Review article **UDC: 616.832-004:616-097**

ROLE OF TH1 AND TH17 IMMUNE RESPONCES IN PATHOGENESIS OF MULTIPLE SCLEROSIS

Miloš Kostić

Th17 cells are novel cell subpopulation of $CD4^+$ T lymphocytes which dominantly produce proinflammatory cytokine IL-17. Since their discovery in 2003, Th17 cells have been in the focus of interest, because of the numerous data pointing out their crucial significance in pathogenesis of multiple sclerosis and other disorders earlier considered as classical Th1-mediated autoimmune disorders. It is demonstrated, on animal model of multiple sclerosis, also known as experimental autoimmune encephalomyelitis, that Th17 cells are the main mediators of inflammatory process in autoimmunity, since Th1 cells have protective role, which is in contrast to the current concept of multiple sclerosis. Th17 and Th1 cell relationship determine if inflammation initiation and forming of demyelinisation plaque will occur. Inflammation will take place, if relationship between Th17 and Th1 cells is higher than 1 because of disproportional enhanced secretion of IL-17, which is considered to be the main regulator of autoimmune processes in the central nervous system. Discovery of TGF-β and IL-6 involvement in proinflammatory Th17 cells development was also surprising, according to the fact that TGF-β, itself, has anti-inflammatory effects and induces the activation of FOXP3 transcription factor, essential for the generation of regulatory T lymphocytes, the cells important for immunosuppression, development and maintenance of immunotolerance. Functional antagonism and similar differentiation factors of Th17 cells and regulatory T cells are very interesting in the aspect of immunomodulation therapy of autoimmune disorders. The aforementioned discoveries have constructed a novel concept of pathogenic mechanisms involved in multiple sclerosis development, mediated mainly by Th17 cells. That questions our knowledge of drug effect mechanisms that we commonly use today, but also raises the possibility for novel therapeutic approaches. *Acta Medica Medianae 2010;49(4):61-69.*

Key words: *Th17, Th1, regulatory T cells, IL-17, multiple sclerosis*

Department of Immunology, Faculty of Medicine University of Niš, Serbia

Contact: Kostić Miloš
Uroša Predića 6/13, 18000 Niš, Srbija
E-mail: milosh.kostic@hotmail.com

Immunopathogenesis of Multiple Sclerosis

Multiple sclerosis is demyelinating inflammatory disease of the central nervous system characterized by episodical neurologic deficits that are disseminated in time and neuroanatomical location (1). Disease was first described and defined by the French physician Jean-Martin Charcot as early as 1868. Since then, etiopathogenetic mechanism of this disease is a real enigma for any aspect of biomedical science. Great progress in the understanding of multiple sclerosis was made in the last decade. Every aspect of the disease is becoming more complicated every day and therefore the previous concept of multiple sclerosis as a classical Th1-mediated autoimmune disease must be reconsidered.

The current concept of pathogenesis of multiple sclerosis involves the activation of potentially auto-reactive CD4 T lymphocytes at the periphery, outside the structure of the CNS, due to the recognition of certain antigens presented within the MHC class II molecules on the surface of antigen presenting cells that provide the necessary costimulation (2). It is believed that most of these antigens are in the form of peptides of viral origin such as peptides derived from the human herpes virus 6 (HHV6), Epstein-Barr virus (EBV), Chlamydia pneumoniae. Peptide fragments of these micro-organisms activate auto-reactive T lymphocytes specific for myelin basic protein or MBP (Myelin Basic Protein), the most common protein of neurons myelin layer, through two mechanisms: molecular mimicry and functional disturbance of anergy in the proinflammatory enviroment (3). For peripheral activation, genetic constitution of an individual is also important and it is associated with HLA-DR2, DR15 and other DR molecules that are believed to contribute to the molecular mimicry between viruses and MBP peptide (2,3).

Activated auto-reactive T lymphocytes then bond to endothelium of blood-brain barrier with the help of adhesive molecules such as LFA-1 (Lymphocyte Function Associated antigen-1) and VLA-4 (Very Late Activation molecule-4) and migrate into the brain parenchyma (4). For the time being, mechanisms that drive these lymphocytes in the CNS are unknown, nor if additional antigen presentation in the deep neck lymph nodes, where antigens of certain parts of the brain are collected, is required. When these cells encounter in encephalic department with MBP presented by microglial cells, which are antigen presenting cell in CNS, auto-reactive lymphocytes will be reactivated and begin the secretion of cytokines and proinflammatory chemokines. A various number of cytokines and chemokines determine: activation of residual cells such as microglia and astrocytes, recruitment of other immune cells, including monocytes, CD8 T lymphocytes, B lymphocytes, mastocytes from peripheral blood, due to disruption of blood-brain barrier semi-permeability and determine the formation of inflammatory lesion (2).

