

Cytokines as Mediators of Neuroinflammation in Experimental Autoimmune Encephalomyelitis

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Abstract—Cytokines play a pivotal role in maintaining homeostasis of the immune system and in regulation of the immune response. Cytokine dysregulation is often associated with development of various pathological conditions, including autoimmunity. Recent studies have provided insights into the cytokine signaling pathways that are involved not only in pathogenesis of autoimmune neuroinflammatory disorders, such as multiple sclerosis, but also in neurodegenerative states, for example, Alzheimer's disease. Understanding the exact molecular mechanisms of disease pathogenesis and evaluation of relevant experimental animal models are necessary for development of effective therapeutic approaches.

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In developed countries, aging of the population is a risk factor for development of central nervous system (CNS) disorders. Despite the steadily increasing number of patients with these serious diseases, up to now no effective therapy for such neuropathologies has been developed. For a long time, it was believed that the CNS does not interact with the immune system because of its immune privilege being maintained, in particular, by the blood-brain barrier (BBB). The BBB is a physical border between the CNS and immune cells circulating in the

blood and soluble factors produced by them. This barrier is maintained by tight junctions between the brain endothelial cells, their basal membrane, pericytes and astrocytes. However, recent studies in neuroimmunology have shown that an interaction of the immune system components with the CNS is required for maintaining homeostasis and that homeostatic imbalance caused by inflammatory processes may lead to various pathologies. Thus, immune dysregulation and development of pathological processes trigger the effector immune response to CNS-specific antigens that is mediated by infiltration of the circulating immune cells into the CNS and by activation of the CNS-resident cells. In the inflammatory microenvironment the infiltrating and resident cells of the CNS secrete cytokines which in steady state regulate the immune response and homeostasis. Proinflammatory cytokines play a pivotal role in the pathogenesis of autoimmune diseases, in particular, of those associated with pathology of the nervous system. For instance, increased levels of many proinflammatory cytokines, such as interleukins 6, 1, 17 (IL-6, IL-1, IL-17) and tumor necrosis factor (TNF), were found in the cere-

Abbreviations: AD, Alzheimer's disease; BBB, blood-brain barrier; CCR, chemokine receptor; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; GM-CSF, granulocyte-macrophage colony-stimulating factor; gp130, glycoprotein 130; IFN, interferon; IL, interleukin; IL-1R1, IL-1 β receptor subunit; IL-6R, interleukin-6 receptor; LT, lymphotoxin; MOG, myelin oligodendrocyte protein; MS, multiple sclerosis; NK-cells, natural killer cells; TGF β , transforming growth factor beta; Th, T helper; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; Tregs, regulatory T cells.

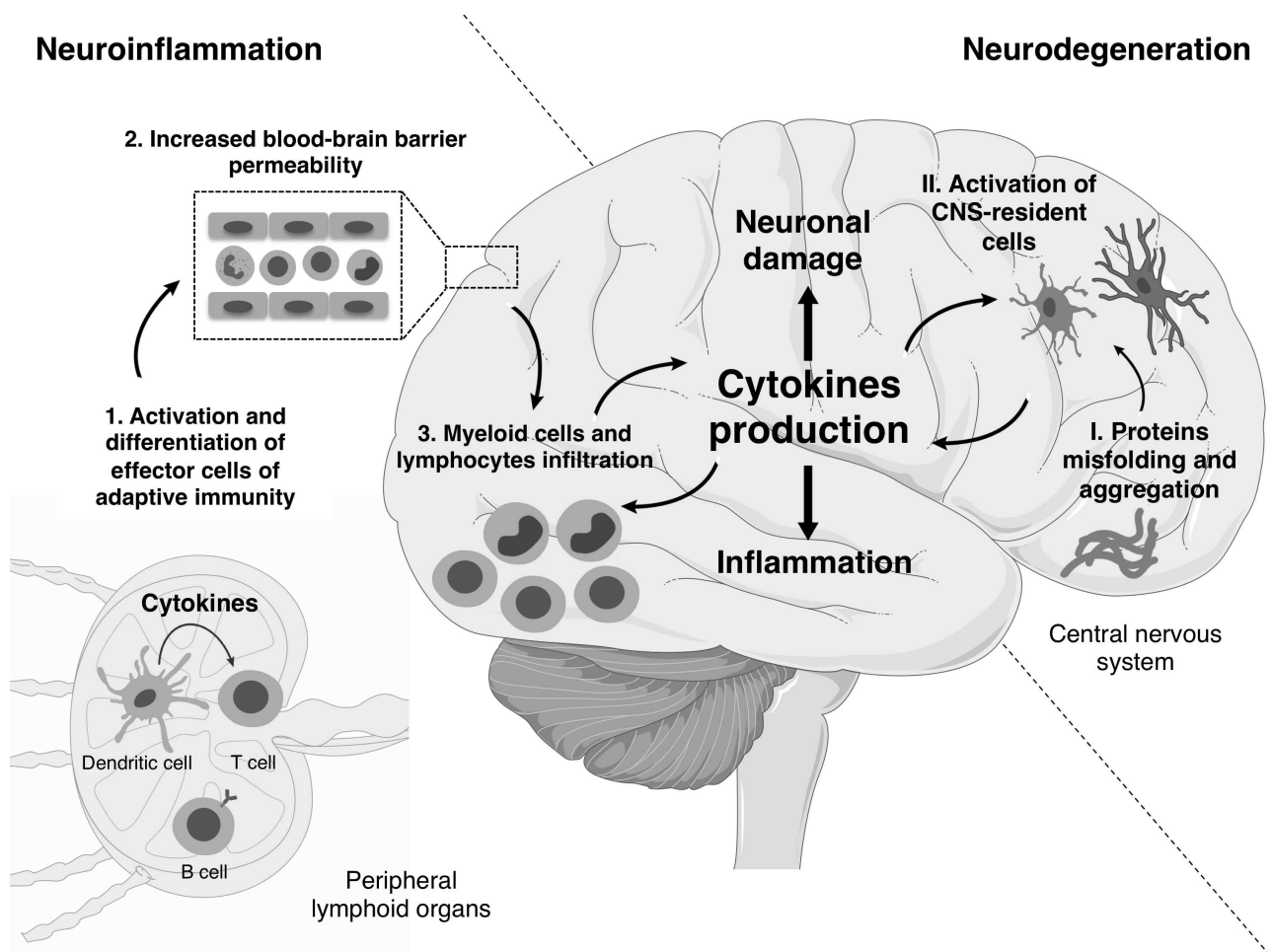
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brospinal fluid and in demyelinating plaques of patients with multiple sclerosis (MS) suggesting their possible involvement in the pathogenesis of MS [1, 2]. The roles of various cytokines in neuroinflammation and neurodegeneration have been investigated in the mouse experimental models using reverse genetics approach.

