THE DUAL ROLE OF TRANSFORMING GROWTH FACTOR BETA IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS AND EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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ABSTRACT

Transforming growth factor beta (TGF-β) is a pleiotropic cytokine that is involved in numerous pathological processes. TGF-β functions in the immune system are to perform the regulation of lymphocyte differentiation, survival and destruction. In autoimmune diseases of the central nervous system, like multiple sclerosis (MS) and the animal model of experimental autoimmune encephalomyelitis (EAE), depending on the context, TGF-β has paradoxically pro- and anti-inflammatory effects, explaining the difficulties in creating therapeutic strategies targeting TGF-β. This review presents the progresses that have been made on the understanding of TGF-β in the pathogenesis of both EAE and MS.

Keywords: transforming growth factor beta, multiple sclerosis, experimental autoimmune encephalomyelitis, pathogenesis

INTRODUCTION

Transforming growth factor beta (TGF-β) is a cytokine that is secreted by cells of the immune (mainly macrophages) system but also by other non-hematopoietic cells. Most tissues have high expression of the genes encoding TGF-β, that contrasts with other anti-inflammatory cytokines (IL-10), whose expression is minimal in tissues that are unstimulated and need triggering by commensal or pathogenic flora (1,2).

TGF-β has many different roles in controlling cellular differentiation and proliferation. Many diseases *benefit* in some degree from TGF-β: autoimmune diseases (depending on the context, pro-inflammatory properties or of potent immune suppressor), infections (protects against damages caused by the immune system, but promotes chronic infections), cancer (tumor suppressor at the beginning of tumor genesis, then supports tumor growth and metastasis), asthma (promote allergen tolerance), diabetes, heart diseases, graft-versus-host disease, AIDS, Marfan syndrome, other neurodegenerative diseases like Parkinson's and Alzheimer disease etc. (1,2).

TGF-β is a protein found in three isoforms: TGF-β 1, TGF-β 2 and TGF-β 3. TGF-β is secreted in a latent form of two polypeptides that need a serum proteinases (plasmin) catalyze the release of active TGF-β from this complex, the process taking place on the surface of macrophages. Macrophages are activated by inflammatory stimuli that increase the release of active TGF-β by stimulating the plasmin. Macrophages can also endocytose latent TGF-β complexes that are IgG-bound. Under certain circumstances, macrophages release active TGF-β into the extracellular fluid. On the other hand, macrophages might be inhibited by TGF-β by the negative effect on the expression of the inducible form of nitric oxide synthetase (3).

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The main characteristic of all three isoforms of TGF-β is that their effect depends by the state of the target cell together with the presence of other growth factors and cytokines (3).

TGF-β1 is the first member of a superfamily of proteins called the TGF-β that includes: anti-müllerian hormone, activin, bone morphogenetic protein, decapentaplegic inhibins, and Vg-1. Some cells that secrete TGF-β have receptors for TGF-β (autocrine signaling). The signaling of TGF-β is done through heteromeric complexes of type I and II transmembranale receptor serine/threonine kinase. The role of TGF-β have been studied in many diseases, a special interest being shown in the autoimmune diseases of the central nervous system (CNS) like multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) (1-3).

ROLE OF TGF-β IN MULTIPLE SCLEROSIS PATHOGENESIS

MS is characterized by multiple inflammatory demyelination and axonal lesions in the CNS. The pathogenesis of MS is not totally understood. It is well known the immunological, clinical and therapeutic heterogeneity of MS. This heterogeneity of the clinical course may relate to changes that occur in the adaptive and innate immune system over the course of MS. However, T cells play a central role. Activated T cells were found both in active and in chronic MS lesions in all four histopathological subtypes of MS. T cells are divided into 2 groups on the basis of expression of surface molecules: CD4 or CD8. CD4+ cells provide help for B cell differentiation and are called *T* helper (Th). On the contrary, CD8+ cells are called cytotoxic T cells being involved in class I-restricted lysis of antigen-specific target (4).

The CD4+/Th cells play an important role in the immune response, mainly by producing of cytokines that send secondary signals to other cells from the immune cascade. Five major types of Th cells have been described: a) Th1 produce pro-inflammatory cytokines like interleukine-2 (IL-2), interferon-γ (IFN- γ), tumor necrosis factor α (TNF α); b) Th2 cells produce mainly anti-inflammatory cytokines like IL-4, IL-5, IL-10, IL-13; c) Th9 (produce IL-9, IL10); d) regulatory T cells (Tregs) will be discussed later; e) Th17 produce IL-17, a powerful proinflammatory cytokine. Th17 are formed by the differentiation of naïve Th cells in the initial presence of TGF-β and IL-6 and followed by IL-23 that play an important role for survival and at the end of the Th-17differentiation process (4,5). The modern paradigm of neuroimmunology is built around Th-17. In the presence TGF-β, IL-6 and IL-23, Th-17 cells produce IL-17. Until now, the use of a monoclonal antibody against IL-23 called ustekinumab was disapointing in humans (6).

