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Anti TNFalpha immunomodulators and susceptibility to infectious diseases

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Anti TNFalpha immunomodulators and susceptibility to infectious diseases

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Anti TNFalpha immunomodulators and susceptibility to infectious diseases

Abstract

Tumor necrosis factor-α is a pleiotropic cytokine involved in a variety of immunological processes. It plays a major role in protective immunity, intervening in the control of infections, especially in the defense against intracellular microorganisms. However, it can also be implicated in a number of pathological processes, as is the case of autoimmune diseases.

The introduction of anti-TNF-α biological therapy constituted a major progress in the treatment of many autoimmune diseases. However, as the usage of these drugs expanded several reports of infections have been increasingly reported in patients taking these medications. Nonetheless, the current evidence does not offer undoubtable proof of a causal relationship between pharmacologic TNF-α's inactivation and the emergence of different infections.

In this work, the authors review TNF-α's general biological functions and mechanisms of action, and summarize the evidences found in literature regarding the role and importance of TNF in the immune defense against different microorganisms, particularly *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Listeria monocitogenis*, *Histoplasma capsulatum*, *Candida*, *Leishmania*, *Hepatitis B virus* and *Herpes simplex virus*.

Key Words

Biological Therapy; Drug-Related Side Effects and Adverse Reactions; Immunotherapy; Infection; Tumor Necrosis Factor-alpha

Introduction

Tumor Necrosis Factor-α (TNF-α) is a cytokine with multiple effects of importance in inflammation and immune functions.[1] In fact, it has been found to contribute to the immune defense against several pathogens, including virus (e.g. *Herpes virus*, *Hepatitis virus*), bacteria (such as *mycobacteria* and intracellular bacteria, like *Listeria*), fungi (e.g. *Candida*, *Aspergillus*, *Blastomyces* and *Histoplasma*) and parasites.[2] Moreover, due to its strong pro-inflammatory and immunostimulatory activities, TNF is, in general, an important mediator of progression of many autoimmune diseases, including rheumatoid arthritis and inflammatory bowel disease; significant clinical improvement can be achieved in these contexts when patients are treated with TNF-α neutralizing agents.[3]

Development of TNF inhibitors and its introduction in clinical practice was a significant milestone for the treatment of selected autoimmune diseases. Currently, there are five approved anti-TNFα agents, including infliximab, etanercept, adalimumab, certolizumab pegol and golimumab.[4,5] All of the approved anti-TNF agents, except for eternacept, are full-length bivalent IgG monoclonal antibodies or monovalent Fab antibody fragments. Etanercept is a genetically engineered fusion protein consisting of an Fc fragment of human IgG1 fused to a dimer of the extracellular part of human TNF-receptor (TNFR) 2. Infliximab is a chimeric protein, 25% of which are constituted by mouse-derived amino acids, with human-derived amino acids forming the remaining 75%. Certolizumab is a humanized protein, containing amino acid sequences derived from a mouse's anti-TNF monoclonal antibody, inserted into human V_H

and V_L domains; the hinge region of certolizumab is covalently linked to polyethileno glycol, which ensures better solubility and half-life prolongation; additionally, since it is a Fab fragment and has no Fc region, it lacks effector functions. Adalimumab and golimumab are fully human monoclonal antibodies.

TNF inhibitors revolutionized the treatment of many autoimmune conditions, being especially advantageous to the patients resistant to first-line treatments. Due to their ability to decrease the inflammatory parameters associated with the autoimmune disease, anti-TNF drugs allowed these patients, who otherwise would have a more active disease, to have a beter quality of life.

However, ever since the introduction of anti-TNF drugs, several reports have claimed an increased incidence of infectious diseases in these patients, suggesting a probable association of this findings to the therapy in question; among the reported, are included opportunist infections, such as *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Leishmania*, *Toxoplasma gondii*, *Histoplasma capsulatum*, *Candida* and *Aspergillus* infections. [2,3,6]

Therefore, in order to prevent these secondary infections, it is essential to improve our knowledge of TNF-α's actions and the mechanisms through which it influences the immune responses to different pathogens. Several experimental studies have already been performed in this area, mostly using animal and *in vitro* models, in order to understand the link between TNF and infection. This review of the literature aims to comprehensibly summarize the mechanisms through which TNF contributes to the immunological defense against different pathogens, as well as to revise the consequences of its inhibition using biologic anti-TNF drugs.