NEURODEGENERATIVE DISEASES AND NEUROINFLAMMATION

Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are the most frequent and best studied neurodegenerative diseases. It is thought that initiation of neurodegenerative diseases is

associated with the accumulation of misfolded proteins in the brain causing neuronal damage in different brain regions [3, 4]. The role of the immune system in the pathogenesis of these diseases is intensively studied, particularly, in mouse models. Thus, systemic inflammation in mice may promote the accumulation of a β -amyloid precursor that further results in development of pathological processes specific for AD: the accumulation of β -amyloid plaques and activation of microglia [5]. Genetic studies have revealed the association between predisposition to AD and mutations in the gene encoding the triggering receptor expressed on myeloid cells 2 (TREM2) which is present on the microglia cell surface [6, 7]. It has been shown that in a mouse model of AD microglia acquires a protective phenotype in a two-step process due



Role of cytokines in the development of neuroinflammatory and neurodegenerative diseases. During the neuroinflammation, priming occurs in peripheral lymphoid organs with subsequent differentiation of effector T cells due to cytokines being released by antigen-presenting cells (1). Then the increased BBB permeability promotes the infiltration of lymphoid and myeloid cells into the CNS (2, 3). The infiltrating cells recruit other immune cells from the periphery through the production of cytokines and also activate CNS-resident cells driving inflammation in the CNS. The development of neurodegenerative diseases is characterized by accumulation of protein aggregates (I). This process results in the activation of stromal cells and phagocytes of the CNS, i.e., astrocytes and microglia, respectively (II). Subsequently, astrocytes and microglia secrete cytokines, and this chronic CNS-intrinsic cytokine production is associated with loss of tissue function. The effector phase of neuroinflammatory and neurodegenerative diseases is characterized by neuronal damage and local inflammation in the CNS. (Materials of the Servier Medical Art were used for the figure)

to increased expression of genes responsible for phagocytosis of the accumulated β -amyloid plaques [8]. Another study found that the microglia phenotype depends on activation of TREM2–APOE signaling pathway which is associated with the pathogenic role of the microglia in the neurodegeneration model [9]. Recent data have identified that the inflammation, mainly initiated by activated myeloid cells, plays an important role in the pathogenesis of AD. Thus, the immune system is believed to play an important role in the pathogenesis of this neurodegenerative disorder [10].

Mechanisms of development and pathogenesis of neurodegenerative and neuroinflammatory diseases are different. First, the main causes of neurodegenerative diseases are protein aggregates of β -amyloid, α -synuclein and a mutant form of superoxide dismutase 1 (SOD1) in the case of AD, PD, and the hereditary form of ALS, respectively [11]. On the other hand, in the classical neuroinflammatory disease, i.e., multiple sclerosis, pathological processes are mainly mediated by cells of the immune system. Second, in proteinopathies, proinflammatory cytokines are mainly produced by myeloid cells, predominantly, CNS-resident cells, whereas in neuroinflammation cytokines are produced by both T cells and myeloid cells [12, 13]. Third, in neuroinflammation the BBB is destroyed during the early stages of disease onset leading to infiltration of the immune cells that later will support the inflammation in the CNS (figure).

MECHANISMS OF MULTIPLE SCLEROSIS DEVELOPMENT

Multiple sclerosis (MS) is a complex autoimmune disease characterized by damage of the myelin sheath in numerous inflammatory lesions. To date, MS affects approximately 2.5 million people worldwide, and the average age of MS diagnosis is 30. In the majority of patients, clinical manifestations include disorders of motor, cognitive, and visual functions, as well as the loss of sensitivity [14]. The development of MS is contributed by both genetic and environmental factors (e.g., the Epstein–Barr virus infection) that results further in development of the autoimmune response directed against the myelin sheath of nerve fibers [15].