Inflammatory lesion, pathognomonic for multiple sclerosis, is demyelization plaque, oval in shape, which stretches along the centrally placed blood vessel. In these lesions, demyelization, oligodendrocyte destruction and axonal damage of neurons may occur, induced by many processes involving free radicals, TNF-α, direct deposition of complement, complement activation in classical manner, antibody dependent cellular cytotoxicity mediated by NK cells, myelin phagocytosis by microglial cells, cell-lyses mediated by cytotoxic CD8 T lymphocytes, secretion of proteases commonly metalloproteases, oligodendrocyte apoptosis (3). Virtually, all effector mechanisms of the immune system are involved in the formation of demyelization plaques in multiple sclerosis and it is believed that they are all orchestrated primarily by auto-reactive CD4 T lymphocytes (2-4).

The aforesaid inflammatory events last from several days to two weeks. After that, the residual cells as well as infiltrated T cells begin variable secretion of growth factors: neurotrophic factor, platelet growth factor, fibroblast growth factor and others. Oligodendrocyte precursors that are still present in the adult CNS are also activated and begin remyelization of damaged internodal areas of axons, but the thickness of the novel myelin layer will never restitute to the previous level, thus nerve impulse conductivity will always be slower in remyelizated areas. Repaired myelin sheath differ from the adult, maturated sheath not only in thickness jet in composition, because it has the certain isoforms of MBP that are considered to be functionally insufficient and vulnerable to inflammation (3).

The modern concept of multiple sclerosis pathogenesis gives the central roll of "main player" to CD4 T lymphocytes, which conduct and control all immune effector mechanisms involved in the genesis and development of the disease. For more than thirty years it has been known that CD4 T cells after antigen stimulation can differentiate into two main subpopulations, with a clearly defined profile of cytokine secretion and functions in the immune system, referred to as Th1 and Th2 cells (5). Th1 cells primarily produce interferon-γ (IFN-γ), which acts as the most potent macrophage activator in the cellular immunity (6). However, Th1 cells are thought to be responsible for a number of tissue-specific autoimmune diseases such as arthritis, psoriasis, and inflammatory intestinal diseases including multiple sclerosis (7). Th2 cells produce primarily interleukins IL-4, IL-5 and IL-13 that are important for humoral immunity because they stimulate the production of IgE immunoglobulin class and activate eosinophils, which are crucial for antihelminthic protection and development of allergic diseases (5). It is considered that many diseases, the etiopathogenesis of which is based on immunological mechanisms, are just the consequence of unbalance Th1/Th2 immune response. Today, this hypothesis is called into question, because of the discovery of regulatory T lymphocytes and brand new CD4 T cell subpopulations marked as Th17 cells. A large body of evidence indicates to the involvement of these cell lines of CD4 T lymphocytes in the pathogenesis of many allergic and autoimmune diseases including multiple sclerosis, what could challenge a simple concept of multiple sclerosis as a classical Th1-mediated autoimmune diseases.

Profiles of Th Cells Involved in Phatogenesis of Multiple Sclerosis

In multiple sclerosis, there are numerous abnormalities in the immune system function but many authors regard Th immune response, namely Th1, Th17 and regulatory T cells as the factor of great importance in the development and pathogenesis of this autoimmune disease.

Th1 cells are generated in the primary immune response, during naive T lymphocytes activation in the presence of interleukin 12 (IL-12), produced by myeloid dendritic cells, and interferons produced by plasmocytoid dendritic cells and NK cells (7). IL-12 has hetrodimeric structure; it is composed of two subunits P35 and P40, and activates signaling molecule STAT4, jet interferons conduct signals through STAT-1 molecule (6). Signals sent by IL-12 and interferons through STAT4 and STAT1 increase expressiveness of transcription factor T-bet, essential for the differentiation of naive T lymphocytes into Th1 cells. Th1 cells have a heterogeneous cytokine secretion profile that includes firstly interferon γ (IFN-γ), tumor necrosis factor β (TNF-β) or lymphotoxin and interleukin 2 (IL-2) (8). Physiologically, the mentioned cytokines regulate cellular immune response against tumor cells, intracellular viruses and bacteria by macrophage and cytotoxic T lymphocyte activation. However, Th1 cells are thought to be responsible for a number of tissue-specific autoimmune diseases such as arthritis, psoriasis, and inflammatory intestinal diseases including multiple sclerosis (7) (Figure 1).

