deficient macrophages. BCG infection resulted in logarithmic growth of the intracellular bacilli, while recombinant BCG-expressing TNF (BCG-TNF) led to bacillary killing associated with production of NO. Therefore, the expression of NOS2 requires TNF and hence TNF contributes indirectly to inhibition of growth [40].

IFNγ has been shown to be an essential component of immunity to tuberculosis by activating infected host macrophages to directly inhibit the replication of Mtb [2]. Although IFNγ-inducible NOS2 is considered to be a principal effector mechanism, other pathways exist. MacMicking *et al*. described a 47-kilodalton (p47) guanosine triphosphatase family member, LRG-47 that acts independently of NOS2 to protect against infection [41]. Mice lacking LRG-47 failed to control Mtb replication, unlike those missing the related p47 guanosine triphosphatases IRG-47 or IGTP. Defective bacterial killing in IFNγ-activated LRG-47-deficient macrophages was associated with impaired maturation of Mtb-containing phagosomes, vesicles that otherwise induce LRG-47 in wild-type cells. Thus, LRG-47 may serve as a critical vacuolar trafficking component used to dispose of intracellular pathogens such as Mtb [41]. The TNF-dependence of LRG-47 activation has not yet been investigated.

Mtb has developed several mechanisms to escape eradication, including inhibition of phagosome maturation, as reviewed recently [42]. Mycobacteria, by blocking Ca^{2+} signaling and phagosome maturation in human macrophages or by inhibiting sphingosine kinase, may allow their escape from eradication by phagocytes [43-45].

Mycobacteria induce apoptosis of macrophages, causing the release of apoptotic vesicles that carry mycobacterial antigens to uninfected APC, which is indispensable for subsequent cross-presentation of antigens, through MHC-I and CD1b, to T cells. This new "detour" pathway for presentation of antigens from a phagosome-contained

pathogen shows the functional significance of infection-induced apoptosis in the activation of CD8 T cells specific for both protein and glycolipid antigens in tuberculosis that carry mycobacterial antigens to uninfected antigen-presenting cells [46].

Finally, we demonstrated that activation of TLR-MyD88 signaling is critical for inducing an innate immune response, with the production of TNF and other proinflammatory cytokines and for controlling infection [47-49]. There are several possibilities as to how TLR- and MyD88-dependent signaling may be involved in resistance to TB. Firstly, in the absence of MyD88, production of TNF and other critical cytokines is abrogated [47, 49] while upregulation of costimulatory molecules occurs successfully. Secondly, TLR/MyD88-dependent signaling is required for phagosome maturation [50]. In summary, TNF participates in resistance to mycobacteria in the following ways: (1) activation of macrophages, (2) induction of chemokines and cell recruitment, (3) activation of T cells, (4) killing by macrophages, T and other cells and (5) signals from TLR/MyD88/IL-1R pathway contribute to the host response.

HYPERINFLAMMATORY SYNDROME - DELETERIOUS TNF

TNF has long been regarded as a critical cytokine involved in antimicrobial Th1 immunity. We and others have shown that mice lacking TNF or TNF signaling quickly succumb to Mtb infection, with respiratory failure due necrotic pneumonia [16, 20]. TNFR1 has been shown as an important player in caseous necrosis of granulomas. Tissue destruction may be the result of an uncontrolled type 1 immune response characterized by expansion of activated and antigen-specific CD4 and CD8 T cells, and overproduction of IFNγ and IL-12 as shown recently [51]. In support of an exaggerated type 1 response is the fact that depletion of CD4 and CD8 T cells decreased IFNγ levels, prevented granuloma and tissue necrosis, and prolonged the survival of TNF-deficient mice. Early reconstitution of TNF by gene transfer reduced the frequency of antigen-specific T cells and improved survival. TNF controlled the type 1 immune activation at least in part, by suppressing T cell proliferation, and this suppression involved both TNF receptor p55 and TNF receptor p75. Heightened type 1 immune activation also occurred in TNF-deficient mice treated with dead mycobacteria, live, replication-deficient mycobacteria, mycobacterial cell wall components or heat killed *C. parvum* [52]. These studies and our unpublished results suggest that TNF has a regulatory role on the expression of Th1 cytokine preventing a detrimental, type 1 immune response [51]. Therefore, absence of TNF results in an uncontrolled Th1 cytokine response.