Fundamental mechanisms of the disease pathophysiology were studied in a mouse model of multiple sclerosis – experimental autoimmune encephalomyelitis (EAE) [16, 17]. It should be noted that EAE induction can be both “active” or “passive”. “Active” EAE is induced by immunization with a CNS-specific antigen in complete Freund’s adjuvant with the subsequent injection of pertussis toxin. “Passive” EAE requires adoptive transfer of neuroantigen-specific T cells into recipient mice leading to development of the immune pathology [18]. Depending on the genetic strain of mice, myelin basic

protein (MBP), myelin oligodendrocyte protein (MOG) or proteolipid protein (PLP) are used as antigens for the disease induction. Moreover, a model of spontaneous EAE development was established using transgenic mice with overexpression of the antigen-specific T- or B-cell receptor [19]. However, it should be noted that EAE is not an ideal model of multiple sclerosis. First, mice on C57BL/6 background, a commonly used mouse strain in immunological studies, show a monophasic disease course, whereas the most frequent MS type is relapsing-remitting MS characterized by periods of exacerbation and recovery. Second, the composition of key cell populations involved in the adaptive arm of immune response during EAE can vary, depending on the chosen antigen or on the mouse strain. Thus, when the disease is induced with the MOG₃₅₋₅₅-peptide, the T cell response in C57BL/6 mice is characterized mainly by CD4⁺ T cells, whereas in humans CD8⁺ T cells and B cells are also involved [20, 21]. In clinical studies, the efficiency of B-cell depletion has been already approved, whereas the role of B cells in EAE requires further investigation. It has been demonstrated that B cells can modulate the severity of the disease by their antigen-presenting function, as well as via secretion of anti-inflammatory cytokines IL-10 and IL-35 [22, 23]. Moreover, EAE in C57BL/6 mice is characterized by more severe tissue damage of spinal cord than the brain. Nevertheless, despite the obvious limitations, EAE remains the most frequent experimental model of MS, also used in preclinical trials.

It was shown in mouse models that the escape of autoreactive T cells from thymic deletion to the periphery and the loss of immune tolerance can lead to the development of T-cell mediated immune response directed against CNS-specific autoantigens [24]. For many years, the CNS was thought to be an immunologically privileged organ, but more recently lymphatic vessels responsible for drainage of the CNS and cerebrospinal fluid were found in the brain meninges [25]. This led to the revision of the existing hypotheses about the mechanisms of neuroinflammation. It is now understood that neuroinflammation can be initiated by antigens from the CNS entering the draining lymph nodes in a soluble form or as a result of the transfer by antigen-presenting cells. Moreover, viral antigens mimicking CNS-specific autoantigens also can induce an immune response in the periphery [26].

The next stage of the disease development is associated with T-cell priming and is accompanied by differentiation of CD4⁺ T cells into various effector populations in the draining lymph nodes. Thus, in the EAE model main pathogenic CD4⁺ T cell subpopulations are Th1 and Th17, and their adoptive transfer can induce the disease in naive mice [27]. In this case, FoxP3⁺ regulatory T cells play a protective role, and their deletion leads to exacerbation of the disease [28]. The pathogenic and protective properties of different T cell subsets are considerably determined by production of certain cytokines. In

Specific features of experimental autoimmune encephalomyelitis in mice with genetic or pharmacological inhibition of cytokines or their receptors

Cytokine	Inhibition	EAE model	Phenotype	Reference
1	2	3	4	5
GM-CSF	genetic	MOG/CFA	resistance	[37]
GM-CSFR β (CSF2RB)	genetic	MOG/CFA	resistance	[38]
IFN γ	pharmacological	SCH/CFA	high susceptibility, increased mortality	[39]
	genetic	MBP/CFA	high susceptibility	[40]
IFN γ R1	genetic	MOG/CFA	high susceptibility, increased mortality	[41]
IL-1 β	genetic	MOG/CFA	resistance	[42]
IL-1R1	genetic	MOG/CFA	resistance	[42, 43]
	conditional deletion in CD4 ⁺ T cells	MOG/CFA	diminished clinical symptoms	[44]
IL-6	genetic	MOG/CFA	resistance	[45, 46]
	conditional deletion in CD11c ⁺ dendritic cells	MOG/CFA	resistance	[47]
IL-6R	pharmacological	MOG/CFA	diminished clinical symptoms, delayed disease onset	[48]
IL-6R β (gp130)	conditional deletion in CD4 ⁺ T cells	MOG/CFA	resistance	[49, 50]
IL-10	conditional deletion in B cells	MOG/CFA	chronic disease	[22]
IL-12p35	genetic	MOG/CFA	high susceptibility	[51, 52]
IL-12p40	genetic	MOG/CFA	resistance	[51]
IL-23p19, IL-23R	genetic	MOG/CFA	resistance	[52, 53]
IL-35	conditional deletion in B cells	MOG/CFA	chronic disease	[23]
IL-17A	genetic	MOG/CFA	diminished clinical symptoms, delayed disease onset	[54]
LT α	genetic	MOG/CFA	resistance	[55]
	genetic	MOG/CFA	susceptibility	unpublished data
LT β	genetic	MOG/CFA	susceptibility	[55]

Table (Contd.)

