**Cytokines as immunomodulators in tuberculosis therapy**

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Keywords: *M. tuberculosis*, XDR-TB, cytokines, chemokines, monocytes/macrophages, neutrophils, T cells

Short running title: Cytokines in tuberculosis therapy

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**ABSTRACT**

The use of cytokines for therapeutic purposes is limited by their high cost and toxicity. Nevertheless, the emergence of extensively drug-resistant tuberculosis (XDR TB), for which chemotherapy is ineffective, has again made cytokine-based therapy attractive as one of the last available options. The results of clinical trials treating pulmonary tuberculosis with cytokines have not been encouraging, making it clear that therapeutic strategies utilizing a single cytokine are inadequate. To develop effective cytokine-based XDR TB therapies, more basic research will be needed to achieve a better understanding of how cytokines promote a successful immune response. We not only have to investigate cytokines already known to participate in tuberculosis, but also the role of other cytokines and chemokines that may enhance both the mycobacterial killing activity of effector cells and the restriction of bacterial intracellular multiplication. There are already several patents involving cytokines for therapeutic use, in the hope of stimulating the immune system in a variety of infectious diseases, including tuberculosis. The validity of these patents needs to be reassessed from a clinical standpoint, and new applications of patents concerning cytokines potentially useful in XDR TB treatment should be encouraged.

## INTRODUCTION

*Mycobacterium tuberculosis* is an extraordinarily successful human pathogen that has plagued humankind for thousands to millions of years [1]. Although early theories postulated that this bacterium originated in a domestic animal, current evidence supports the idea that *M. tuberculosis* was initially a human pathogen that later spread to other hosts [2]. If the latter hypothesis is correct, both the bacterium and the human host have had ample time for coevolution and adaptation to one another. It is difficult to estimate the incidence of the disease in Europe before the seventeenth century, but, in the following centuries, tuberculosis reached epidemic levels. The discovery of the causal agent of tuberculosis was announced by Robert Koch in 1882, and anti-tuberculous antibiotics were developed beginning in the 1940s [3]. These advances, together with the implementation of appropriate social and health policies and the natural course of the disease, which spontaneously recedes over time, contributed to decreased incidence levels in industrialized countries in the twentieth century. This declining trend, however, was reversed in the United States after 1985 by the confluence of several factors, including the emergence of human immunodeficiency virus (HIV) infection and the immigration of people from tuberculosis-infected areas [4]. Now tuberculosis is a major global health issue, remaining the leading cause of death by bacterial infection [5]. The disease is transmitted via airborne particles generated by sneezing. It has been estimated that 30 percent of exposed people will become infected resulting in primary tuberculosis that frequently goes undetected. The disease will either progress over the next two years (five percent) or attain a latent form. An additional five percent will become active in the following years. This scheme is very different in HIV patients, who develop active tuberculosis much more frequently [4]. The World Health Organization (WHO) has estimated that in 2006, 9.2 million new cases were detected and 1.7 million people died of tuberculosis [6]. Prompted by this dramatic situation, the WHO developed the Stop TB Partnership, which goal is to halve the global burden of tuberculosis by 2015 and to eliminate the disease as a public health problem by 2050 [7].

## EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS (XDR TB)

Current tuberculosis therapies rely heavily on the administration of antibiotics. A combination of first-line antibiotics (rifampin, isoniazid, pyrazinamide, and ethambutol, RIPE) is the standard treatment, designed to prevent the emergence of drug-

resistant microorganisms [4, 8]. The four drugs are usually prescribed for two months, followed by four months of only rifampin and isoniazid. Despite the fact that shorter treatments are not as effective, patient compliance, even with six-month regimens, is low in many circumstances. The most striking consequence of non-compliance is the development of multi-drug-resistant tuberculosis (MDR TB). This bacterial strain is treated with second-line antibiotics, which can be grouped into six classes: aminoglycosides other than streptomycin (e.g., kanamycin), cyclic polipeptides (e.g., capreomycin), fluoroquinolones (e.g., ciprofloxacin), thioamides (e.g., prothionamide), serine analogs (e.g., cycloserine), and salicylic acid derivatives (e.g., para-aminosalicylic acid). These antibiotics are more toxic, less effective, and less studied than first-line drugs and their use is more difficult and expensive.