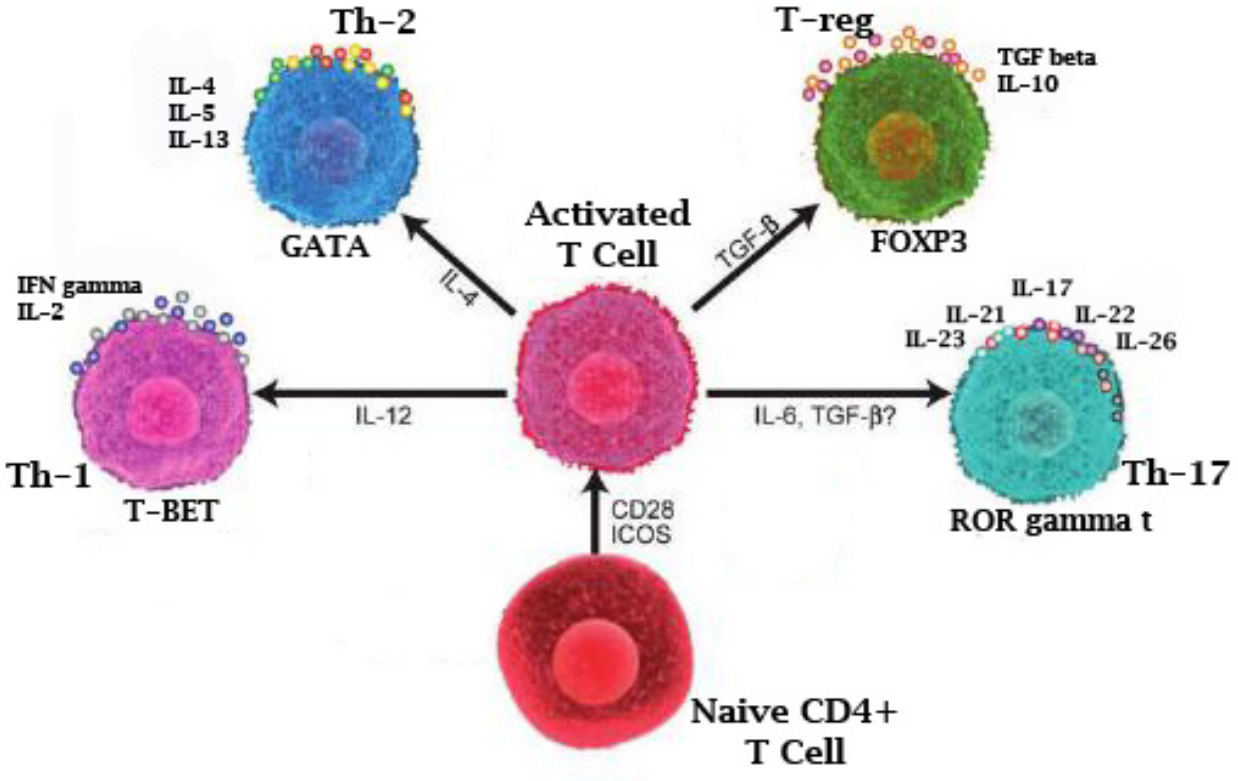

Figure 1. Pathways of differentiation, transcription factors expression and cytokine secretion profile of various subpopulations of CD4 T lymphocytes: Th1 cells are generated under the influence of IL-12, by the T-BET transcription factor expression, and secrete IL-2 and IFN-γ; Th2 cells appear under the influence of IL-4 , by the GATA transcription factor expression and secrete IL-4, IL-5 and IL-13; Regulatory T cells (T-reg) are formed under the influence of TGF-β, by the FOXP3 transcription factor expression and secrete TGF-β and IL-10, Th17 cells likely occur under the influence of TGF-β and IL-6, by the RORγt transcription factor expression and secrete IL-17, IL-21, IL-22 and IL-26.

Regulatory T cells are the second important line of CD4 T lymphocytes involved in the development of multiple sclerosis, which in addition to CD4, express also CD25 molecule. Less commonly, these cells are named Th3 cells. They have the ability to regulate the function of both Th1 and Th2 cells as well as important roll in the maintenance of immune system homeostasis. Regulatory T cells may arise in the thymus from naive T lymphocytes in the presence of IL-2 and transforming growth factor β (TGF-β) when they are marked as natural T regulatory cells. In peripheral lymph organs naive CD4 T cells, in the presence of TGF-β that activates signaling molecule STAT5 and transcription factor FOXP3, can also be converted to the so-called inducible T regulatory cells. Regulatory T cells produce low levels of IL-2 and IFN-γ, but large amounts of IL-10, IL-35 and TGF-β (8). They have an important role in maintaining peripheral immunotolerance and immunosuppression because after differentiation, they migrate to peripheral lymph compartments where suppress the activation and effector functions of autoreactive T lymphocytes, throughout the life of an individual. Considering the immunosuppressive functions and significance in immunotolerance, regulatory T cells are potentially one of the most important line of organism defense from autoimmune diseases, and therefore are in the focus of today's studies (9) (Figure 1).