Pathogenic T CD8+ play a role in in MS and contribute to MS heterogenity. These cells contribute directly to demyelination and axonal loss during inflammation by expression of cytotoxic molecules (tumor necrosis factor-α and related molecules), contribute toward the secretion of IL-17 and IFN-γ (5).

TGF-β is a central cytokine in the induction from the naive CD4+T cells of Tregs cells and combined with other cytokines (IL-6 and IL-23) induces the powerful pro-inflammatory Th-17 cells (6,7,8). The Tregs are a network that form the adaptive immune system that mediate the active suppression of T cells response and maintain the peripheral tolerance. Tregs can be divided into: natural and induced Tregs. Tregs have major transcription factor called Foxp3. Natural Tregs are marked by CD25. The differentiation of Tregs is realized by TGF-β. Induced Tregs can secrete TGF-β (becoming Th3 cells) or IL-10 (called Tr1 cells). Defects in Tregs percentages and function were found in MS and represents one of the main strategies in novel development of MS treatments (9,10).

TGF-β 1 has effects on B cells also by inhibiting the proliferation and immunoglobulin synthesis. The monocytes can be activated by TGF-β 1 and induce the production of pro-inflammatory mediators (3).

The role of TGF-β in the neurobiology of MS is performed also by inhibiting the astrocytes reactivity and proliferation: TGF-β 1 increases the availability of neurite growth promoting molecules, contributes to limit the formation of reactive astrocytes and decreases the microglia and macrophages infiltration. All TGF-β isoforms to astrocytes are localized in areas of chronic demyelination and participate to the formation of chronic MS lesion. TGF-β 2 is found in microglia bordering active lesions (11,12). Concerning the process of oligodentrogenesis and myelination, studies performed in vitro showed that progenitor cells express TGF-β 1 receptors and TGF-β 2 had an antimitogenic effect. These finding proposed that TGF-β has a role in remyelination and regeneration (13).

Many years, TGF-β was associated with disease remission, being found in local tissue components, mainly astrocytes, during the recovery from inflammation within the CNS. TGF-β was considered

an immunosuppressive cytokine and its local production in demyelinated lesions suggested a prominent role in downregulation of the inflammatory response of brain inflammation, together with other cytokines (IL-10) and neurotrophins (14). TGF-β has a well known suppressive effect on naïve T cells functions. In contrast, the effect of TGF-β oh Th1 cells is of enhancing cellular activation, cytokine production, cell proliferation. Another mechanism was described by which TGF-β is able to suppress the encephalitogenicity of Th1 effector is done via IL-10 production (15). TGF-β can have a pronounced inhibitory effect on antigen-specific T cell proliferation without modulating their cytokine production by reducing the cell-cycle rate (16). As encouraging results were found in treating EAE (see below), in 1998 Calabrese et al. used TGB-β in a phase I safety trial but the results were very disappointing both from the clinical efficacy point of view and of adverse effects (see below) (17). Therefore the dichotomy between central and peripheral events needs to be considered with a special interest in the context of both intracerebral and systemic production of cytokines. Rollnik et al (18) found that the biologically active TGF-β 1 is decreased in serum but is increased in CSF of MS. The levels in CSF were significantly higher in remitting phase than in acute/active phase of MS (19).

TGF-β, together with IL-4 and IL-10 may suppress delayed-type hypersensitivity reactions and downregulate macrophage activation and T-cell mediated inflammation. In situ hybridization studies showed mRNA for many cytokines including TGF-β (20). The addition of TGF-β to the Th-17-inducing cytokines determined the marked co-expression of IL-9 (a Th2 cytokine) in IL-17 producing memory cells (21).

MS has a polygenic model of disease. In some MS families, numerous evaluation of common polymorphism within the TGF-β 1 was performed. The results were contradictory. A study found an association between a TGF-β 1 haplotype and a mild MS course, while another study did not find any genetic variation in TGF-β (10,19) . Meoli et al (9) found in MS cases, a reduction in the levels of TGF-β regulated genes. A study from Northern Ireland found no association of TGF-β 1 and 2 gene polymorphisms with MS (22).