MEMBRANE TNF HAS BIOLOGICAL ACTIVITY

Although a key role of TNF in controlling intracellular bacterial infections is uncontested, the function of membrane TNF, which is subsequently cleaved by the metalloproteinase-disintegrin TACE (TNF-converting enzyme [53]) into the secreted trimeric TNF, is unknown in mycobacterial host resistance.

Several biological functions of membrane TNF have been described, such as cytotoxicity, polyclonal activation of B

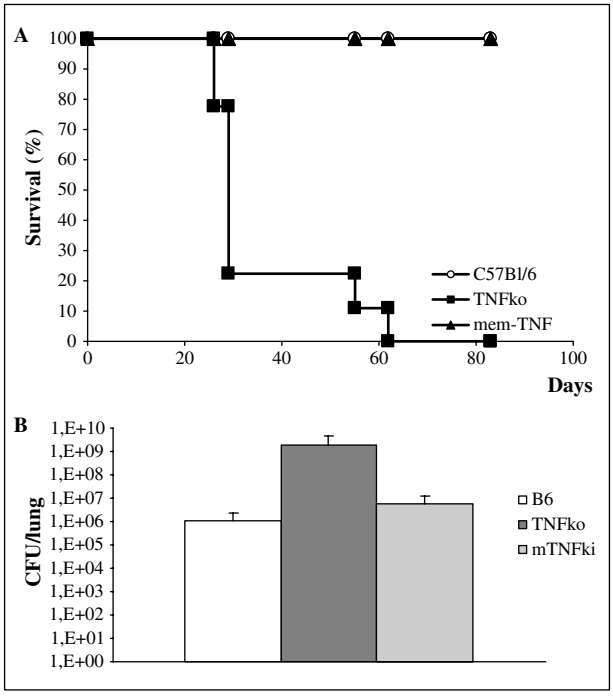

Figure 2

Membrane TNF, unlike complete TNF KO, is able to control acute aerosol infection with Mtb H37Rv.

Membrane TNF KI mice, which lack soluble TNF, complete TNF-deficient and control B6 mice were infected by aerosol at 100cfu of H37Rv and followed for survival (**A**) and bacterial load in the lung (cfu), $p < 0.05$ (**B**). The data are adapted from Fremond *et al*. [59].

cells, induction of IL-10 by monocytes, and ICAM-1 expression on endothelial cells [54-56]. The transgenic expression of membrane TNF suggested an *in vivo* role of membrane TNF [57]. Olleros *et al*. investigated the resistance to mycobacterial infection in membrane TNF transgenic mice against a TNF-LTα-deficient background, and showed that membrane TNF has a partially protective effect [58]. However, transgenic expression of high levels of membrane TNF is artificial and may cause non-physiological effects. The recent generation of a mouse with functional, normally regulated and expressed membrane-bound TNF, which was obtained by knocking-in an uncleavable Δ1-9,K11E TNF allele, represents a major advance and has allowed interesting insights into the role of membrane TNF in lymphoid structure development and inflammation [23]. Δ1-9,K11E TNF knock-in mice [23] were compared against TNF-deficient mice for their host resistance to mycobacterial infection [52]. Our data demonstrate that membrane TNF has important biological functions and substitutes for soluble TNF to a large extent. Membrane TNF knock-in mice were able to recruit and activate macrophages and T cells, generate granulomas and partially control mycobacterial infection, unlike TNF-deficient mice [59, 60]. Unlike TNF-deficient mice, membrane TNF knock-in mice survived an aerosol infection for three months, and controlled infection in the early stage as shown by reduced numbers of viable cells (cfu) in lung *(figure 2)*. However, during the chronic phase of infection, TNF knock-in mice demonstrated reduced bacterial clearance and finally succumbed to infection [59]. The data from the genetic mouse models suggest that membrane-expressed TNF is sufficient and soluble TNF dispensable, in the control of the first phase of TB infection.