1	2	3	4	5
TNF	pharmacological	SCH/CFA, CREAE	inhibition of development	[56]
	genetic	SCH/CFA, PLP/CFA	progression of clinical symptoms	[57]
	genetic	MOG/CFA	progression of clinical symptoms	[58]
	conditional deletion in myeloid cells	MOG/CFA	delayed disease onset	[59]
	conditional deletion in CD4 ⁺ T cells	MOG/CFA	progression of clinical symptoms	[59]
TNFR1	genetic	MOG/CFA	delayed disease onset	[60, 61]
TNFR1/TNFR2	genetic	MOG/CFA	delayed disease onset	[60, 61]
TNFR2	genetic	MOG/CFA	progression of clinical symptoms	[60, 61]

Note: CFA, complete Freund adjuvant; CREAE, chronic relapsing EAE; MBP, myelin basic protein; MOG, myelin oligodendrocyte protein; PLP, proteolipid protein; SCH, spinal cord homogenate.

particular, skewing of T cell differentiation towards protective Th2 subset is the mechanism of action of existing drugs approved for MS treatment [29, 30]. For example, one of the immunomodulatory effects of interferon- β (IFN β) is the inhibition of IL-12 production by T cells, a key factor of Th1 differentiation [31]. Besides CD4⁺ T cells, which are the main regulators in EAE, activation and maturation of CD8⁺ T cells and B cells occur in the periphery [32, 33].

After induction of the adaptive immune response, these cells migrate into the CNS. For this, activation of the vascular endothelium is required through the induction of the signaling cascade $\alpha 4\beta 1$ -integrin/VCAM-1, as well as the increased BBB permeability for the subsequent cell infiltration [34]. The interaction of $\alpha 4\beta 1$ -integrin on the surface of immune cells with the protein on endothelial cells – vascular cell adhesion molecule 1 (VCAM-1) – facilitates the accumulation of lymphocytes in inflammatory lesions in the brain and spinal cord. Therefore, $\alpha 4\beta 1$ -integrin is one of the approved targets for the MS therapy [35]. The activation of the CCR6/CCL20 (chemokine receptor 6/chemokine ligand 20) signaling axis is another mechanism of migration of autoreactive and regulatory T cells into the CNS. Epithelial cells in the CNS constitutively express CCL20 that allows CCR6⁺ Th17 cells to migrate into the CNS [36].

Infiltrated CD4⁺ T cells, as well as the activated CNS-resident cells, microglia and astrocytes, produce many cytokines and chemokines, which, in turn, promote the influx of effector cells and drive the demyelination, axonal damage and the persistent inflammation.

Thus, the pathogenesis of MS is a multi-stage process and involves various populations of T cells, as well as B cells and myeloid cells, both in the periphery and CNS. EAE is the most suitable and well-studied mouse model of MS not only for understanding the fundamental mechanisms of induction and development of the disease but also for clinical studies.

ROLE OF CYTOKINES IN EAE DEVELOPMENT

Cytokines play an important role on all stages of EAE development. The pathogenic or protective function of each cytokine depends on the cellular source, the target cell population, as well as on the phase of the disease when this cytokine has been secreted. Biology of cytokines in MS was mainly studied in the model of MOG-induced EAE. In this model CD4⁺ T cells are key cells implicated into the disease pathogenesis, and, therefore, in this review we conventionally classify the cytokines considering their role in the differentiation of pathogenic T cells. Direct experimental evidences of contribution of different cytokines in EAE development were obtained using reverse genetics approach or pharmacological inhibition and are presented in the table.

Cytokines determining the differentiation of naïve T cells. *IL-6* is a cytokine of the IL-6 family of cytokines characterized by gp130, a transmembrane protein, present as a subunit of the receptor complex and responsible for signal transduction [62]. IL-6 is produced by various cell types of both hematopoietic and stromal origin, i.e.,

fibroblasts, neurons and endothelial cells. On the contrary, IL-6 receptor (IL-6R) expression is restricted to certain cell types, namely, leukocytes, hepatocytes and microglia. IL-6R exists not only in membrane-bound, but also in soluble form (sIL-6R) as a result of proteolytic cleavage by the enzyme ADAM17. This determines the existence of two distinct mechanisms of signal transduction, the classical and trans-signaling cascades, respectively [63].

The "classical" IL-6 receptor complex consists of the cytokine IL-6, α -subunit (IL-6R) that binds IL-6, and β -subunit (gp130), which is responsible for the intracellular signal transduction. In the case of trans-signaling, sIL-6R binds to inactive dimers of the ubiquitously expressed gp130, and thus noticeably widens the spectrum of the IL-6 signaling target cells. Both the classic and trans-signaling activate the Jak-STAT signaling pathway, which predominantly leads to activation of the transcription factor STAT3. The signaling pathway Jak-STAT is tightly controlled, in particular, it is negatively regulated by a suppressor of cytokine signaling 3 (SOCS3) and by phosphatase SHP-2, which inhibit activities of Jak-kinases and activation of STAT3 [64].

It is interesting that IL-6 is one of only a few cytokines which are absolutely crucial for the EAE development. *Il6*-deficient mice are completely resistant to EAE induction [45, 46]. IL-6 in different combinations with IL-1 β , IL-21, IL-23 and the transforming growth factor β (TGF β) directs the differentiation of naïve T cells into pathogenic Th17 cells [65-68]. On the other hand, studies on reporter FoxP3-GFP mice and mice with the conventional knockout of gp130 in T cells demonstrated that IL-6 inhibited the differentiation of T cells into FoxP3⁺ regulatory T cells [49, 69]. CD11c⁺ dendritic cells can induce the differentiation of pathogenic Th17 cells via trans-presentation mechanism shown for the EAE model [47]. Besides that, the positive feedback loop between IL-17A and IL-6 is formed. Thus, injection of MOG-specific Th17 cells into healthy wild type mice leads to the increased production of IL-6 in serum. Adoptive transfer of Th17 cells from the wild type mice with EAE into the IL-6-deficient mice leads to induction of an attenuated disease suggesting a pathogenic role of the IL-17A/IL-6 signaling axis in EAE [70]. Moreover, IL-6 can maintain inflammation in the CNS where astrocytes are the major source of IL-6 [71, 72].