XDR TB is characterized by bacterial resistance against this second line of antibiotics, which may lead to lethal epidemics. Shah *et al.* [9], following WHO guidelines, have defined XDR TB as MDR TB with resistance to at least three of the six classes of second-line drugs. Extensively drug-resistant bacteria are nearly untreatable with currently available drugs and have been detected in all regions of the world, accounting for 0.6 to 15.4 percent of all MDR TB cases, depending on the world region. There are few options available for XDR TB patients, such that their mortality rate is much higher than that of MDR TB patients [9]. Antibiotics like caopreomycin and para-aminosalicylic acid, which were discarded in the past due to their potentially toxic effects, are again being considered as XDR TB treatments. Although the discovery of other antibiotics could provide new tools against drug-resistant bacteria, it is likely that *M. tuberculosis* would develop resistance to these drugs as well.

An alternative method of treating forms of tuberculosis that are not amenable to chemotherapy is strengthening the immune system using immunomodulators. Several pieces of evidence support the viability of this therapeutic approach. When a naïve population that has not had previous contact with the bacterium becomes infected, the resulting epidemic adheres to the following trend. The epidemic begins slowly due to the disease's long incubation time, followed by a rapid rise in mortality over several generations that gradually recedes as the proportion of infection-resistant people increases [10]. Although most people exposed to a high dose of *M. tuberculosis* for enough time will become infected and tuberculin skin test (TST)-positive, there is a small fraction of infected people who will remain TST-negative or take much longer to

test positive [10, 11, 12, 13]. From these reports, we may conclude that some people are able to clear a *M. tuberculosis* infection before the bacteria multiply and adaptive immunity develops. This conclusion is supported by observations made before the introduction of chemotherapy, which clearly state that a proportion of people with active tuberculosis can control the disease and may occasionally be cured [3]. In other words, the human immune system has the ability to keep tuberculosis in check, and only when this mechanism fails do the bacteria survive and multiply. It is thus reasonable to believe that biologic therapeutics that strengthen a weakened immune system may help to control disease progression. Although the high cost of such therapeutics prevents their widespread use for MDR TB, for which there still is the cheaper option of second-line antibiotics, immune-strengthening therapeutics may be the only chance for XDR TB.

## IMMUNE RESPONSE TO *M. TUBERCULOSIS*

In the current model of infection, *M. tuberculosis* invades the alveolar spaces of the lung, where it infects the macrophages that line the pulmonary epithelium. At this point, either the innate immune system destroys the bacteria or the bacteria multiply. Neutrophils migrate early to the site, followed by monocytes that mature into macrophages, giving origin to the tuberculous granuloma. After a few days, some macrophages differentiate to epithelioid cells (activated cells with abundant cytoplasm) and Langhans giant cells (macrophages fused into multinucleated cells). At 2-4 weeks post-infection, cell-mediated immunity arises and new cells are recruited to the site of infection, including lymphocytes, macrophages, and fibroblasts. This cell recruitment results in chronic inflammation and caseation of the granuloma, which develops a necrotic acellular core surrounded by macrophages, epithelioid cells, and Langhans giant cells, accompanied by an outer layer of fibroblasts and lymphocytes. The granuloma may not contain the multiplication of the bacteria, which then disseminate to other areas of the lung [14, 15, 16, 17].

Together with the human model, the murine model of tuberculosis is perhaps best characterized due to its cost efficiency, extensive knowledge of the murine immune system, and the availability of monoclonal antibodies against murine surface markers and cytokines. As a result of numerous mouse studies, the immune response to tuberculosis has been greatly elucidated, though care should be taken before extrapolating from the mouse to the human model [18]. There are important differences

between the two models, from both histopathological (e.g., murine granulomas do not caseate [19]) and molecular points-of-view (e.g., differing functions of molecules like granulysin or reactive nitrogen intermediates [18]). For example, murine macrophages may kill *M. tuberculosis* when activated with interferon-γ (IFN-γ) [20], a capacity that is not shared by human macrophages, as detailed below. Another marked divergence is that the disease is characteristically paucibacillary in the human host (latent infection), but multibacillary and eventually lethal in mice [21]. To simplify our discussion, we will focus on the human model

As already mentioned, the human immune system has the ability to clear or contain *M. tuberculosis* infection. However, this bacterial clearance mechanism is far from understood. There are three effector cells with the potential to kill microorganisms: monocytes/macrophages, neutrophils, and cytotoxic lymphocytes. Yet in vitro experiments have not provided undisputed evidence of successful elimination of *M. tuberculosis* by any of these human cell types.