Th17 cells are newly defined subpopulations of CD4 T lymphocytes, first isolated from peripheral blood of patients with Crohn's disease, as subclass of lymphocytes, that produce large amounts of interleukin 17 (IL-17) and interleukin 23 (IL-23) (6). The conditions that allow differentiation of naive T lymphocytes towards the creation of Th17 cells are not yet precisely defined. Large amount of information was obtained by experimenting on animals, mainly on mice. It was originally believed that IL-23 was the factor that determines the differentiation of Th17 cells. This cytokine has heterodimeric structure; it consists of P40 subunit which is also building part of IL-12, the basic factor of differentiation of Th1 cells, and P19 subunit, unique for IL-23. This data suggested a common origin of Th1 and Th17 cells, however, knockout mice IL-23p19$^{-/-}$ showed normal Th1 response and fully depleted Th17 response, that together with the data that IL-12 and IL-23 using different paths in the signal transduction has just distanced Th17 cells from Th1 cells and highlighted the importance of IL-23 in Th17 cells differentiation (6). Later studies have shown that naive T cells on its surface do not express receptors for IL-23, but also that IL-23 is

required for the secretion of cytokine IL-17, primary cytokine of Th17 cells. It is indicated that the initial differentiation of Th17 cells requires a different cytokine milieu whereas IL-23 is important for the survival and function of Th17 cells (10). Period of intense interest in the Th17 immune response followed the discovery that combination of interleukin 6 (IL-6) and TGF-β in the presence of IL-23 is sufficient to induce the emergence of Th cells that produce IL-17 (6,7,10). Therefore, naive T cells with present TCR and costimulatory signalization, may be subjected to the initial Th17 differentiation in the presence of TGF-β and IL-6. TGF-β and IL-6 act through STAT3 and IRF4 signals that determine the secretion of IL-23 and expression of its receptor on the cell surface. Then, IL-23 in autocrine fashion initiates the activation of transcription factor RORγt (Retinoic acid receptor-related Orphan Receptor γt) and thus secretion of cytokines IL-17, which will complete the program of differentiation and form a stable Th17 phenotype (11). Inter-eukin 21 (IL-21) is identified as a cytokine that in cooperation with TGF-β may also be important for Th17 differentiation, because it increases RORγt transcription factor activity and thus increases IL-23 receptor expression as well as expression of its own receptor (6).

After the identification of Th17 cell differentiation program on mice, there were numerous attempts to establish whether this pattern is obligating for human Th17 cells. Studies have shown that a combination of cytokines TGF-β and IL-6 does not induce Th17 differentiation of human T cell but a precise cytokine profile for the development of Th17 responses in humans is still controversial. Based on gathered data interleukin 1β (IL-1β) and IL-23 are considered to be crucial factors, while the precise role of TGF-β in this process is still incompletely understood and debatable. It is believed that IL-1β or IL-23 alone can induce Th17 differentiation of human, circulating, naive T lymphocytes, whereas TGF-β is not necessary for this process but it promotes it, directly or indirectly in such a manner that it inhibits the differentiation of other Th cell subpopulations (12-15). However, some studies have shown that human naive CD4 T cells obtained from umbilical cord blood in the presence of extremely low concentrations of TGF-β no greater than 25 pg/ml induced the creation of Th17 cells, while high concentrations of TGF-β, 2 ng/ml or greater cause the development of regulatory T lymphocytes (4).

Cytokine secretion profile of Th17 cells is polymorphic. The main cytokine is IL-17 acting through its own IL-17 receptors that are ubiquitous tissue distribution because they can be found on the surface of fibroblast, B and T lymphocytes, mononuclear cell system, bone marrow stromal cells, endothelial cells of blood vessels (5). Stimulated IL-17 receptor activates transcription factor NF-κB (Nuclear Factor-κB) and MAPK (Mitogen-Activated Protein Kinase) signal path, that causes the production of different types of chemokines: CCL2, CCL7, CCL20 and CXCL1, various other cytokines: IL-6, IL -8 as well as certain enzymes: matrix metalloproteases 3 (MMP3) and matrix metalloproteases 13 (MMP13). In summary, all products resulting from IL-17 activity determine neutrophil infiltration and inflammation; therefore, IL-17 is a very potent proinflammatory cytokine (11). In addition to IL-17, Th17 cells produce other cytokines such as IL-22 that stimulates keratinocytes proliferation and expression of S100 antimicrobial peptide family, IL-21 and IL-26 (10). Among the Th17 lymphocyte subpopulations, cells that produce IFN-γ were detected, and on this basis, this cell line is divided into IFN-γ producing Th17 cells (Th17IFNγ) and no IFN-γ producing Th17 cells ($Th17^{IFN\gamma-}$) (16).

Physiologically, Th17 immune response plays a significant role in the host defense against certain extracellular bacteria and fungi, but there are data that suggest that this cell line is included in the immunoprotection from viral pathogens. In contrast to its protective functions, Th17 cells are also considered to be promoters of tissue destruction during inflammatory processes and important for the establishment and development of chronic inflammatory diseases. Th17 cells were first isolated from the patients with Crohn disease, but high expression of IL-23, IL-17 and other cytokines associated with Th17 immune response is detected in the blood and inflamed tissues in patients with various autoimmune diseases such as multiple sclerosis, psoriasis, rheumatoid arthritis (6) (Figure 1).