Thus, during the disease onset, IL-6 regulates the balance between pathogenic Th17 cells and protective Tregs in the periphery. Moreover, IL-6 produced by the CNS-resident cells can be pathogenic due to maintenance of local inflammation through activation of the IL-6 trans-signaling, but at the late stages of the disease it can play the anti-inflammatory role.

IL-1 β . IL-1 β belongs to the IL-1 cytokine family and is produced by monocytes, dendritic cells, microglia, and also by B cells and natural killers (NK cells) [73]. IL-

1 β is synthesized in the cell in the form of inactive precursor. For the secretion of the active form of IL-1 β produced via proteolytic cleavage by caspase-1 or caspase-11, an assembly of a special molecular platform, inflammasome, is required. Recent studies unraveled inflammasome-independent IL-1 β activation pathways [74-76]. IL-1 β receptor consists of two subunits, IL-1R1 and IL-1RAP (IL-1R3), which are expressed on all innate immune cells and also on T cells [77]. The IL-1 receptor family is characterized by the presence of a TIR-domain (Toll/IL-1 receptor) in the cytoplasmic part. Formation of a heterodimeric receptor complex facilitates recruitment of the adaptor protein MyD88 and various components of the signaling cascade leading to activation of the transcription factor NF κ B [78].

Il1b gene-deficient mice are resistant to EAE induction confirming the important role of IL-1 β in EAE development [42]. IL-1R1-deficient mice develop significantly attenuated disease in comparison with the wild type mice due to absence of the antigen-specific Th17 cells [43]. In EAE model IL-1 β is required for polarization of T cells into pathogenic Th17 cells and for their further proliferation [44, 79]. During the early stages of the disease, the IL-1 signaling pathway supports the differentiation into Th17 cells by increasing expression of the transcription factors IRF4 and ROR γ t [65]. IL-1 β in the CNS is mainly produced by neutrophils and monocytes infiltrating into the spinal cord as it was shown on the reporter pro-IL-1 β mice. In this case, the activation of the IL-1R1-signaling in the endothelial cells led to secretion of proinflammatory cytokines and chemokines recruiting myeloid cells into the CNS [42]. It also has been revealed that IL-1 β was synthesized by myeloid cells in the draining lymph nodes after injection of the pertussis toxin, a component needed for the EAE induction. In this case, tissue-specific deletion of IL-1R1 in T cells resulted in a decreased amount of CD4⁺ Th17 cells in the draining lymph nodes, but it only partially influenced the resistance of the mice to the disease induction. It appeared that IL-1 β was necessary for supporting the pathogenicity of Th17 cells producing the granulocyte-macrophage colony-stimulating factor (GM-CSF) [44, 80].

Unlike the IL-1 β , IL-1 α plays insignificant role in EAE [42, 81]. Thus, of the family of IL-1 cytokines, it is the IL-1 β that is the most important for EAE development, and its main pathogenic role is the expansion of GM-CSF⁺ Th17 cells in the periphery.

IL-12 and IL-23. IL-12 and IL-23 are members of the IL-12 cytokine family with the IL-12R β subunit in heterodimeric receptors. Active IL-12 is an IL-12p70 heterodimer consisting of subunits p35 and p40. IL-23 contains the same subunit p40, but unlike IL-12, it is combined with the subunit p19. Both cytokines are secreted mainly by antigen-presenting cells. In accordance with the presence of the common subunit p40, IL-

IL-12 and IL-23 have the common receptor subunit IL-12R β 1, but specific subunits are different – IL-12R β 2 and IL-23R, respectively [82]. IL-12R and IL-23R are expressed on activated T cells, dendritic cells and NK cells. Moreover, IL-23R is also expressed on Th17 cells and $\gamma\delta$ T cells [83]. As in the case of IL-6, triggering of receptors of the IL-12 family of cytokines leads to activation of the Jak–STAT signaling pathway, but the consequences of the cytokine binding to the receptor are distinct. Thus, IL-12 binding to the receptor leads to activation of the transcription factor STAT4, whereas IL-23 mediates the STAT3- and STAT4-signaling pathways [84].

IL-12 is one of the cytokines which determines Th1 cell differentiation. Initially it was thought that Th1 cells present the main pathogenic subpopulation of T cells in the EAE model. This concept was partially associated with complete resistance to EAE following ablation of *I12p40* (a subunit of the heterodimeric complex of IL-12) [51]. However, it was surprising that deletion of the gene encoding the second component of IL-12, *I12p35*, led to progression of the disease. Later, it was established that the resistance to EAE was due to the absence of IL-23, but not of IL-12, because p40 is the common subunit for both IL-12 and IL-23. This conclusion was experimentally confirmed by the resistant phenotype of the *I123p19*-deficient mice, similarly to that of *I123r* knock-out mice, characterized by a decreased number of IL-17⁺ cells as compared to wild type mice [52, 53]. It was shown that IL-23 was necessary for stabilizing the phenotype of pathogenic Th17 cells, and in the absence of IL-23/IL-23R-signaling Th17 cells were unable for the terminal differentiation and were more prone to cell death [68, 85]. Thus, IL-12 and IL-23 are required for the development and supporting the phenotype of pathogenic Th1 and Th17 cells.