**Monocytes/macrophages**

Several attempts to activate human macrophages against mycobacteria have been made, but the usual outcome is either unrestricted bacterial growth or limited growth inhibition. In early experiments, Douvas *et al.* [22] observed that both unactivated and IFN-γ-activated macrophages did not prevent multiplication of *M. tuberculosis*. Other modulators, such as calcitriol, have been claimed to activate monocytes against *M. tuberculosis* [23], but these results have not been confirmed by other groups [24, 25]. It has also been observed that tumor necrosis factor-α (TNF-α) does not help monocytes to restrict the intracellular growth of bacteria [25]. Although this review will focus on *M. tuberculosis*, we want to briefly mention an interesting in vitro study of the *M. bovis* strain BCG, which also belongs to the tuberculosis complex. In this study, the authors concluded that no single cytokine tested, including interleukin-1 (IL-1) to -15 (with the exception of IL-9, -11, and -14) and colony-stimulating factors (CSFs), could induce killing activity against BCG in human macrophages [26].

Macrophage activation has also been attempted by coculture with lymphocytes and natural killer (NK) cells. Silver *et al.* observed growth inhibition in human monocytes cultivated in the presence of $CD4^+$ T cells [27], while Bonecini-Almeida *et al.* were able to induce microbicidal activity in macrophages by incubating with primed lymphocytes and IFN-γ [28]. Similarly, Yoneda and Ellner described induction of

microbicidal activity in macrophages by incubation with NK cells [29], although Brill *et al.* found that while the NK cells induced some microbicidal activity, it was only before 24 hours, after which surviving bacteria continued to multiply [30].

Induction of killing activity has also been reported for several molecules, including Toll-like receptor 2 (TLR2) ligands, like the 19-kDa lipoprotein of *M. tuberculosis* [31], adenosine triphosphate (ATP) [32], or sphingosine 1-phosphate [33]. Boechat *et al.* have also reported controlling mycobacterial growth by increasing the density of the macrophage culture [34]. Unfortunately, the problem with all of these claims is that it has been difficult to replicate the results, possibly due to variations in bacterial species or strains, infection methods, or cell sources. For instance, we and others have not identified any microbicidal activity in macrophages against non-pathogenic mycobacteria like *M. phlei* or *M. gordonae* [35, 36].

**Neutrophils**

Investigators have paid neutrophils little attention because of their early disappearance from the nascent tuberculous granuloma. The inverse association between peripheral blood neutrophils count and TB risk has provided, however, evidence of their in vivo importance in stemming TB infection [37]. However, no clear anti-mycobacterial activity has been detected in these cells in vitro. While some groups have observed successful activation of neutrophils against *M. tuberculosis* [38, 39, 40, 41], other authors have been unable to confirm these results [36, 42, 43]. Nevertheless, a recent report has suggested an interesting interaction between macrophages and neutrophils, in which apoptotic neutrophils are phagocyted by infected macrophages. The authors concluded that acquisition of the granules present in the apoptotic neutrophils confers antimicrobial activity on the macrophages [44]. This kind of complex model may provide a more realistic view of the immune response to *M. tuberculosis*, and may also explain the lack of microbicidal activity when isolated cells are infected in vitro. Successful innate immune responses against mycobacteria may require the coordinated participation of several cell types exposed to a specific milieu of cytokines. The identification of these multifaceted events will therefore be very complicated.