However, during the chronic phase, membrane TNF alone is not sufficient and soluble TNF seems to be required for disease control, which merits further investigation.

USE OF MUTANT MYCOBACTERIA TO INVESTIGATE VIRULENCE AND DISSEMINATION

Several mutant mycobacteria were recently generated, which has allowed the analysis of those molecules critical for pathogen survival or virulence. A few examples are mentioned here since they have relevance to TNF, virulence, and persistent TB infection.

A biologically-active lipid species, a polyketide, synthase-derived phenolic glycolipid (PGL), produced by a subset of Mtb isolates belonging to the virulent W-Beijing Mtb family, has been found to cause "hypervirulence" in mice [61]. Disruption of PGL synthesis results in loss of virulence, but not enhanced bacterial clearance in mice, and correlates with increased production of TNF, IL-6 and IL-12 production *in vitro* [61].

Persistent infection is associated with metabolic changes of the TB bacilli, which includes isocitrate lyase (ICL) activation, an enzyme essential for the metabolism of fatty acids. Disruption of the *icl* genes in Mtb reduced bacterial persistence and attenuated virulence in mice, but had no effect on bacterial growth during the acute phase of infection [62]. This and other data suggest that metabolism of Mtb *in vivo* is profoundly influenced by the host response to infection, an observation which may be important for the understanding and treatment of chronic TB infection.

The Mtb *mce1* operon encodes several membrane proteins which are apparently required for Mtb entrance into host cells [63]. An *mce1* mutant Mtb strain displayed uncontrolled growth *in vivo* and killed the mice more rapidly than the wild-type Mtb strain [63]. Murine macrophages infected *ex vivo* with the mutant strain produced less TNF, IL-6, monocyte chemoattractant protein 1 and nitrite. These observations indicate that the *mce1* operon mutant is unable to stimulate T helper 1-type immunity in mice. The hypervirulence of the mutant strain may result from its inability to enter host cells and to stimulate a proinflammatory response that would otherwise induce organized granuloma formation and control the infection without killing the organism. The *mce1* operon of Mtb may be involved in modulating the host inflammatory response in such a way that the bacterium can enter a persistent state without being eliminated or causing disease in the host [63].

Another mechanism of defense against oxidative and nitrosative stress is the mycobacterial proteasome [64]. As indicated before, macrophages control Mtb in part by the production of nitric oxide and other RNI. To identify genes that Mtb requires to resist RNI, an Mtb transposon mutant library was screened for hypersusceptibility to acidified nitrite. Among the mutants identified were insertions of proteasome-associated genes. An Mtb mutant deficient in a presumptive proteasomal adenosine triphosphatase was attenuated in mice, suggesting that the mycobacterial proteasome serves as a defense against oxidative or nitrosative stress [64].

In an attempt to identify a virulence factor involved in the extrapulmonary dissemination of Mtb, Pethe *et al*. disrupted the *hbhA* gene in Mtb and BCG, which encodes the heparin-binding haemagglutinin adhesin (HBHA). The mutant strain grew normally in the lung, but was severely impaired in spleen colonization. Coating wild-type mycobacteria with anti-HBHA antibodies also impaired dissemination after intranasal infection. HBHA enhances binding of Mtb to epithelial cells and is required for extrapulmonary dissemination [65]. These few examples demonstrate that investigations into the biology of Mtb mutants will provide interesting and novel insights in the pathogenesis and persistence of TB infection.

MODEL OF LATENT/PERSISTENT INFECTION AND REACTIVATION OF TB INFECTION

Over a million patients with rheumatic arthritis are currently on TNF blockers, which is often associated with reactivation of a clinically silent infection. In fact, this is a major infectious complication and has been predicted by preclinical studies.