Th Immune Respons in Multiple Sclerosis

The intense interest in the Th17 cell line of CD4 T lymphocytes is followed by thedemonstration of their potential importance in the pathogenesis of tissue-specific autoimmune diseases. This has led to reconsideration of the classical paradigm by which Th1 cells are considered to be pathogenic cells, the initiators of tissue destruction in auto-immune diseases. The concept of this paradigm is called into question after series of studies carried on knockout mice in which experimental auto-immune encephalomyelitis (EAE), animal model of multiple sclerosis was induced. Testing was based on the fact that IL-12 (a major factor of Th1 cells differentiation) and IL-23 (an important factor of Th17 cells differentiation) have a common P40 subunit, while IL-12 has in addition to P40, P35 subunit, and IL-23 in addition to P40, has P19 subunit. P40 gene deletions resulted in IL-12 and IL-23 deficiency and therefore the Th1 and Th17 cells depletion and these mice were resistant to EAE. When P35 gene deleted, only Th1 cells were depleted and interesting, mice were not protected from EAE, yet developed a more severe form of the disease. P19 gene deletion caused only Th17 cells deficiency and mice were resistant to EAE or

developed much milder form of the disease. Based on these results it was concluded that Th17 cells have a pathogenic role in animal models of autoimmune disease, while Th1 cells have protective role (7). In support of this hypothesis goes the fact that during EAE, Th17 cell infiltration happens first, and it induces the development of clinical symptoms of disease. All this events coincide with the activation of CD11 microglial cells and local production of IL-1β, TNF-α and IL-6 in the CNS. In contrast, significant infiltration of Th1 cells is detected only after the development of clinical manifestations of disease. It was also found that in microglial and MBP-specific Th mixed cell culture, Th17 cells are more potent inductors of pro-inflammatorny cytokine production than Th1 cells, although both Th cell subclasses lead to increasing expression of class II MHC and costimulatory molecules on the microglial cells surface (16).

Other data that verify the significance of Th17 cells in the pathogenetic mechanisms of autoimmune processes were also obtained on animal models. They are related to the fact that TGF-β in combination with IL-6 determines naive T lymphocytes differentiation into Th17 cells, whose expansion leads to exacerbation of EAE in animal models. Involvement of TGF-β in the creation of Th17 cells was surprising, according to the fact that TGF-β alone has anti-inflammatory effects and induce activation of FOXP3 transcription factor, which is essential for the generation of regulatory T lymphocytes, that are important for immuno-suppression, and immunotolerance establishment and maintenance. Experiments on IL-6 deficient mice have demonstrated that the animals are protected from EAE and do not develop a competent Th17 immune response. In summary, the data have suggested the existence of natural antagonism between Th17 cells and regulatory T lymphocytes. In the absence of inflammatory environment, TGF-β produced in the immune system will determine suppression of effector autoreactive T lymphocytes and the development of regulatory T lymphocytes to maintain immuno-tolerance. However, in case of infection and inflammation, proinflammatory cytokine IL-6 produced by innate immunity components suppresses the development of regulatory T lymphocytes and induce the development of proinflammatory T cell response predominantly mediated by Th17 cells (9). Such antagonism has become particularly interesting in terms of autoimmune diseases pathogenesis and significance of chronic infection in this process. Further research has shown that regulatory T cell population does not change dramatically in peripheral compartments of immune system during EAE, while in the CNS there is a significant difference in the frequency and function of these cells that is in correlation with the clinical presentation of disease. In the CNS, the level of Th17 cells is very high in the peak of disease while dramatically reducing in the recovery phase. However, parallel with regression of clinical symptoms, in the stages of recovery the frequency of myelin-specific regulatory T cell is significantly increased. Therefore, it was concluded that during EAE, myelin-specific T regulatory cells target and accumulate in the CNS structures and form stable resident population of regulatory cells. Even more, there is a precise correlation between the clinical presentation of EAE and the relationship between specific regulatory T cells and Th17 cells in the CNS, but this does not apply on peripheral lymph nodes. Analysis of the dynamics of cytokine secretion have shown that regulatory T cells in the CNS predominantly produce IL-10, anti-inflammatory cytokine, whose level is also correlated with the clinical course of disease (9).

Findings obtained on the EAE, animal model of multiple sclerosis were also tested in the human population. It was indeed shown that in the sclerotic plaques and cerebrospinal fluid of patients with multiple sclerosis, there are elevated levels of IL-17, the main cytokine of Th17 cell (17). It is generally known that microglial cells act both as antigen-presenting cells and effector cells, and that they are included in the process of inflammatory demyelization of CNS, therefore, the research is conducted in order to determine the effect of IL-17 on human microglial cells, and contribution of Th17 cells in neuron inflammatory demyelization. The results demonstrated that after exposure to IL-17, microglial cell begins production of proinflammatory substances such as: IL-6, macrophage inflammatory protein 2 (MIP-2), nitric oxide (NO), a variety of neutrophil chemotaxis factors and adhesion molecules. These data additionally suggest patho-genicity of Th17 cells in multiple sclerosis (8).