**Cytotoxic lymphocytes**

A third kind of effector cell has been suggested to participate in the direct killing of *M. tuberculosis*: namely cytotoxic lymphocytes, including T cells and natural killer T (NKT) cells. The killing mechanism of these $CD8^+$ T cells is based on the lytic activity of antimicrobial peptides [45]. The molecule responsible for this cytotoxic activity is granulysin, which acts against both extracellular and intracellular bacteria. The participation of another protein, perforin, is also necessary for killing intracellular bacteria, since it grants granulysin access to the cytosol by creating pores in the cell membrane [46]. Other cells, including γδ lymphocytes [47] and NKT cells [48], also demonstrate similar antimicrobial abilities.

Both the innate and acquired immune responses make use of these effector cells to eliminate *M. tuberculosis*. The difficulties of observing mycobactericidal activity in vitro, however, have hampered our ability to identify the specific mechanisms that render a proportion of people tuberculosis-resistant. Nevertheless, the knowledge that we have acquired from these in vitro studies has encouraged us to develop therapeutic approaches based on immunomodulators.

## POTENTIAL ALTERNATIVES TO CHEMOTHERAPY IN TUBERCULOSIS: IMMUNOMODULATORS

For decades, chemotherapy was deemed the appropriate way to treat tuberculosis. Although MDR TB has a poor prognosis, there are some effective antibiotics also for these drug-resistant strains. However, there are few chemotherapy options for XDR TB, so its mortality rate is high. Immunomodulation is now emerging as one promising therapeutic alternative. This approach is based on the belief that a particular microbe causes disease in an organism due to the host's susceptibility [49], rather than due to the characteristics of the microbe alone. Bolstering the weakened immune system of the host may thus restore the equilibrium broken by the infection. Immunomodulators could be used for this purpose (immunostimulators), although they may be also useful in decreasing an exacerbated immune response (immunosuppresors). Some of the current immunomodulators comprise microbial products, drugs of natural or synthetic origin, or proteins derived from the immune system [50]. Microbial products like bacterial lysates have already been used to prevent recurrent infection in the lungs. Vaccines, usually made with microbial products, are also intended to promote the adaptive response to infection. Drugs with immunomodulatory properties include analog peptides of thymic hormones, muramylpeptides, or glucans [50]. Probiotics (live

bacteria derived from the human gastrointestinal tract) may be used to inhibit inflammatory responses to infectious disease at mucosal surfaces. Corticosteroids may also be used as an adjunct to these antimicrobial therapies [49]. Antibiotics, besides their anti-infective capacity, may also have immunomodulatory properties [51]. The remainder of this review will focus on one last group of immunomodulators derived from the immune system, known as cytokines.

## IMMUNOMODULATION BY CYTOKINES

Cytokines are small proteins that may influence cell behaviour or properties in an autocrine or paracrine manner. Cytokine-secreting cells may belong to the immune system, although cytokines are produced by other cell types as well. The biological activities of each cytokine are manifold and may have profound influences on the immune response. The roles of cytokines may be classified based on the secretion pattern of two types of T helper cells, Th1 and Th2 cells. Th1 cells secrete IFN-γ, IL-2, and lymphotoxin, while Th2 cells produce IL-4, -5, -6, -9, -10 and -13 [52]. In tuberculosis, the protective immune response is considered cellular, driven by Th1 cells secreting IFN-γ as discussed earlier. The central role of Th1 cytokines in tuberculosis may be confirmed by analyzing individuals who are highly susceptible to mycobacteriosis. Some of these patients have genetic mutations in the genes for IL-12/23, IFN-γ, or their respective receptors [53]. In recent years, there is also a mounting interest in regulatory T cells, which are hypothesized to participate in the suppression of antigen-specific immune responses against infectious diseases like tuberculosis [54]. The contribution of several cytokines to the development of tuberculosis and their possible clinical applications as immunomodulators will be described below. For the sake of brevity, we will restrict our discussion to studies of *M. tuberculosis*. Patents related to these cytokines and their use as therapeutic tools in tuberculosis will also be discussed (Table 1).

### IFN-γ

In early studies, macrophages, the main cell target of *M. tuberculosis*, were found to be activated by a poorly characterized macrophage activation factor (MAF) that was present in lymphocyte culture supernatants [55]. MAF enhanced the antimicrobial properties of macrophages [56, 57] and was later identified as IFN-γ [58], which was initially described as an agent inhibiting viral replication [59]. The role of IFN-γ in fighting human tuberculosis has been controversial, since it does not activate

human monocytes/macrophages against mycobacteria in vitro [22, 36]. However, its importance is now indisputable, based on the finding that mutations in the IFN-γ receptor genes make the patient highly susceptible to mycobacteriosis [53].