Regarding TGF-β subtypes in MS, TGF-β 1 is the major subtype implicated in MS pathology and is always present in the plasma mainly binding to other components, creating a source of immunosuppression. TGF-β 1 has multiple functions in MS pathogenesis: a) suppresses B cells, T cells and other cells; b) terminates immune responses; c) stimulating the production of regulatory T cells (Treg), determines the production of type 1 inflammatory cytokines; d) contributes in the shift from effector to memory cells; e) inhibits leukocytes adhesion to endothelium; f) downregulates adhesion molecules; g) maintain a state of immune tolerance (2,19).

TGF-β IN THE PATHOGENESIS OF EAE

MS and EAE were considered Th1-mediated diseases. The discovery of IL-23, the IL-12 related cytokine that shares the subunit p40 (an essential inducer of Th1 cells development), contributed to the re-thinking of the previous work done with the p40 knockout mice. Description of the Th-17 cells (stimulated by IL-23) with the production of the proinflammatory cytokines (IL-17) is found in an important number of EAE studies (23).

In EAE, TGF-β 1 synthesis is taking place in glial cells also. TGF-β induced signaling in the brain were activated several days before the clinical onset of EAE. Later, TGF-β was activated in neurons, in infiltrating T cells in inflammatory lesions but also in meningeal and perivascular infiltrates (23).

TGF-β is involved both in the disease onset but also in the clearance of CNS inflammation by apoptosis induction in T cells. Also, it is known that apoptotic T cells release important amounts of TGF-β. For this reason, it is difficult to know whether increased levels of TGF-β are a cause or a consequence of local apoptosis (14).

TGF-β is a major mediator associated with EAE and the early production of TGF-β in the CNS may create a favorable environment for the initiation of inflammation (18,19).

TGF-β INVOLVEMENT IN EAE AND MS TREATMENT

In the EAE condition, many experimental trials have been made using the knowledge about the immunosuppressant effect of TGF-β given alone without the presence of pro-inflammatory Il-6 and IL-23. Since 1991, Johns et al. (24) focused on the capacity of intravenous TGF-β to influence the clinical evolution of EAE. They obtained successful results when treating EAE with TGF-β and on the contrary, the aggravation of EAE when antibodies to TGF-β were used. These findings lead to promising results at that moment (25). Later, Quintana et al (26) induced TGF-β dependent Tregs cells by injecting a ligand that bind the aryl hydro-

carbon receptor (the mice transcription factor equivalent to Foxp3 in humans) resulting in induced functional Tregs that decreased the production of Th-17 cells, obtaining a suppression of EAE. Chen et al (27) have described the new CD8+ population that exhibited regulatory properties in EAE mice in a TGF-β and IFN-gamma dependent manner. Oral tolerance strategies are realized by oral application in EAE rats of myelin basic protein. The mechanism underlying this therapy include activation of immune T cells that secrete cytokines with an anti-inflammatory effect like TGF-β, IL-4, IL-10 and therefore inhibiting the autodistructive Th1 cells (23).

A number of 12 MS patients were treated 2-3 times a week with TGF-β by the group conducted by Calabresi et al (17) for a period of 4 weeks. The results were disappointing, no change in clinical and magnetic resonance imaging (MRI) was found. A decrease in the renal glomerular filtration determined the conclusion that high-dose TGF-β is not a safe and active treatment for MS.

In 271 MS patients treated with interferon-β1a (IFN-β) Lunemann et al (28) found a downregulation of serum levels of TGF-β 1 4 weeks from therapy onset that remained constantly low after one year. Other study, having approximately the same objectives, found after 6 months of IFN-β1a therapy of 20 MS patients, a significant increase of TGF-β 1 serum levels measured by enzyme linked immunoabsorbent assay. The results suggest that serum levels of TGF-β 1 are upregulated by 6 months if IFN-β1a (29). If we were to find a link between the 2 studies, we may suppose that INF-β initially decreases the serum levels of TGF-β 1 and after some months, increases the peripheral TGF-β 1.

CONCLUSIONS

TGF-β is an important pleiotropic cytokine, with both stimulatory and inhibitory effects on cell differentiation and growth. In certain circumstances, TGF-β exerts protective effects on the EAE and of MS but in other cases, paradoxically, TGF-β has a pro-inflammatory role and induces pathogenic Th-17 cells. Understanding the function and regulation of TGF-beta during immune responses offers therapeutic promise for the control of MS.

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