Overview of TNF's functions

TNF is a pleiotropic factor synthetized mainly by activated macrophages in response to various stimuli (bacteria, viruses, complement factors, ischemia, hypoxia, injury and cytokines, such as IL-1, IL-17, TNF, GM-CSF or IFN-γ)[7], but also by a broad variety of other tissues, including lymphoid cells, mast cells, endothelial cells, fibroblasts and neuronal tissue. [7,8,3] It functions as a key regulator of leukocyte trafficking and its effects include increased vascular permeability, migration and maturation of dendritic cells, antigen processing, activation of macrophages and nitric oxide production, induction of its own secretion and upregulation of other molecules, such as: proinflammatory cytokines (e.g., INF-γ in T-lymphocytes and NK-cells), chemokines, prostaglandins, adhesion molecules and major histocompatibility complex molecules. [9,10] The proteins induced by TNF include IL-6, IL-8, GM-CSF, M-CSF, plasminogen's activator inhibitor and platelet activating factor; among new antigens appearing on the endothelial membrane are the adhesion molecule E-selectine, membrane-bound IL-1 and enhancement of lymphocyte adhesion molecule ICAM-1 and VCAM-1; the enzymes inducible nitric oxide synthase (iNOS) and COX-2 are also activated.[11,12]

TNF directs leucocyte movement through several mechanisms, including its action on the vascular endothelium and its ability to establish gradients for chemokines, such as CCL2 (MCP1), CCL3 (MIP1a), CCL4 (MIP1b), CCL5 (RANTES), CXCL10, and CXCL13.[13] Furthermore, its signal results in leukocytes' cytoskeletal re-organization, filopodium formation and macropinocitosis, ultimately enabling the process of leukocyte migration. [9]

TNF has a cytotoxic action and causes necrosis and lysis of the target cell; nonetheless, this cytokine can also induce cell death through other pathways. In fact, in some cell types, TNF's action leads to apoptosis. The induction of apoptosis by TNF-α is mainly mediated by activation of TNFR1, which results in activation of pro-caspase 8. [14,3] Additionally, on a number of cells, especially primary fibroblasts, TNF exerts a mitogenic activity, presumably through induction of proteins that promote cell cycle. [14,12] IL-10 is induced by TNF-α and, in turn, also inhibits its synthesis, therefore being involved in a negative feedback loop.[4]

There are two biological forms of TNF: transmembrane-TNF and soluble-TNF; both forms function physiologically by interacting with the TNF-receptors 1 and 2. Membrane-bound TNF may be cleaved by tumor necrosis factor alpha-converting enzyme, a matrix metalloprotease, which leads to the release of soluble TNF.[8] TNF-α binds as homotrimers to TNF receptor 1 (p55) and TNF receptor 2 (p75). [15] TNF-receptors are present on nearly all cell types, with a few exceptions (for example, erythrocytes); TNFR1 seems to be ubiquitous and occurs, among others, on epithelial cells and fibroblasts; on the other hand, TNFR2 seems to be more restricted to immune system cells (for example, it is strongly expressed upon T-cells' induction). [12] When TNF binds to TNFR1, the receptor's conformation is changed in order that its death domain can interact with TNFR-associated factor containing death domains (TRADD), which in turn recruit TNFR-associated factors (TRAFs), including TRAF2 and TRAF5, as well as the cellular inhibitor of apoptosis proteins 1 and 2 (c-IAP1/2), in order to form the TNF-receptor signaling complex (TNF-RSC). Subsequent steps lead to translocation of NF-kB to the nucleus, where it initiates the transcription of more than 200 NF-kB-dependent genes, including cell-survival genes, pro-inflammatory cytokines, chemokines, growth-factors and TNF-α itself; TNF signalling also activates a heterodimer called Activation Protein 1 (AP-1), which is a critical transcription factor itself and has similar functions to NF-kB. [14,3] TNFR2 does not contain a death domain, but is able to form a complex with TRAF2 and TRAF5 upon stimulation with TNF, leading to activation of NF-kB and AP-1. [14,3] Therefore, there is a complex interaction and cross-talk between TNFR1 and TNFR2 signaling.[16]

TNF and granuloma formation/ defense against *Micobacterium tuberculosis*

TNF is believed to be essential in the maintenance of granulomas.[17,18] In fact, as both experimental and observational studies have shown, its downregulation could lead to an increased susceptibility to granulomatous infections, such as tuberculosis, histoplasmosis or listeriosis. [17,18]

TNF has been shown to be fundamentally involved in cellular immunity against intracellular pathogens, as exemplified by its role in regulating immunity against mycobacterium species, particularly in tuberculosis by *Micobacterium tuberculosis* (*M. tuberculosis*). [19]

The first contact of the host with *M. tuberculosis* can have three possible outcomes: the person becomes infected and develops symptoms of the disease (primary infection); the immune system is capable of controlling the bacteria, eliminating it from the host; the immune system can partially control the pathogen and *M. tuberculosis* becomes dormant (latent infection), although symptoms may possibly develop sometime in the future (secondary infection). [20] Not everyone with a latent infection will manifest the clinical disease, and only about 10% of them will have a reactivation of tuberculosis. [20]

Infection by *M. tuberculosis* results in the recruitment of host macrophages, which phagocyte the bacteria and then transit into deeper tissues. There, the infected macrophages initiate the inflammatory response, which includes production of TNF, IL-12, IL-1, IL-6 and various chemokines [21], along with recruitment of additional macrophages and other immune cells (e.g. monocytes, neutrophils and dendritic cells). [22] At this point, the bacilli are likely taken up by dendritic cells, which then traffic to lung-draining lymph nodes and prime naive T-cells. [22] Effector T-cells move to the lungs to help forming tightly aggregated immune structures, called granulomas; this is one of the TNF-α major functions.[16,20] On the effector side, TNF supports apoptosis of infected macrophages, activated cytotoxic T-cell are able to kill the bacilli through phagocytosis, and there is an induction of reactive nitrogen intermediates' (RNI) production.[16,13]