Cytokines produced by effector cells. *IL-17* is the most studied member of the IL-17 cytokine family. Th17 cells are a classic producer of IL-17A, however, IL-17A also can be secreted by CD8⁺ T cells and by the group 3 innate lymphoid cells 3 (ILC3), $\gamma\delta$ T cells and invariant NK cells (NKT cells) [86]. IL-17A functions as a homo- or heterodimer in the complex with IL-17F. The receptor to IL-17A consists of the IL-17RA subunit, which is common for all receptors of the IL-17R family, and the IL-17RC subunit. The IL-17RA subunit is expressed on all hemopoietic cells which defines the large spectrum of IL-17 target cell populations [87]. All IL-17R receptors have a conservative domain SEFIR (similar expression of fibroblast growth factor and IL-17R), which is required for the intracellular signal transduction through the adaptor protein Act1. The IL-17A/IL-17R interaction results in activation of NF κ B transcription factor [88]. Normally, IL-17A-producing cells participate in the control of bacterial infections, but dysregulation of this cytokine is associated with various autoimmune diseases,

such as psoriasis, rheumatoid arthritis and multiple sclerosis [89].

The role of IL-17 in EAE is insufficiently understood. Selective deletion of the IL-17A subunit leads to a slight improvement in the course of the disease, whereas the deletion of the IL-17F subunit does not affect EAE development [54]. Although T cells are important source of IL-17A in EAE, induced overexpression of IL-17A in CD4⁺ T cells does not lead to exacerbation of the disease [54]. Moreover, IL-17A-producing Th17 cells show plasticity, so that IL-17A produced by Th17 cells can play a pathogenic role during the early stages of EAE, but with disease progression the so-called ex-Th17 cells in the CNS secrete interferon- γ (IFN γ), TNF, and GM-CSF but not IL-17A [90]. As it has been demonstrated by the transcriptome analysis, the population of Th17 cells is heterogenous, and Th1-like cells, which are present in the CNS at the peak of the disease, are thought to be the most pathogenic [91]. Although IL-17A is a pivotal effector cytokine of Th17 cells, the contribution of IL-17A to the pathogenicity of Th17 subset is not clear, as well as the role of IL-17A from different sources and at different stages of the disease.

The binding of IL-17A to IL-17R induces production of many cytokines and expression of chemokines by CNS-resident cells leading to inflammation and increased immune cell recruitment to the CNS [92]. It was shown that activation of IL-17R on the brain endothelial cells facilitated their activation and the subsequent disruption of the BBB [93]. Further studies elucidating the cellular sources of this cytokine and the role of various ligand and receptor subunits are required for understanding IL-17 mediated neuroinflammation and possible clinical application.

IFN γ . IFN γ is the type II interferon which can be produced by activated T cells, NK and NKT cells [94]. IFN γ is an effector cytokine of Th1 cells and an activator of macrophages. The IFN γ dimer binds to the receptor consisting of two subunits – the α -subunit IFN γ R1, which is ubiquitously expressed and interacts with the ligand, and the β -subunit IFN γ R2, whose expression is inducible and is necessary for the signal transduction. The classical IFN γ signaling cascade acts through phosphorylation and activation of the receptor-bound Jak kinases, and that results in phosphorylation and activation of the transcription factor STAT1 [95].

The role of IFN γ in EAE is contradictory [96]. It was assumed that neutralization of the pivotal Th1 effector cytokine should protect mice against EAE development. In contrast, neutralization of IFN γ with monoclonal antibodies resulted in EAE exacerbation, and genetic inactivation of *Ifng* in mice caused a disease with massive immune cell infiltration into the CNS [39, 40]. Perhaps, such effects were due to the fact that systemic IFN γ ablation affected IFN γ -producing cells, which played a protective role [97]. The deletion of *Ifngr1* in 129/Sv mice,

which are resistant to EAE induction, led to development of more severe disease and increased mortality than in the wild type mice [41]. Naves et al. [98] have revealed that injection of IFN γ at the initiation stage of EAE promoted the disease pathogenesis, whereas IFN γ injection at the effector stage had protective effect [98]. Thus, the suppressive role of IFN γ during the effector stage can be a possible mechanism of exacerbated disease in *Irfng*-deficient mice. In the absence of IFN γ , activated CD4⁺ T cells can be expanded in the periphery and in the CNS, and this expansion can be accompanied by reduced expression of the transcription factor FoxP3 in regulatory T cells [99]. In this case, the adoptive transfer of regulatory T cells, differentiated in the presence of IFN γ and anti-CD3 *in vitro*, can promote suppressive functions of regulatory T cells and the further EAE inhibition [100]. Thus, the protective effect of IFN γ is a result of its action on the suppressor activity of Tregs, while at the initiation stage of EAE IFN γ plays the pathogenic role.