IFN-γ is the prototypical product of Th1 cells, promoting the secretion of additional Th1 cytokines, like IL-12, and inhibiting the synthesis of Th2 cytokines, such as IL-4. IFN-γ and IL-12 coordinate pathogen recognition by innate immune cells and the induction of adaptive immunity by means of a positive feedback loop, which amplifies the Th1 response. More specifically, pathogen-associated molecular patterns recognized by macrophages, dendritic cells, and neutrophils induce the production of IL-12, which in turn stimulates T and NK cells to produce IFN-γ. This cytokine provokes several changes in macrophages, including the up-regulation of class I and II antigen-presentation pathways and the activation of microbicidal effector functions, which are of particular interest in tuberculosis. These microbicidal functions include the induction of the NADPH-dependent phagocyte oxidase system; the production of reactive nitrogen intermediates, which is important in mice but plays an uncertain role in humans; and the promotion of antibody-dependent cell-mediated cytotoxicity and tryptophan depletion in activated cells [60]. This positive feedback loop may be enhanced by IL-18, a cytokine that shares some biological activities with IL-12.

The biological activity of IFN-γ renders it a good candidate for treating tuberculosis. However, few studies have been published investigating its clinical use [61]. The first trial was performed by Condos *et al.* using aerosolized IFN-γ in only five patients [62]. After initial improvement, characterized by negative acid-fast bacillus smears, the treatment was discontinued and the patients became bacillus-positive again. Several more clinical trials of IFN-γ have been conducted since, but usually with eight or fewer MDR TB patients and in conjunction with chemotherapy. Results have been variable: in one study, intramuscular IFN-γ improved patients' conditions [63], but in others, the results were predominantly negative, whether IFN-γ was administered as an aerosol [64], intravenously [65], or subcutaneously [66]. Additionally, a controlled trial of inhaled adjunctive IFN-γ, initiated by InterMune, was halted prematurely due to a lack of efficacy [61]. Regarding the reports with good outcomes, we must be aware that small sample sizes tend to exaggerate intervention effects, especially when there is inadequate or no double-blinding [67], as in these studies. Currently, another controlled clinical trial, started in April 2005, is being sponsored by the National Heart, Lung, and

Blood Institute. This trial is analyzing the effects of IFN-γ on cavitary pulmonary tuberculosis and comparing the following treatments: IRPE anti-tuberculous therapy, aerosolized IFN-γ, and subcutaneous IFN-γ [68].

While no clear conclusions may be obtained from these data, it seems that IFN-γ by itself is not very effective for bolstering the immune response to pulmonary tuberculosis. This observation is unexpected, given the established importance of this cytokine in tuberculosis. Larger controlled studies will be necessary to confirm this finding, which is already supported by the inability of IFN-γ to activate human macrophages in vitro [22, 36]. Thus, while IFN-γ is necessary in the immune response against tuberculosis, it may not be sufficient to clear the infection.

**IFN-α and IFN-ω**

Immunologists concerned with tuberculosis treatment have concentrated their attention on IFN-γ, in part because IFN-α/β are classically associated with antiviral activities. Nevertheless, the importance of the latter interferons in non-viral infections is increasingly being recognized. These cytokines may affect the function of dendritic and Th1 cells, the cross-priming of $CD8^+$ T cells, or the activation/deactivation of macrophages [69]. IFN-α is, in fact, the name of a family of proteins codified by 12 different genes, with each protein exhibiting a distinct profile of activities. The proteins most often used clinically, IFN-α2a, IFN-α2b, and IFN-α2c, are encoded by the gene *IFNA2* and are already commercially available [70]. The immunological activity of other proteins in the IFN-α family makes them attractive candidates for use in tuberculosis therapy.