The downregulation of the immune system can play a part in the *M. tuberculosis*'s reactivation, as probably does the inhibitory action of an array of cytokines essential in tuberculosis control. Patients taking anti-TNF drugs have increased risk of reactivating latent tuberculosis or developing a tuberculosis infection *de novo*. [23]

Early experimental works evidenced TNF-α's importance in the defense against *Mycobacterium* infection by showing that experimental models of TNF's inhibition lead to greater susceptibility to infection by this pathogen. Mice lacking TNF-α have been shown to have a much higher susceptibility to *M. tuberculosis* and to succumb to infection, with higher loads of bacteria, when compared to wild-type mice.[24,25] Furthermore, the cellular recruitment has been shown to be significantly delayed in the TNF-negative mice, with higher inflammatory cell levels and appearance of granulomas at a later time during the infectious process, in comparison to wild-type mice.[25,24] The induction of chemokines (MIP-1bβ, MIP-2, MCP-1 and eotaxin) has also been shown to follow a similar pattern: at first it is delayed in mice lacking TNF, but after some time it peaks, reaching higher levels than in wild-type mice. [25]

Nevertheless, although some experimental studies [24,25] showed that formation of granulomas in TNF-depleted mice occurs in equal numbers to control mice, their characteristics differed from those in the latter group: the cells did not form tight clusters; and the granulomas were less organized and lacked differentiated cells (e.g. epithelioid cells). Furthermore, some studies found that granulomas formed without TNF signaling displayed a larger size, which was thought to be caused by an increase in macrophages, possibly lead by the higher bacterial burden observed in this context. [26]

Other *in vivo* studies that followed have led to the prevailing view that TNF is responsible for granuloma formation and maintenance. [26] This hypothesis is consistent with its well-known role in orchestrating macrophage trafficking and leukocytes movement during inflammation. However, subsequent experimentation showed that TNF is likely unnecessary for the formation of granulomas; additionally, TNF's absence was shown to be responsible for the loss of previously formed granuloma, as it resulted in increased granuloma expansion and accelerated necrosis of participating macrophages due to higher intracellular bacterial burdens.[26]

There have also been studies evaluating the production of nitric oxide and RNI, considering this is a known mechanism by which macrophages control mycobacteria. [24] iNOS seems to be induced by INF-γ, while TNF-α exerts a co-stimulatory activation.[20,16] When macrophages lacking TNF-receptor are

stimulated with TNF and IFN- γ, they do not evidence an increase in the production of nitric oxide, as opposed to wild-type macrophages that respond to this stimuli. [24] While wild-type macrophages produce substantial spontaneous RNI upon infection, macrophages lacking TNF-receptor do not do so in the absence of alternative stimulation.[24,16] Nevertheless, a few days after the beginning of infection, TNF-negative mice and control-mice produced almost equivalent amounts of spontaneous RNI. [24] Furthermore, other experimental works also found no difference in expression of iNOS or RNI between knock-out and control groups. [16]

Classically, granulomas are thought to preform three basic functions: they are a structure that physically gathers pathogens and immune cells together, facilitating the interaction of immune cells in order to kill the bacteria; they form a barrier that prevents the spread of bacilli to other areas; and they also prevent the spreading of inflammation. [22,21]

However, contradicting these theories, granulomas have recently been hypothesized to contribute to the spreading of bacteria and growth of bacterial burden.[27,26] Indeed it seems that bacillus benefit from this structure, which appears to work as a "shelter" until conditions are favorable for growth and reactivation. In fact, although a large portion of the bacilli within the granuloma can likely be killed, a few are able to survive and under certain conditions they may reactivate.[21] In the absence of TNF, studies have shown an increased death of granuloma macrophages by a non-apoptotic way, which was associated with a higher mycobacterial burden. Regardless of whether increased macrophage death is a primary or secondary effect of the loss of TNF signaling, it confers a further growth advantage to the bacteria by rendering them extracellular. Additionally, bacteria released from dead granuloma macrophages can subsequently be ingested by other macrophages, thus perpetuating the infection. [26]

Rather than being a cytokine responsible for starting and upregulating the immune response against intracellular pathogens, experiments with mycobacterium have suggested that TNF is actually important in its downregulation; this suggests that the lack of TNF would ultimately lead to an increased Th1 immune response, with higher levels of IFN-γ and IL-12. [16,28] In an experimental model of mycobacterial infection, it was found that TNF-deficient mice had an immune response characterized by expansion and activation of $CD4^+$ and $CD8^+$ T-cells, overproduction of IFN-γ and IL-12, and simultaneous disintegration and degradation of granuloma and lung structure.[29] TNF was concluded to be a critical negative regulator of cellular immunity, at least in part by suppressing T-cell proliferation during intracellular mycobacterial infection. It became clear that most of the pathology stemmed not from the increased number of mycobacteria but most likely from a deregulation of the immune response to infection.[30]