GM-CSF. GM-CSF is a member of the superfamily of colony-stimulating factors (CSF), however, recent studies have implicated that its major function is the immune regulation *in vivo*. In fact, GM-CSF directs the differentiation of hematopoietic precursors into granulocytes and monocytes, but, unlike the other members of the CSF superfamily, GM-CSF participates in a crosstalk between myeloid cells and lymphocytes during inflammation [101, 102]. GM-CSF is secreted by various cell types, i.e. cells of myeloid and lymphoid origin as well as by stromal cells [103]. The heterodimeric GM-CSF receptor consists of the ligand-binding α -subunit and the signal-transducing β -subunit, which is common for GM-CSF, IL-3 and IL-5 [104]. GM-CSFR signaling triggers activation of the Jak–STAT pathway due to phosphorylation of the Jak2 kinase and the transcription factor STAT5. In addition to the Jak–STAT-signaling, other signaling pathways can also be activated, including the MAP-kinase and PI3K–Akt-signaling cascades, as well as NF κ B [105].

GM-CSF is required for EAE development. Mice lacking GM-CSF are resistant to EAE induction with decreased cellular infiltration of the CNS [37]. At least partially, GM-CSF is involved in regulation of the balance between effector T cells and Treg cells, because neutralization of Tregs using monoclonal antibodies to CD25 makes the GM-CSF-deficient mice susceptible to the EAE development [106]. Following BBB disruption and leukocyte infiltration into the CNS, GM-CSF produced by T cells affects cells of the myeloid origin, i.e. activates microglia and recruits circulating proinflammatory Ly6C⁺ monocytes into the CNS [107, 108]. Codarri et al. have shown that the secretion of GM-CSF by Th1 and Th17 cells is sufficient for EAE initiation, and that the GM-CSF production depends on the expression of IL-23/IL-23R and transcription factor ROR γ t [109]. Recent evidence also suggests that IL-1 β and IL-23, but not IL-

6 and TGF β , can induce the expression of GM-CSF in Th17 cells [80]. Complete GM-CSF receptor deletion provides the resistance to EAE. This phenotype is also reproduced in mice with tissue-specific deletion of *Csfr2b* in Ly6C^{hi}CCR2⁺ monocytes but not in dendritic cells or neutrophils. It is important that GM-CSF is not required for the development of proinflammatory Ly6C^{hi} monocytes but is needed for their effector functions [38]. Conditional overexpression of GM-CSF in CD4⁺ T cells facilitates CNS infiltration by myeloid cells, which produce such proinflammatory mediators as IL-1 β and reactive oxygen species [110]. Taken together, GM-CSF in the EAE model is produced by T cells and promotes the recruitment of different myeloid cells into the CNS, which further support local inflammation by production of various proinflammatory cytokines.

TNF and lymphotoxin (LT). TNF is a member of TNF superfamily, which is characterized by signaling of homo- or heterotrimeric ligands through homotrimeric receptors. TNF is produced mainly by myeloid cells, but also by B cells, activated T cells, as well as CNS-resident cells – astrocytes and microglia. TNF is a transmembrane protein (tmTNF), which is released in a soluble form (sTNF) following proteolytic cleavage by TACE (ADAM17). TNF binds to two receptors expressed on the cell membrane or to their soluble forms produced via the same mechanism as sTNF. The TNFR1 receptor (p55, CD120a) is present nearly on all cell types, and TNFR2 (p75, CD120b) is expressed on the cells of the hematopoietic origin, especially, leukocytes and B cells [111]. TNF binding to its receptors leads to recruitment of intracellular adaptor proteins activating different signaling pathways. The intracellular part of TNFR1 contains “death domain”, therefore, the functional outcomes of TNF–TNFR1 signaling are cell survival or cell death. TNFR2 does not contain this domain, therefore, the signal transduction from TNFR2 leads to activation of the transcription factor NF κ B which stimulates the cell survival and proliferation [112, 113].

According to published works, the role of TNF in EAE development is contradictory. On the one hand, the pharmacological inhibition of TNF improved clinical symptoms of EAE, because TNF may play the pathogenic role by inducing chemokine expression in the CNS [56, 114]. On the other hand, data obtained using reverse genetics approach revealed a more complicated picture [115]. Mice lacking *Tnf* develop more severe disease than the wild type mice (i), mice with *Tnfr1(p55)* deletion showed delayed disease onset and diminished disease progression (ii). In contrast, genetic inactivation of *Tnfr2(p75)* led to exacerbated EAE with the massive infiltration of CD4⁺ T cells into the CNS and increased production of Th1 effector cytokines (iii) [57, 58, 60]. Double knockout TNFRp55/p75^{-/-} mice have been shown to develop attenuated disease, suggesting a pathogenic role of TNFR1 and a protective role of TNFR2 in

EAE [61]. The recent studies have demonstrated that selective inhibition of TNFR1 protected mice against EAE development. Moreover, a protective function of TNFR2 was also shown in the neurodegeneration model *in vivo* [116, 117].