In order to investigate the factors leading to reactivation of chronic or treatment-controlled latent infection, several experimental investigations have been undertaken. Two basic models have been described to date, of which the Cornell model was the first reported [66, 67]. Upon intravenous administration of *M. tuberculosis* H37Rv and treatment with pyrazinamide and isoniazid (INH) for 12 weeks, mice seemed to be able to clear the bacilli from organs, but a substantial proportion of mice experienced spontaneously reactivation of acute disease. Since the Cornell model has been published, a few variations on this model have been reported as reviewed recently [68, 69]. The alternative model that has been described, also known as the low-dose model [70], involves low dose infection with tubercle bacilli in the absence of treatment, with the ensuing infection exclusively controlled by the host [68, 69]. Although considered to reflect the human host response better, bacterial numbers in the organs of these mice remain high during the chronic persistent phase of infection. To date, both of these models have yielded significant information on the immune effectors participating in latent or chronic persistent and reactivated tuberculosis.

We have established the first low dose aerosol infection model of drug-induced, latent and reactivatable tuberculosis infection using rifampicin (RFM) and isoniazide (INH) [68]. In this model, latency is defined as undetectable levels of bacilli in mouse organs for a prolonged period of time. Mice are infected by low aerosol dose (10cfu), and 2 weeks later treated with INH and rifampicin for 4 weeks, after which all treatment is stopped *(figure 3A)*. The bacterial load in the lung reached about 10^5 cfu per lung 2 weeks after aerosol exposure, and was reduced to undetectable levels after 8 weeks. Reactivation of infection could be achieved by inhibiting macrophage NOS2 by aminoguanidine [69, 70] or with antibody and other inhibitors [68-74].

This low dose aerosol model of latent infection, with a four week treatment period, demonstrated spontaneous reactivation, the TNF-deficient mice succumbing to uncontrolled infection and acute pneumonia, with about 10^9 cfu in the lung *(figure 3B)*, while wild-type mice developed subclinical reactivation [75]. Administration of neutralizing TNF antibody or soluble TNF receptor such as Enbrel (not shown), induced a comparable reactivation of latent infection as observed in TNF-deficient mice *(figure 3B)*.