Regarding latent infection, some studies evidenced that TNF-α is expressed in *Mycobacterium tuberculosis* infected tissues throughout the quiescent phase of infection. [31] TNF has not only been involved in response to acute *mycobacterium* infection, but has also been shown to play a role in chronic persistent tuberculosis and its reactivation.[31] On TNF-α-deficient mice with chronic persistent tuberculosis, receiving anti-tuberculosis drugs, the drugs' cessation resulted in a massive spontaneous reactivation of the infection, with necrotic pneumonia and death; contrary to that, wild-type mice displayed mild subclinical reactivation. [31]. Experiments using anti-TNF antibodies or soluble TNF-receptor were able to reactivate latent infection. Disease recrudescence was associated with: moderately

increased bacterial burden and 100% mortality; altered levels of specific genes in the lungs (increased IL-10 and decreased iNOS expression); and severe pulmonary infiltration of inflammatory cells. [16,31]

TNF-α's role in other bacterial infections

Listeria monocytogenes is a facultative intracellular coccobacillus that is particularly prevalent in immunocompromised patients; immunity in this context is due to rapid activation of neutrophils and macrophages, followed by activation of specific $CD4^+$ and $CD8^+$ T-cells. [8] TNF has an essential protective role in *Listeria* infections, since TNF-depleted mice are unable to control the growth of *Listeria*.[8] Transmembrane TNF is sufficient to establish protective immunity against a primary low-dose *Listeria* infection (despite increased hepatic inflammation); however, soluble TNF is required for optimal control of cellular inflammation and resistance to a primary high-dose infection; moreover, in the case of a high-dose infection and lack of soluble TNF, a delay in leukocytes infiltration and, more specifically, a delayed T-cell response, is observed. By contrast, membrane TNF alone is sufficient for resolution of a secondary high-dose infection and for transfer of protective immunity, from mice immune to *Listeria* to TNF-deficient mice, through memory T-cells. [8]

Host defense against *Streptococcus pneumoniae* is also dependent on TNF-α's action. In TNF-α knock-out mice infected with *Streptococcus pneumoniae*, it was observed an early and high mortality, along with increased bacterial counts in blood and lungs.[32] Similar observations had previously been made in TNF-α-depleted mice infected with a lethal *Streptococcus pneumoniae* strain (serotype 3).[33] In both these studies, the knock-out mice's lung histopathology did not significantly differ from the control group's.[32,33] On the other hand, histopathology of the knock-out mice's spleen revealed a severe white pulp depletion and increased apoptosis, in comparison to wild-type mice; mice infected with a lethal strain showed exacerbated liver damage in the context of disseminated infection. [33] In this animal model, the absence of the cytokine influenced the extrapulmonary pathology to a greater extent than the pulmonary pathology, and sepsis and systemic organ damage were probably the cause of death in these animals. [32] Cases of exacerbated *Legionella* infection have been reported in patients taking anti-TNF drugs, constituting one of infectious diseases probably linked to this therapy.

TNF-α's role in viral infections

TNF-α is also implied in the defense against virus. Viral components can be recognized by the innate immune system, leading to release of TNF-α; this is the case of *Herpes simplex virus* (*HSV) type 1*, when recognized by Toll-like receptors 2 and 3. [35] Anti-TNF therapy is frequently associated with worse clinical manifestations, especially in chronic infections (including latent viral infections, such as *Varicella zoster virus* or *HSV*, which are often reactivated in this context). [9] One of TNF-α's properties is its ability to inhibit replication of a number of RNA and DNA virus. [1,34,15] Several authors have shown that, when both TNF and IFN-γ are present simultaneously, they induce a synergistic response which inhibits viral-gene expression and lowers viral titers to a greater extent than treatment with each cytokine alone; this synergistic antiviral activity state has been shown to be effective against a diverse array of viruses, including *HSV1*, *HSV2*, *murine Cytomegalovirus* and *Adenovirus*. [35] *HSV2*'s replication is inhibited after co-stimulation with IFN-γ and TNF-α; it has been evidenced that TNF alone is unable to

inhibit *HSV2*'s replication, but rather acts to enhance IFN-γ activity in this context. These cytokines act together by inducing indoleamine 2,3-dioxygenase, which cleaves the essential amino acid L-tryptophan to kyurenine, causing L-tryptophan's availability for protein synthesis to decrease and, therefore, limiting *HSV2*'s replication.[36] Other mechanisms proposed to explain the synergic effect of these two cytokines in virus control include: increased TNF-receptor expression, as well as an increased binding of TNF to the cell surface, both occurring after exposure to INF-γ; and TNF-induced secretion of INF-β, which then acts along with INF-γ. [35] Treatment with TNF has been shown to prolong the survival of mice acutely infected with *HSV* [1], and decreased levels of TNF-α have been associated with *HVS1* reactivation from latency in humans; this could possibly be explained by a shift to a Th2-immune response. [7]