Few trials have been performed using IFN-α for tuberculosis treatment. Giosuè *et al*. administered aerosolized IFN-α to patients already being treated with chemotherapy and observed an improvement in their condition [71, 72], although their sputum analysis remained positive. In another report of five patients treated subcutaneously with IFN-α2b, two patients became culture-negative, one had clinical improvements, and two did not respond [73]. As in the case of the IFN-γ trials, the number of treated patients is too small to render the results conclusive. Furthermore, recent reports indicate that problems may arise in patients with concomitant tuberculosis and other diseases who are treated with IFN-α, although no clear correlation between the cytokine-based therapy and these negative outcomes has been established [74, 75].

Little information is available about IFN-ω, which shares several biological functions with the IFN-α/β family [70]. A recent patent, however, proposes its use in treating intracellular bacterial diseases, including tuberculosis [76]. Yet as we have already indicated for IFN-α, while many cytokines have the potential to be useful in tuberculosis therapy, a lack of biological information and research may prevent their clinical use. This situation will be observed for several cytokine-related patents that are later mentioned in this review.

**TNF-α**

One of the most important proinflammatory cytokines identified in the immune response to tuberculosis is TNF-α. Infection of monocytes/macrophages and dendritic cells with *M. tuberculosis* in vitro induces the production of TNF-α, which is also present at the site of disease in tuberculous patients. Nevertheless, no association has been found between TNF-α gene polymorphism and disease susceptibility [77]. The role of TNF-α in tuberculosis is unclear, but there is evidence that supports its role in coordinating the formation of the tuberculous granuloma by inducing the production of chemokines and chemokine receptors [78]. However, the therapeutic use of TNF-α to promote granuloma formation, which considered a positive defence mechanism in tuberculosis, is dangerous for patients. This cytokine participates in severe inflammatory conditions and may have toxic effects [79]. Nevertheless, its importance in fighting infection is underscored by the use of TNF-α-blocking agents, which promote the development of several infectious diseases, and in particular tuberculosis [80].

**GM-CSF**

In an early communication, it was reported that human macrophages activated with granulocyte macrophage CSF (GM-CSF) exhibited antimicrobial activity against *M. tuberculosis* [81], but we are neither aware of any further study confirming this result, nor have we been able to replicate this finding. Nevertheless, GM-CSF induction of antimicrobial activities in both neutrophils and macrophages, as well as the secretion of proinflammatory cytokines, has been shown [82]. Due to these properties, the use of GM-CSF in the treatment of infections should be investigated, as recommended by a recent patent [83]. Thus far, one phase-II controlled clinical trial of GM-CSF used in conjunction with chemotherapy has been performed. Results showed no major

differences between the treatment and control groups, although faster clearance of acid-fast bacilli in sputum was observed in the GM-CSF treatment group [84].

**IL-12**

IL-12 and IFN-γ contribute to a positive induction loop that enhances the activation state of macrophages, as explained earlier. Its importance in fighting tuberculosis is critical, as may be deduced from studies examining mutations in genes of the IL-12/23-IFN γ system [53], which revealed that the subunit IL-12p40 (which is also part of IL-23) was necessary for protective IFN-γ responses to *M. tuberculosis* to arise. Additional investigations have found that the chemokine responsiveness of lung dendritic cells was also reduced in the absence of IL-12p40 [85].

IL-12 is raising enormous interest among immunologists researching tuberculosis, which is reflected by the large number of patents concerning IL-12 for the treatment of this and similar diseases. The purpose of one of these patents is to enhance the immunostimulatory effect of IL-12 in vaccine therapy [86]. To reduce the cytokine's toxicity, the authors propose to co-administer a nitric oxide-neutralizing agent. Another patent seeks to encourage a similar immunomodulatory effect in neonates by using IL-12 as a vaccine co-adjuvant [87]. Other patents intend to improve the bioactivity of the cytokine by administering it together with other agents, such as IFN-α, which may synergize with IL-12. The authors of one patent suggest that the use of sub-optimal doses of both cytokines may promote effective protection against bacterial diseases [88]. Another option is the co-administration of IL-12 and thalidomide [89], which has recognized anti-inflammatory and immunomodulatory effects, including suppression of TNF-α production and elevation of IFN-γ [90]. Yet another interesting patent describes the use of a mutated subunit of IL-12p40 as a DNA vaccine adjuvant. The indicated mutation affects Asn-222, a glycosylation site, in the hope of promoting the expression of active IL-12p70, the IL-12 protein constituted by the IL-12p40 and IL-12p35 subunits, while decreasing the secretion of IL-12p40, a subunit that may antagonize IL-12p70 responses at high levels [91].