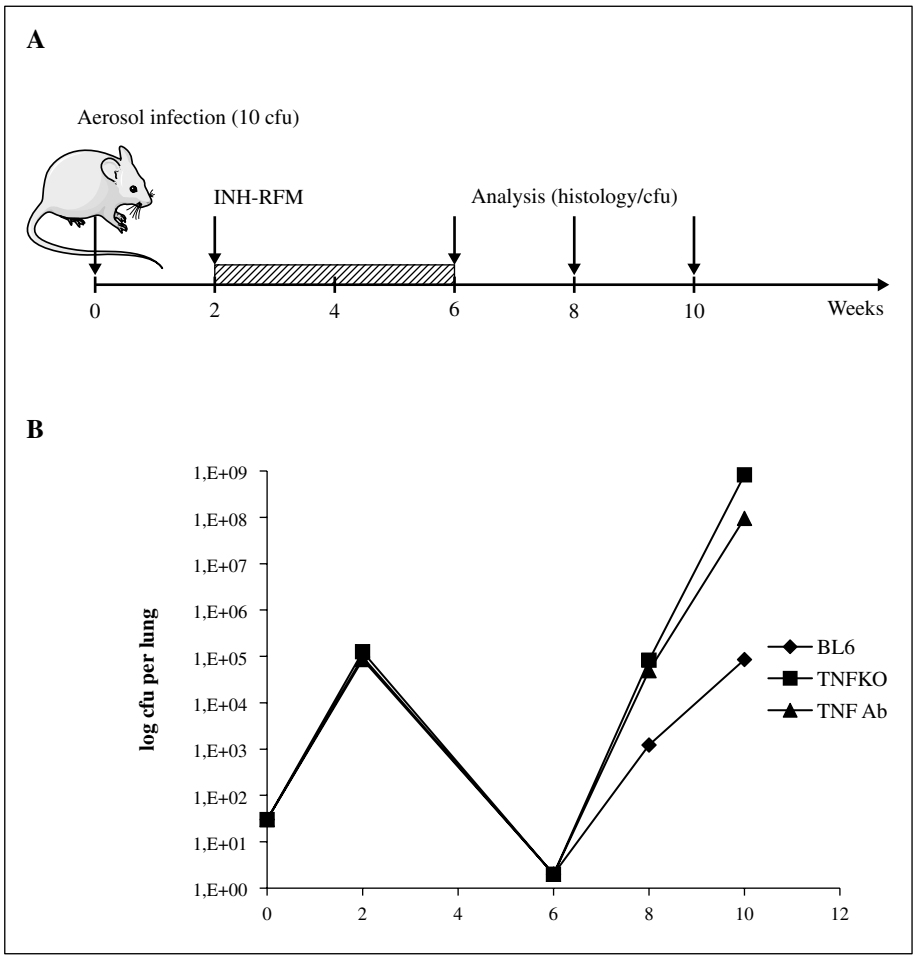

Figure 3

Reactivation of latent TB infection by TNF neutralization.
A) Model of low dose aerosol infection with treatment, is followed by reactivation of infection. Mice are infected by aerosol with 10cfu Mtb strain H37Rv, the mice are treated from weeks 3 to 6 with INH and RFM, and then left untreated.
B) Spontaneous reactivation of latent TB infection in TNF-deficient mice, and reactivation by neutralizing TNF antibody in B6 mice as compared to spontaneous subclinical reactivation in B6 control mice (n = 6 mice per group, $p < 0.05$). Figure modified from Botha [75] and unpublished data (Ryffel *et al.*).

A novel approach for TNF neutralization by competing for natural TNF using dominant negative mutant TNF monomers has been proposed. This approach may have the advantage of sparing membrane-expressed TNF, which is not accessible to the mutant TNF monomers [76]. Therefore, our experimental model allows testing for the potential risk of this and other novel TNF-neutralizing therapies inducing reactivation of silent/latent TB infection.

CLINICAL REACTIVATION OF TB INFECTION

Patients suffering from severe rheumatoid arthritis benefit from therapies using neutralizing TNF antibody, infliximab, or soluble TNF-R, etanercept, but the major complication of TNF-neutralizing therapy is reactivation of previous, clinically silent TB infection which may develop within 12 weeks of therapy [77, 78]. A substantial percentage of these patients develop atypical extrapulmonary TB infection (disseminated disease, lymph node disease, peritoneal and pleural disease). The frequency of tuberculosis associated with infliximab therapy was much higher than the reported frequency of other opportunistic infections associated with this drug. Indeed, active tuberculosis may develop soon after initiation of treatment with infliximab or etanercept [77-79].

Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) represents a global health issue in Southern Africa and Asia, and the major complication is reactivation of Mtb infection in endemic TB areas (http://www.who.int). However, this is not the scope of this review; suffice to recall that depletion of CD4 cells and cytokines allows the reactivation of latent/persistent infection. In addition to the personal tragedy of individuals affected with both diseases, the costs of TB and HIV/AIDS are tremendous for the Third World. There is an urgent need for a substantial increase in health sector investment to expand access to preventive and curative health services. International economic support is critical to control TB and HIV/AIDS infection [80].

PERSPECTIVES

In conclusion, TNF is critical and non-redundant in the formation of microbiocidal granulomas and the control of Mtb infection. TNF can neither be replaced by other TNF

family members nor other proinflammatory cytokines. The reason for this requirement for several cytokines is not understood. We propose that TNF and other cytokines are working in sequence, although their exact sequential functions remain elusive.

An important notion is the fact that latent mycobacterial infection can be reactivated by TNF-neutralizing therapies. The finding that membrane TNF confers partial protection and abrogates the hyperinflammatory syndrome is significant, as sparing membrane TNF in TNF-neutralizing therapy used in rheumatic arthritis or Crohn's disease may diminish the infectious complications and the reactivation of latent TB infection. Obviously, the most significant cause of reactivation is HIV infection with depletion of CD4 T cells, TNF and related cytokines.

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