One of the most compelling evidences for an antiviral role for TNF is the demonstration that different virus families encode factors which target TNF-dependent activities: *Adenovirus* have been shown to encode multiple genes that can block TNF's cytotoxic effects; several of *Poxviruses* encode soluble versions of TNF-receptors and disruption of the viral TNFR gene in *Myxoma virus* resulted in reduced virulence *in vivo*; overexpression of viral genes encoding homologues of death effector domains, involved in p55-mediated signaling, inhibited TNF-induced apoptosis, same as occurs in the genome of *Molluscum contagiosum* [37]

In the pulmonary intersticium, *Influenza virus* infects macrophages and stimulates these cells to release a number of cytokines, including TNF-α. An experiment *in vitro* has shown that TNF-α has a dose-dependent antiviral effect in *influenza virus'* replication.[1] In this study, it was demonstrated that TNF decreases *influenza's* viral proteins, which appears to occur at an early stage of the virus' replication (namely during the translation phase).[1]

Hepatitis B can be controlled by the action of cytotoxic T-cells that target the infected hepatocytes and induce their death. [38,39] Moreover, cytotoxic T-cells can also control the viral infection through the secretion of different cytokines, namely IFN-γ and TNF-α. [38] These two cytokines contribute to the destruction of the viral genome and proteins hosted within the hepatocytes, doing so without cytolysis and therefore contributing to the process of virus elimination from the host hepatic cells. [40,41,38,39] These cytokines are also thought to be involved in the unspecific immune response that develops before T-cell cytotoxic response. [42,43]

Although IFN-γ and cytotoxic T-cells are normally seen as the major factors responsible for the destruction of infected hepatocytes and viral DNA, different studies have confirmed that TNF-α plays a role in controlling *Hepatitis B virus' (HBV)* infection in the liver. [38,40,41]

One of TNF-α's non-cytotoxic roles is the downregulation of *HBV*-nucleocapside levels in the cytoplasm of infected hepatocytes.[43] Additionally under TNFα's influence, *HBV*-DNA levels in the nucleus have been shown to be decreased, which could be a result to of possible DNA translocation from cytoplasmic nucleocapsides into the nucleus. These effects could be mediated through the activation of NF-kB by TNF and the different products of the subsequent NF-kB-induced transcription[42,43] The *HBV*-nucleocapside downregulation does not appear to be mediated by a reduction in core proteins, suggesting that TNF does not degrade core proteins and rather compromises nucleocapside assembly and/or stability. [42,43] Nonetheless, the *HVB*-mRNA levels do not appear to be significantly influenced

by TNF. Therefore, *HVB-mRNA* reduction does not constitute a suitable cause for the diminution of DNA viral replication and capsid levels. [42]

Alongside IFN-γ, TNF-α was also found to be responsible for the deamination of covalently closed circular *HBV*-DNA (cccDNA). Apurinic/apyrimidinic sites in the cccDNA (introduced through cleavage of uracils by uracil-DNA glycosylase) are recognized and digested by endonucleases, one of which, A3B endonuclease, was found to be upregulated in infected hepatocytes under TNF stimulation. Furthermore, DNA deamination by TNF-α was found to be almost completely inhibited when this endonuclease is neutralized. [40]

TNF-α may also induce a negative regulation of *HBV* replication in hepatocytes through another molecular pathway. In fact this cytokine was shown to increase the expression of p22-FLIP in cells infected with *HBV*. [41] p22-FLIP was proven to exert an antiviral effect *in vitro*, suppressing *HBV* replication; furthermore, its inhibition blocks the antiviral effects of TNF-α.[41] This anti-viral effect is thought to be mediated by the suppression of viral enhancers and copromoter (Enh 1 and Enh 11/Cp), which in turn occurs through the induction of their inhibitor HNF3β and reduction of their stimulator HNF4α.[41]

The control of hepatitis B by TNF can be summarized to two major effects: intervention on innate and adaptive immunity. Due to its involvement in innate immunity, TNF-α may be responsible for the activation of T-cells and, therefore, the development of adaptive immunity. Confirming this effect, TNF-α's blockade with antibodies in the context of an *HBV* infection model was shown to result in an impaired T-cell activation. [44] In this model, the TNF inactivation also resulted in an impaired *HBV* clearance, inducing an elevation of serum *HBV* viral-load and a sustained *HBV* viral antigen expression. The sustained elevation of serum HBsAg and *HBV*-DNA levels varied in a dose-dependent response, with higher anti-TNF dosages also being shown to correlate with an increased T-cell exhaustion. [44] Furthermore, early TNF blockade led to persistent *HBV* clearance with enhanced T-cell exhaustion and maintained high *HBV* viral load.[44]

These experiments confirmed that both IFN-γ and TNF-α are key factors in non-cytolytic inhibition of *HBV* and, most importantly, are able to trigger nuclear *HBV*-DNA degradation in an additive fashion, with IFN-γ playing the dominant role in this context.[41] Alternative mechanisms under TNF influence and yet to be discovered may be involved in HBV elimination; nevertheless, as opposed to IFN-γ, TNF does not appear to exert is effects through iNOS activity.[42]

Cytokines other than these two have been implicated in decreasing *HBV* replication; IL-2, for example, was shown to decrease HBV replication, in part by inducing the production of TNF.[42]