Understanding TNF biology in the context of EAE is difficult not only because of its interaction with two receptors and the presence of two related ligands (see below), but also because role of TNF depends on its cellular source. Mice with genetic ablation of TNF in myeloid cells, despite delayed disease onset, had no improvement during the late stages of EAE, indicating that the role of TNF from this source was mainly pathogenic [59]. Moreover, in experiments with the inducible TNF deletion in microglia, it was convincingly shown that TNF from monocytes, but not from the CX₃CR1⁺ microglia, is crucial for EAE development [118]. It is interesting that functions of TNFR2 are different depending on its expression on microglia or on monocytes/macrophages. Deletion of TNFR2 on CX₃CR1⁺ microglia increased the leukocyte infiltration into the CNS, whereas in the *LysM^{Cre}:Tnfrsf1b^{fl/fl}* mice with inactivated TNFR2 in monocytes/macrophages the disease severity was reduced [119]. TNFR2 is expressed on various suppressor cells, including protective regulatory T cells, and TNFR2 ablation in FoxP3⁺ Tregs resulted in progression of clinical symptoms of the disease with decreased suppressor properties of Tregs (Atretkhany et al., in print). These results emphasize that protective role of TNFR2 in the EAE model, at least partially, is associated with the signaling in Tregs.

Another member of TNF superfamily, lymphotoxin (LT α_3), can also bind to TNFR1 and TNFR2. The lymphotoxin exists in the soluble and transmembrane forms (LT α_3 and LT $\alpha_1\beta_2$, respectively), and each form interacts with different receptors. The homotrimer LT α_3 binds to TNFR1 and TNFR2, while the heterotrimer LT $\alpha_1\beta_2$ can bind only to LT β R [120]. Unlike TNF, the lymphotoxin is produced by a limited number of cell types, namely, by activated T cells, B cells, and ILC [121]. It is difficult to interpret data about the role of lymphotoxin in EAE, because the genes *Lta*, *Ltb* and *Tnf* are closely linked in the TNF/LT locus, limiting the possibilities of independent editing of these genes. Another reason is their overlapping signaling through the corresponding receptors. Moreover, *Lta*^{-/-} and *LtbR*^{-/-} mice do not have lymph nodes, making it difficult to interpret the data when dissecting the molecular mechanisms of the disease pathogenesis [122]. Earlier studies of pharmacological inhibition of TNF and LT α_3 concluded that these cytokines play a pathogenic role, because injection of the antibodies prevented the development of the passively induced EAE [114]. The genetic deletion of *Lta* leading to the absence of both the soluble and membrane-bound forms of lymphotoxin resulted in the complete resistance of mice to EAE. Nonetheless, it was found that the ablation

only of the *Ltb* gene did not reproduce the phenotype associated with the resistance. Therefore, it was concluded that just the soluble form of lymphotoxin was required for EAE [55]. Note that in the cited work the mice with the conventional knockout of *Lta* were used, and later it was shown that these mice have defects in TNF production by some cell types, thus making doubtful the unambiguous interpretation of the reported phenotype [122]. According to our observations, *Lta* knockout mice with normal TNF production were fully susceptible to EAE (unpublished data). Moreover, injection of antibodies specific to LT α caused the depletion of LT α -expressing cells, i.e., the cells with the membrane form of the lymphotoxin, and also weakened clinical symptoms of the disease in these mice [123]. However, the heterotrimeric structure of the membrane-bound lymphotoxin makes it impossible to separate effects of the soluble and membrane-bound forms using the pharmacological inhibition. It was also shown that Th17 cells facilitated the development of tertiary lymphoid organs in the CNS, and the interaction between LT $\alpha_1\beta_2$ on Th17 cells and LT β R on stromal cells was necessary for neuroinflammation [124].

Taken together, the role of TNF in EAE can be both pathogenic and protective, depending on the cellular source of TNF, as well as the target population and the type of receptor involved, and lastly, on the stage of the disease. On the other hand, the role of lymphotoxin has to be revised questioning some earlier studies, in which pharmacological and genetic inhibition of lymphotoxin in EAE, may have also affected TNF.

CYTOKINES AND THERAPY OF MULTIPLE SCLEROSIS

Cytokines play an important role in the development of autoimmune diseases, including multiple sclerosis (MS). The detection of cytokines in the cerebrospinal fluid or in demyelinated lesions of MS patients suggested that anti-cytokine therapy could be one of the main approaches for the treatment of MS, like in the case of rheumatoid arthritis. However, it should be pointed out that such therapy does not lead to clearance of autoreactive T cells, which are the causative agent of the disease. Clinical trials of TNF and IL-12/23 inhibitors were not successful in MS therapy, and at present, there are no antagonist or agonist of the cytokine signaling among the FDA-approved drugs for MS [125, 126]. On the other hand, neutralization of IL-17A with the secukinumab has been effective during the second phase of clinical trials and remains promising for further evaluation of the effect of this blocker in the therapy of MS [127].

The failure of existing anti-cytokine therapies can be partially attributed to the fact that systemic inhibition of an individual cytokine will result in blocking the pathogenic functions, but inevitably in blocking important pro-

tective functions. Therefore, more specific therapy should be considered, including inhibition of the cytokine from a defined pathogenic cell type, inhibition (or stimulation) of a specific receptor or signaling cascade in case if this cytokine is characterized by activation of signaling pathways through several receptor complexes [128, 129]. Another possible explanation of the inefficiency of anti-TNF therapy may be due to existing polymorphism of the gene encoding TNFR1, which is shown to be associated with the development of MS [130, 131]. More accurate evaluation of precise molecular mechanisms of cytokine regulation and their signaling pathways can drive the development of new therapeutics. On the other hand, it should be noted that immunotherapy with specific antibodies remains a promising trend in the therapy of MS, and $\alpha\beta 1$ -integrin, CD52 and CD20 can be used as targets [35, 132, 133].

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