In multiple sclerosis, the localization of CNS lesions can be extremely variable and that is an important factor that determines the clinical presentation of disease. Differences in the distri-bution of lesions are associated with the structure of HLA molecules, suggesting that MBP-specific T cells do not infiltrate entire brain parenchyma, although MBP is widely present, but a certain places where they will induce the development of demyeli-zation plaque. Analysis of lymphocytes infiltrate showed that it consists of Th1 and TH17 cells and that the relationship between these two cell subsets decides whether the initiation of inflammation in that area will take place. Inflammation will develop when the relationship between Th17 and Th1 cells is greater than 1, because there is a dispropor-tionate increase in IL-17 secretion, which is now considered to be a major regulator of autoimmune processes in the CNS. IL-17 induces the activation of enzyme matrix metalloprotease 3 (MMP-3) and recruits neutrophils to the site of inflammation. Neutrophil activation increases enzyme activity in the extracellular matrix as well as various other types of metalloproteases, proteases and gelati-nases that contributes to the violation of blood-brain barrier selective permeability, and additionally increases neutrophil infiltration. Increased protease activity attracts significant number of monocytes or macrophages to the place of inflammation resulting

in myelin destruction and axonal damage. Intriguing, in the spinal cord, inflammation development occurs when the relationship between Th17 and Th1 cells is less than 1, indicating that during inflammation in the brain, pathogenic cells are Th17 cells while in the autoimmune processes that affect the spinal cord, those are Th1 cells. The reasons for this paradoxical pathogenetic process of autoimmunity in the brain and spinal cord are still unexplained (18).

In addition to abnormalities in the Th17 and Th1 immune response in multiple sclerosis, some irregularities in the level of regulatory T lymphocytes were also recorded. It was found that patients suffering from multiple sclerosis did not show significant variation of regulatory T lymphocytes number compared to healthy individuals, but in vitro experiments, the insufficiency of their suppression function was demonstrated (19). Later studies have shown that there are actually two subtypes of regulatory T cells, and that their ratio is violated in multiple sclerosis. Subclassification of these cells was performed on the basis of programmed death receptor 1 (PD-1) surface expression, on: $PD1^+$ and $PD1^-$ regulatory T cells. In short, PD-1 is present in the intracellular domain of naive T regulatory cell, after its activation PD-1 is expressed on the cell surface that creates memory T regulatory cells. $PD1^-$ subclass makes the most of circulating regulatory T cells in healthy people and it is the holder of a strong immunosuppressive effect 20, while $PD1^+$ subclass is short living and it is quickly removed by apoptosis (21). It is shown that in the remittent phase of multiple sclerosis $PD1^-$ regulatory T lymphocytes are significantly increased in cerebrospinal fluid compared to healthy individuals corresponding to the so-called "Autoimmune Status" characteristic of multiple sclerosis, because the disease can be kept under control only if there is higher suppressive regulatory T cell function. Also, it has been shown that the number of these cells is significantly increased in the peripheral blood of patients in the remission stage, compared to the relapsing phase of disease when there is increased number of $PD1^+$ regulatory T lymphocytes. These data suggest that $PD1^-$ regulatory T cells do not have much influence on multiple sclerosis development, yet they act as some sort of protective mechanism that allows body to maintain immunotolerance and control disease. In other words, qualitative-quantitative alteration of $PD1^-$ T lymphocytes is the reflection of the illness rather than its cause (20,21).

Despite numerous enthusiastic results suggesting that Th17 cells have pathogenic features in the development of multiple sclerosis while Th1 cells are protective, caution is recommended, because for the period of 150 years of disease researching, it is clear that its etiopathogenetic mechanisms cannot be observed just from one aspect, and characterized by uncomplicated polarization and simplification. In addition, recent researches indicate that Th17 cells are pathogenic only in the specific form of multiple sclerosis that is characterized mainly by granulocyte infiltration, while still there is intact importance of Th1 cells in the development of multiple sclerosis that is characterized by predominantly mononuclear cell infiltration (7).

Therapeutic Implications of Novel Pathogenetic Concept of Multiple Sclerosis

Starting from the fact that virtually all effector mechanisms of the immune system are involved in the pathogenesis of multiple sclerosis, in order to achieve control of disease activity and progression, guidelines that base immunotherapy on: autoreactive Th1 cell and B lymphocyte depletion, regulatory T lymphocyte promotion, innate immunity modification, neuroprotection and remyelization promotion, are given (22). Today, multiple sclerosis therapeutic approach depends on the stage of disease; in the relapse phase, pulse doses of corticosteroids are applied with the idea of immunosuppression, while in the stage of remission, the so-called disease modifying drugs are applied in the forefront IFN-β (Betaferon®, Avonex®, Rebif®) and glatiramer acetate (Copaxone®) with the idea of extending remission and relapse reduction (1). New findings constructed the system of pathogenetic mechanism of multiple sclerosis as primarily Th17 cell mediated disease, and questioned the acting mechanisms of the drugs commonly used today, but also opened up possibilities for new therapeutic approaches.