TNF-α's role in fungal infections

Histoplasma capsulatum fungus' infection is associated with a prompt and vigorous release of TNF, which does not necessarily mean that TNF is implicated in the immune response against this agent. Nevertheless, when TNF is neutralized, this infection has a fatal outcome, with a markedly increased burden of *Histoplasma capsulatum* evidenced in different animal models, regardless of it being a primary or secondary infection. [2,45] Therefore, TNF-α is necessary for protection in the context of both primary and secondary *Histoplasma* infections. [45,46] In primary infections, TNF appears to be essential in the

first 5 days; in fact, experiments show that neutralization of TNF-α on the seventh day of infection dampened the host response transiently, but did not lead to uncontrolled infection. [46]

In infected mice, T-cells were found to constitute the largest proportion of TNF-α-producing cells.[46] After TNF-α's blockade there wasn't an alteration in the number of T-cells expressing IFN-γ; on the other hand, T-cells expressing a memory phenotype appear to be decreased in this context; nevertheless, the number of activated T-cells and T-cell expansion do not seem to be influenced. [46]

During secondary infections, TNF's blockade appears to be associated with an upregulation of IL-10 and IL-4, both of which are known to diminish protective immunity to *Histoplasma capsulatum*; in primary infection, TNF's blockade correlates with a decrease in nitric oxide, which may be related to the impaired immunity.[2,45] In both primary and secondary infections, T-cells from infected mice given neutralizing anti-TNF antibodies are unable to transfer protective immunity, what may be explained by an increase in regulatory T-cells.[46] Mice receiving anti-TNF antibodies also showed an increased population of $CD4^+CD25^+$ T-cells in both primary and secondary infections, an effect which was not otherwise demonstrated in mice lacking other important regulatory cytokines (such as IFN-γ and GM-CSF), or receiving higher inoculum of *Histoplasma*. However, despite that, the presence of cells expressing natural regulatory T-cell markers didn't differ from the control group. The population of $CD4^+CD25^+$ T-cells treated with anti-TNF antibodies dampened the proliferation of *Histoplasma*-specific T-cells when in presence of antigenic extract of *Histoplasma in vitro*, and the suppressive effect was reversed with anti-IL-10 antibodies; similar results were found *in vivo* after transferring these cells. Therefore, IL-10 can be mediate the dampened proliferation of *Histoplasma*-specific T-cells. Furthermore, the neutralization of CD25 (and thus CD25 expressing cells) improved the survival and diminished fungal burden of anti-TNF treated mice, particularly in primary infections. [47]

Protective immunity against other fungi, such as occurs in candida infections and aspergillosis, can be related to TNF-α's function. For example, when *Candida albicans* yeasts were treated with TNF-α *in vitro*, this cytokine lead to impaired morphological transformation to the hyphal form of the fungus, even at adequate CO_2 conditions. To be noted that the hypha is characteristically the invasive and resistant form of *C. albicans*, and phagocytes (neutrophils and macrophages) easily ingest the yeast form and short filaments of *Candida*. [48] TNF-α synthesis is increased during fungal infection, and TNF-knock-out mice or mice receiving antibodies against this cytokine show diminished survival when challenged with *C. albicans* intravenously.[6] Additionally, maximal endothelial expression of VCAM-1, E-selectin (leukocyte adhesion molecules) and IL-8 (neutrophilic chemoattractant) in response to *C. albicans in vitro* requires TNF-α.[6] Therefore, TNF probably stimulates recruitment of polymorphonuclear leukocytes and, moreover, enhances phagocytic activity and oxidative burst during *candida* infection. [48,6] Concerning immunity to *Candida*, TNF-receptor 1 signaling has been shown to be the principal mediator of TNF-α stimulation and is likely to mediate the neutrophilic augmentation of fungus phagocytosis and elimination.[6,48]

TNF-α's role in defense against *Leishmania*

Leishmania, an intracellular parasite, is also controlled by the immune system in a TNF-dependent manner. [49,50] While there are many *Leshmania* species, TNF seems to play a role transversal to them;