**IL-2**

For a long time, IL-2 has been used to induce T-cell expansion in vitro and has been assumed to do the same in vivo. Under this assumption, therapeutic IL-2-based strategies were designed. However, in the last few years, a new role is being assigned to

this cytokine: the development and peripheral expansion of $CD4^+$ $CD25^+$ regulatory T cells, which suppress T-cell responses [92]. This may explain why no clear benefits were reaped from IL-2 therapy for tuberculous patients [93, 94, 95, 96]. A patent was recently issued in which the authors describe a protocol to enhance the immune response by intermittent administration of IL-2, combined with other therapies [97]. Yet a similar regimen of IL-2 administration was implemented in one of the cited trials with no success [93].

**IL-4 and TGF-β**

Other cytokines are important in the persistence of tuberculosis because they have anti-inflammatory properties and are characteristic of a Th2 response [77]. Some patents attempt to antagonize the activity of these cytokines. In one patent, the authors intend to inhibit IL-4 by fusing the IL-4 receptor to the Fc portion of an antibody, thus blocking IL-4 binding [98]. The proposed therapeutic regimen is completed by co-administration of IFN-γ. Similarly, another patent aims to inhibit TGF-β by blocking the cytokine or its receptor using antibodies [99].

**Other interleukins**

Several other patents attempt to use interleukins to treat microbial infections, including tuberculosis. We will briefly mention patents that implement IL-7, -11, -16, and -17.

The best-understood role of IL-7 is its participation in T-cell development. In humans, a defect in the IL-7 receptor results in severe combined immunodeficiency (SCID) and is being studied for therapeutic use in HIV infection [100]. IL-7 has been shown to be present in significant amounts in the bronchoalveolar fluid of patients with acquired immunodeficiency syndrome (AIDS)-associated tuberculosis [101]. This interleukin also promotes the secretion of several proinflammatory cytokines by human monocytes [102], which is the basis of one patent that proposes the co-administration of IL-7 and IFN-γ for therapeutic purposes [103].

In addition to its roles in platelet formation and inhibition of epithelial-cell multiplication, IL-11 modulates cytokine production by monocytes/macrophages, down-regulating the production of TNF-α, IL-1β, and IL-12 [104]. The role of IL-11 in the enhancement of T-cell cytotoxic activity and the down-regulation of human

monocytes is utilized in one patent for clinical applications, including the treatment of tuberculosis [105].

Lymphocyte chemoattractant factor, also known as IL-16, attracts $CD4^+$ cells, including T cells, certain monocytes, eosinophils, and other cells. Although IL-16 does not induce T-cell proliferation by itself, it augments this effect in cooperation with IL-2 and IL-15 [106]. Significantly elevated levels of IL-16 have been detected in tuberculosis patients [107]. A patent concerned with treating disorders typified by a granulomatous immune reaction, like tuberculosis, has been issued. The authors consider the granuloma a hyperresponsive immune reaction that may be mitigated by the use of antagonists or antibodies against IL-16 [108].

Unlike the last few cytokines, the role of IL-17 in tuberculosis disease progression is better characterized. IL-17 is a proinflammatory cytokine that promotes the secretion of chemokines that attract neutrophils and other leukocytes. However, the implication of this cytokine in the formation of the tuberculous granuloma is still unclear [109]. The utilisation of reagents that modulate the function of IL-17, which may be of interest in tuberculosis, is exemplified by a patent focused on IL-17 receptor-like proteins [110].

**CCL13**

Chemokine ligand 13 (CCL13) is a protein that promotes leukocyte chemotaxis. Few attempts have been made to test chemokines as therapeutic agents, although their critical involvement in the immune response is widely acknowledged. Only a handful of chemokines have been analyzed in the context of tuberculosis, but their importance is clear [78]. These chemokines not only regulate the cellular traffic that controls granuloma formation, but also may activate macrophages to restrict mycobacterial growth [111]. Some chemokines have similarities to defensins, exhibiting antimicrobial activity [112]. Several years ago, two patents suggested the use of chemokine β10 (MCP-4, CCL13) for treating infective diseases, including tuberculosis, given its participation in the regulation of monocyte chemotaxis [113, 114].