IFN-β is now widely applied in the multiple sclerosis treatment, its effectiveness is proven by numerous clinical trials while its acting mechanism remains unclear (21). There are data indicating that IFN-β achieves its effect by just inhibiting Th17 cell differentiation. It is believed that IFN-β acts on dendritic cells and B lymphocytes by stimulating them to secrete Th17 suppressive cytokines, IL-27 and IL-12, which activate STAT1 by phosphorylation, and so inhibits the Th17 phenotype differentiation. IFN-β promotes the secretion of anti-inflammatory cytokine, IL-10 by T and B lymphocytes, and that represents a significant additional immunoregulatory mehanism (23). According to some authors, Th17 cells are highly selective targets for interferon therapy (24). Effects of IFN-β on the regulatory T lymphocytes levels are also examined, however, it has been showed that IFN-β does not alter naive PD1- regulatory T lymphocytes levels in the blood of patients with multiple sclerosis (20).

Glatiramer acetate induces Th2 polarization of immune response, and in accordance with the cross-regulation, it determines attenuation of Th1-mediated immune response, witch is the main purpose of its application in the treatment of multiple sclerosis (25). Drug effects on Th17 cells are not yet fully known, but according to data encountered in literature, decreased phosphorylation and thus decreased activation of STAT3 are induced, which causes decreased expression of

RORγt transcription factor necessary for Th17 differentiation completion (26). Drug effect research on regulatory T lymphocytes have shown that glatiramer acetate has the ability to increase FOXP3 transcription factor expression and thus enlarges the population of naive $PD1^-$ regulatory T lymphocytes in the blood of patients. This could be another pharmacological action of glatiramer acetate in multiple sclerosis, but also important fact that will allow this drug to be used in other autoimmune diseases (20).

There are numerous attempts to identify other therapeutic targets that will selectively inhibit Th17 immune response formation and its pathogenic role in multiple sclerosis. From immunological point of view, perhaps the most interesting study is related to the IL-17 immunization. It is based on the postulate that mice treated with neutralizing anti-IL-17 antibodies suppress the autoimmune inflammatory process in EAE model, even more IL-17 deficient knockout mice are protected from EAE-a. The next step of research tested whether vaccination, in general, has any influence on the emergence and development of the disease. IL-17 is chemically bound to virus resembling particles, VLP (Virus-Like Particles), and injected without adjuvant three times within 28 days. It is demonstrated that VLP-IL-17 immunization reduces the incidence of disease in mice which were vaccinated before attempting to induce EAE. Also, mice with developed EAE after vaccination showed a regression of the disease. Similar results were obtained by using the vaccine in which the IL-17 was bound to ovalbumin. During the study, the dynamics and stability of antibodies induced by vaccination was tracked and it was noted that there was a high level of anti-IL-17 antibody, which decreased relatively slow, with half-life of about five months, indicating that anti-cytokine vaccination may provide a long-term inhibition of IL-17 cytokine function (27). There were attempts with anti-cytokine therapy that target cytokine IL-6 and it showed encouraging results as well. It is found that blocked IL-6 activity, by using anti-IL-6 receptor monoclonal antibody (anti-IL-6R At), inhibits the development of EAE by suppression of MBP-specific Th17 and Th1 cells and thus reduces the lymphocytic infiltration of the CNS (28). Similar results were obtained by targeting cytokine IL-23 (29). Today's studies of multiple sclerosis therapeutic opportunities are also made towards the identification of cytokines or chemokines responsible for Th17 cells migration in demyelization plaques. Among the many tested chemokine/chemokine receptor systems, it was discovered that CCL-2/CCR-2 interaction is essential for the development of EAE. Concentration of CCL-2 in demyelization plaques is manifold increased, and IL-17 stimulates the blood-brain barrier endothelium to produce exactly CCL-2. This suggests the possibility that CCL-2/CCR-2 system is responsible for Th17 cells migration in the plaques, and sets this system as a therapeutic target in multiple sclerosis (17). Taken together, these results indicate that anti-cytokine/chemokine therapy, whether based on the immunization or on the use of monoclonal antibodies, should be considered as a new possibility of treating people with multiple sclerosis.

Beside the anti-cytokine therapy, nowadays studies also try to identify substances that can specifically suppress the Th17 response by other mechanisms, and that is the case with agonists of nuclear receptor PPARγ (Peroxisome Proliferator-Activated Receptor γ). Consideration is given to rosiglitazone (Avandia®), advanced form of thia-zolidinedione, which is already in use for diabetes mellitus type II treatment, and there are implica-tions for its use in Alzheimer's disease. Some studies have shown that nuclear receptor PPARγ is a key negative regulator of mousy and human Th17 cells, because its activation selectively suppress Th17 differentiation, while it realizes no effect on Th1, Th2 and regulatory T cell differentiation. It is believed that Th17 differentiation control via nuclear receptor PPARγ includes inhibition of TGF-β/IL-6 induced expression of RORγt transcription factor in naive T lymphocytes (30).