nonetheless, it also appears that different regulation mechanisms might be involved in the protective immune response against the different species.[51] Upon infection, a specific immune response is mounted after the transition of dendritic cells from peripheral tissue to draining lymph nodes, and the presence of TNF at the site of challenge is essential to achieve an effective migration of sufficient number of antigen-presenting dendritic cells. [50] After that, the T-cells are activated and produce IFN-γ, which in turn is synergistically supported by TNF-α, and drives macrophages to upregulate the enzyme iNOS to produce large amounts of the effector molecule, nitric oxide, essential for the resolution of *Leishmania major* infections *in vivo*.[49] For example, after infection with a *Leishmania major* substrain, TNF-negative mice were unable to mount a protective adaptive immune response. [50] In one study, the authors correlate this deficient protective response with: a reduction in the dermal infiltrate of inflammatory cells and a consequent diminution of inflammatory cytokines (such as IFN-γ); a lack of upregulation of chemokine CCL21, known to be involved in proinflammatory-cells migration (namely the orchestration of mature $CCR7^+$ dendritic-cells migration to draining lymph nodes). [50] The authors hypothesize this findings could be related to a previously found involvement of TNF-α in dendritic cells maturation and, thus, CCR7 expression. Therefore, TNF and CCL21 locally expressed in the skin would enhance specific antigen presentation by increasing the number of dendritic cells that migrate to local lymph nodes.[50] In another study, with dendritic-cells cultured *in vitro*, the authors found them to have a differential activation, distinguishing cells infected by *Leishmania braziliensis* from bystander dendritic cells. In fact, bystander-cells were shown to be activated in this context (contrary to the infected cells), producing IL-12, TNF-α and class II CD80 and CD86 surface markers, and thus being capable of initiating a T-cell response. These findings were replicated with other *Leishmania* species (*major* and *mexicana*). [51] The inhibition of TNF prevented bystander dendritic cells' activation; therefore, TNF was shown to be essential for their activation to the mature form, which in turn permits antigen presentation and T-cell activation. [51] However, while the lack of T-cell activation seems to be a suitable mechanism to prevent *Leishmania* immune resistance, a recent work with mice models infected with *Leishmania major* demonstrated that, in the presence of a strong T-helper 1 response, along with significantly elevated IFN-γ expression in lymph nodes of TNF-null mice, and relatively strong expression of iNOS in the draining lymph nodes, TNF's absence prevented an effective immune response. [49] In this study, TNF-receptor 1 was proven the most important receptor in the protective immune response, while wild-type mice and mice expressing only the membrane-TNF form, controlled the infection and showed a comparable course. On the other hand, TNF-receptor 2-deficient mice developed a large skin lesion, similarly to what was evidenced by TNF-receptor 1-deficient mice; nevertheless, as opposed to the latter group, they were ultimately able to control the infection. [49]

Conclusion

Biological treatment with anti-TNF-α drugs seems to be related to the emergence of infectious diseases. After the introduction of these drugs, multiple reports have emerged of infections concomitant to the anti-TNF-α therapy.[52] Nonetheless, so far, tuberculosis was the only infection shown to present a high correlation with anti-TNF-α therapy, and the existence of a cause-effect for other infections is still dubious. [53] Additionally, there are many confounding factors potentially influencing these

observations: generally, the patients taking these drugs are already immunologically suppressed with other agents; moreover, their own pathology could influence the immunological system, and thus the immune control of infections.[54] Furthermore, it is hard to find a control population for these individuals, as the emergence of infections is greatly related to the patient's background: if an infectious disease has a low frequency in a specific population, individuals from that population (including the patients with neutralized-TNF-α) will consequently have a low probability of catching said infection; thus making it difficult to prove statistically significant increases in infections in patients treated with anti-TNF-α.

Our research has returned a significant amount of articles reporting various infections in patients taking TNF-α suppressing drugs. In fact, respiratory infections are amongst the most common reported adverse effects of TNF-α's inhibitory therapy; virus like *Influenza* and *Adenovirus* may be frequently involved as well. [9] Nonetheless, although most results show a higher incidence of infections among these patients, it does not seem to be a clear consensus in literature regarding the existence of a significant increase in susceptibility to infection as a consequence of anti-TNF-α therapy. Despite that, doctors usually approach this problem in clinical practice, and several guidelines include recommendations to apply preventive measures prior to initiating a patient on anti-TNFα therapy, namely screening for tuberculosis and HBV, along with subsequent treatment if necessary.[55]

Ever since TNF-α was made available, many experimental studies have correlated this cytokine with immune protection against an array of pathogens. Nevertheless, considering clinical studies' limitations, they cannot provide definite evidence that the increasing incidence of infections results from TNF-α's neutralization; experimental studies on this subject are therefore essential to gather further evidence on the importance of TNF-α in immune protection. However, the experimental models used so far in this context present some limitations when extrapolating the findings to human physiology. Nonetheless, experiments show that TNF's presence is strongly correlated with the inhibition of some microorganisms; furthermore, TNF's inhibition was associated with an increased burden of microorganisms; these findings suggest that this cytokine is very important for the regulation of the immune system and, additionally, that it is likely of great importance in controlling pathogens in human hosts. Supporting these hypothesis, several studies using human subjects evidenced that susceptibility to different infections is significantly influenced by whether the individual carries a TNF-α polymorphism.

The authors of this paper proposed themselves to collect the experimental evidence in favor of TNF-α's role in infection control and to comprehensibly summarize its pathways and biological actions. However, in view of the large amount of information collected on the subject over the last decades, and the fact that, for methodological reasons, the authors only considered works published after 1995, it is possible that many important works were excluded, and this article may thus reflect only a fraction of the information already established on TNF-α's functions in infection control. Nevertheless, this review includes information concerning several experimental models in which TNF-α exerts an essential role, and the authors attempted to list all the pathogens in regard to which there seemed to be evidence supporting TNF-α's intervention.

Nonetheless, there is still much to be clarified regarding TNF-α's mechanisms and functions. In fact, its signaling appears to result in hundreds of different outcomes, probably dependent of cellular factors

and kinetic characteristics; additionally, in some cases, TNF seems to favor the proliferation of microorganisms, rather than controlling the infection (e.g. in *HIV* infection).[56] Moreover, the pathways through which it controls infections and whether they are cytotoxic, are not yet fully understood.