## CURRENT & FUTURE DEVELOPMENTS

In this review we have described only cytokines with patented uses that may have therapeutic relevance to tuberculosis, with the exception of TNF-α, for which no patents have been issued. There are several other cytokines that are known to be

important in tuberculosis, including IL-10, -18, and -23, which we have not mentioned. Few of these have been studied in clinical trials as co-adjuvants of chemotherapy, and results have not been impressive. It seems unlikely that the administration of a single cytokine will be sufficient to fight *M. tuberculosis*, which is a very sophisticated microorganism that has adapted admirably to its human host. Although more clinical trials are necessary, designed based on our current knowledge, we must also encourage further basic research. We need to better understand the sequence of events that take place after the bacterium reaches the alveolar space, as well as the mechanisms used by the adaptive immune system to check mycobacterial multiplication in 90% of infected people. Although cytokines like IFN-γ, IL-2, or GM-CSF are known to be important in the immune response to tuberculosis, clinical experience has demonstrated that they are not enough to contain active tuberculosis. Thus, even though IFN-γ is used as a surrogate marker of protective immunity, it has a small predictive value, and the importance of other cytokines that concur in the defence mechanisms, like IL-17, may need to be considered [115]. The in-depth analysis of other cytokines, like IFN-α/β, and chemokines may yield new therapeutic tools. As already discussed, the occasional efficacy of the immune system in fighting tuberculosis infection may guarantee that elucidation of the immune response will inform tuberculosis treatment. Cytokines have enormous potential in clinical use, but they are multifaceted proteins, some of which are toxic at high doses, that need to be accurately administered to provide therapeutic benefits with little harm. High cost precludes their use in MDR TB, which may be controlled by cheaper antibiotics, but the cytokine-based therapies may be one of the last options in XDR TB.

**ACKNOWLEDGMENTS**

Dr. Rivero-Lezcano is a member of the Foundation Institute of Studies in Health Sciences and participates in the SACYL research program. This work was supported by grants LE07/04 from Junta de Castilla y León and PI051288 from Instituto de Salud Carlos III-FIS (Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica). The author has no potential conflict of interest to declare.

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**Table 1. Patented uses of cytokines with applications in tuberculosis.**

| Cytokine | Function | Patent number[a] | Clinical trials references |
|---|---|---|---|
| IFN-γ | Induces macrophage antimicrobial activity | | [61], [62], [63], [64], [65], [66], [68] |
| IFN-α | Influences dendritic and Th1 lymphocytes. Activates macrophages | | [71], [72], [73] |
| IFN-ω | Shares functional properties with IFN-α | WO05039614 | |
| GM-CSF | Stimulates antimicrobial activities in both macrophages and neutrophils | US5162111 | [84] |
| IL-12 | Induces T and NK cells to produce IFN-γ | US20026375944, US5985264, US5928636, WO000072836, US20077253151 | |
| IL-2 | Promotes expansion of T cells, including $CD4^+$ $CD25^+$ regulatory T cells | US20016190656 | [93], [94], [95], [96] |
| IL-4 | Anti-inflammatory properties. Characteristic of a Th2 response | US20016210661 | |
| TGF-β | Anti-inflammatory properties | US5730976 | |
| IL-7 | Participates in T cell development and promotes the secretion of proinflammatory cytokines by monocytes | US5681557 | |
| IL-11 | Modulates cytokine production by monocytes/macrophages | WO049937322 | |
| IL-16 | Attracts $CD4^+$ cells, including $CD4^+$ monocytes | US20006159463 | |
| IL-17 | Proinflammatory cytokine that promotes the secretion of chemokines | US20067094566 | |
| CCL13 | Regulates cellular traffic | WO031467, WO01094557 | |

[a]The first two letters in the patent number correspond to Patent Corporation Treaty (PCT) contracting states: US, United States of America; WO, World Intellectual Property Organization.