The link between vitamin D deficiency and multiple sclerosis has long been established, but in terms of Th17 immune response, vitamin A and its metabolite active form, trans retinoic acid isomer, ATRA (All Trans Retinoic Acid), has attracted particular attention (31). Recent studies have shown that ATRA significantly affects the fate of naive T lymphocytes activated in the presence of TGF-β, in the manner that inhibits development of Th17 cells by reducing expression of RORγt, and promotes the development of T regulatory lymphocytes (32). Even more, it is demonstrated that in vitro generated regulatory T cells in the presence of TGF -β and ATRA, were effective in preventing EAE, whereas regulatory T cells generated in the absence of ATRA were only partially effective. This suggests that ATRA and its synthetic analogues may be significant additive treatment for multiple sclerosis because they alter Th17 cells and T regulatory lymphocytes balans (31,32).

Anti-Th17 therapy practice in multiple sclerosis is still at the stage of clinical trials and of course that a further study are needed, so this therapy concept can be considered as standard, but so far, the experimental data obtained on EAE models, give very encouraging results.

Conclusion

By the discovery and long-term study of Th17 cells, it became clear that Th17 rather than Th1 cells are essential for the development of experimental autoimmune encephalomyelitis, animal models of multiple sclerosis. TGF-β and IL-6 are essential for Th17 cell differentiation, while IL-23 is essential for cell survival. Th17 differentiated cells secrete cytokine milieu in which a central place is taken by proinflammatory cytokine IL-17. Cellular infiltrate in demyelization plaques, lesions pathognomonic for multiple sclerosis, is mainly composed of Th17 cells. Under

the influence of IL-17, here present microglial cells begin to produce proinflammatory substances such as: IL-6, macrophage inflammatory protein 2 (MIP-2), nitric oxide (NO), a variety of neutrophil chemotaxis factors and adhesion molecules. These data strongly suggest pathogenicity of Th17 cells in multiple sclerosis. It is, therefore, necessary to review pathogenic concepts of multiple sclerosis as classical Th1 mediated autoimmune disease, because some findings suggest even protective role of these cells. Novel concept, in which Th17 cells have a central pathogenetic role could contribute to a better understanding of the disease and identify new fields of research for treatment options in terms of IL-17 immunization and other forms of anti-cytokine therapy.

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ULOGA TH1 I TH17 IMUNOG ODGOVORA U PATOGENEZI MULTIPLE SKLEROZE

Miloš Kostić

Th17 ćelije su novootkrivena subpopulacija $CD4^+$ T limfocita koja se predominantno karakteriše produkcijom proinflamatornog citokina IL-17. Od njihovog otkrića, 2003. godine, Th17 ćelije su pobudile veliku pažnju, zbog mnogobrojnih dokaza koji ukazuju na njihovu ključnu ulogu u patogenezi multiple skleroze i drugih bolesti koje su ranije smatrane klasičnim Th1 posredovanim autoimunim bolestima. Na animalnom modelu multiple skleroze, eksperimentalnom autoimunom encefalomijelitisu, demonstrirano je da su Th17 ćelije nosioci inflamatornog procesa u autoimunosti, dok Th1 ćelije imaju protektivnu ulogu, što je u suprotnosti sa dosadašnjim konceptom multiple skleroze. Odnos između Th17 i Th1 ćelija decidira da li će doći do inicijacije inflamacije i nastanka demijelinizacionog plaka. Inflamacija nastaje kada odnos između Th17 i Th1 ćelija bude veći od 1, jer tada postoji disproporcionalni porast sekrecije IL-17, koji se danas smatra glavnim regulatorom autoimunih procesa u CNS-u. Otkriće uključenosti TGF-β i IL-6 u proces nastanka proinflamatornih Th17 ćelija je takođe iznenađujuće, s obzirom da TGF-β samostalno ima antiinflamatorna dejstva i indukuje aktivaciju FOXP3 transkripcionog faktora, esencijalnog za generisanje regulatornih T limfocita, koji su značajni za imunosupresiju, uspostavljanje i održavanje imunotolerancije. Funkcionalni antagonizam, uz slične diferencijacijske faktore Th17 ćelija i regulatornih T limfocita postao je posebno interesantan sa aspekta imunomodulatorne terapije autoimunih poremećaja. Nova saznanja su konstruisala sistem patogenetskog mehanizma multiple skleroze posredovan prevashodno Th17 ćelijama, što je dovelo u pitanje mehanizme delovanja lekova danas, ali i otvorilo mogućnosti za nove terapijske pristupe. *Acta Medica Medianae 2010;49(4):61-69.*

***Ključne reči**: Th17, Th1, regulatorni T limfociti, IL-17, multipla skleroza*