The comprehension on how TNF exerts its effects could be of great value, potentially offering new ways to improve infections' control; furthermore, assuming TNF's inhibition does increase susceptibility to infections, this knowledge could be helpful in preventing infections in individuals taking anti-TNF-α drugs.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

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- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London
- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007
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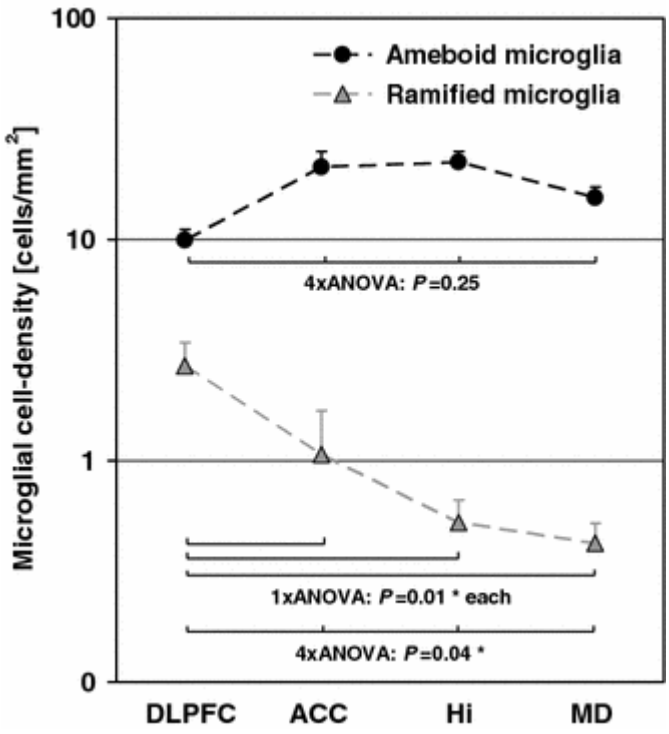

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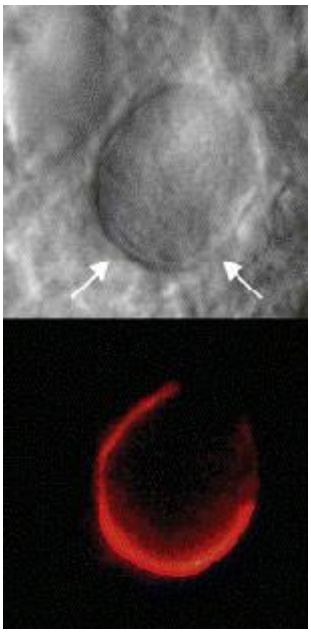

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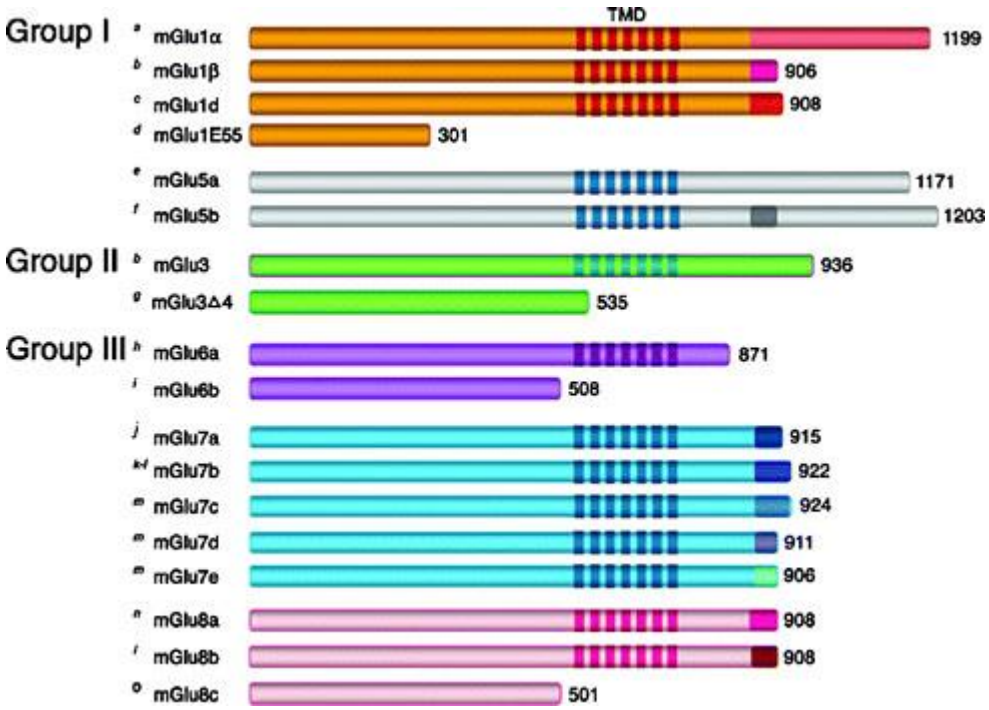

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