## Autoimmunity in acute ischemic stroke: The dark side or the light one?

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Autoimmunity in acute ischemic stroke: the dark side or the light one?

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Abstract: The article presents a synopsis of current data on the mechanisms of blood-brain barrier alteration and autoimmune response in acute ischemic stroke. The ischemic stroke is accompanied by aseptic inflammation, that alters the brain tissue and exposes co-stimulatory molecules of immune system as well as neuronal antigens. Disruption of the blood-brain barrier contributes to the leakage of brain autoantigens and induction of secondary autoimmune response to neuronal antigens and long-term inflammation. At the
same time, the stroke-induced immune activation may promote reparation phenomena in brain.

**Key words:** stroke, blood-brain barrier, autoimmunity, innate immunity, inflammation, cell death.

The incidence of stroke is decreasing over time, yet stroke remains one of the global leading causes of death and disability, and as the population ages, the burden of this disease becomes tremendous [1–3]. Ischemic stroke is the most common (83%) type of stroke and up to 67% of cases result from two main causes: thrombosis and embolism [3, 4]. According to the Helsingborg Declaration on European stroke strategies (2006), the priority area for research and development is, among others, the development of the new methods of ischemic stroke treatment. For these purposes, it is necessary to study the mechanisms of formation of infarction core and periinfarction area, including the immune response, because the acute ischemia promotes the local and systemic inflammatory reaction as well as autoreactive immune response [5–8].

The research of the contribution of the immune system to pathogenesis of cerebrovascular disease was initiated by I.V. Gannushkina in the middle of the XXth century [9]. Up to date the experimental studies didn’t allow to determine the entire role of the immune cells in pathogenesis and outcome of ischemic stroke [7]. Thus the research of the reduction of the ischemic brain injury through the target regulation of the immune response is promising [10,11].

The immunological privilege of the central nervous system (CNS) is the concept, that implies the isolation of the central nervous system from the immune system by the blood-brain barrier (BBB) [12]. The brain antigens are usually isolated from the immune system,
The autoimmune response to these antigens promotes extensive damage of the brain cells. The stroke patients have the increased levels of circulating antibodies to neurofilaments and components of N-methyl-D-aspartate (NMDA) [13].

![Cell interaction under intact (A) and altered (B) blood-brain barrier](image)

**Fig.** Cell interaction under intact (A) and altered (B) blood-brain barrier

The BBB provides structural and immune isolation of the CNS. It consists of three main components: endothelium of microvessels of the brain, pericytes and processes of astrocytes. The endothelial cells in the brain microcirculatory vessels have specific morphological and functional characteristics (deep interendothelial connections, absence of pores and fenestrae between endotheliocytes, solid basal membrane, etc.), which provide the barrier function and the transport of substances through the BBB. The strong interendothelial contacts of intact BBB limit the diffusion of the substances more than 10-15 nm in diameter into the brain. The structure of BBB is preserved in most parts of the brain, except for the hypothalamic-pituitary region, where the basal membrane has pericapillary spaces, and the barrier is abundantly fenestrated [14, 15]. Although self-tolerance is facilitated by relative isolation and sequestration of brain antigens, the whole concept of immunologically privileged antigens “behind barriers” should not be overvalued because “there is no hiding
place” completely excluding immune influences in the body. All locations are principally accessible for local and/or systemic immune effectors. The last decades researches showed, that the real amount of cloistered autoantigens is substantially limited, and their isolation is not absolute. So the contribution of the BBB alteration into the pathogenesis of autoimmune disorders was overestimated. The alteration of blood-tissue barriers and expression of cell adhesion molecules on endothelium promotes the migration of immune effectors into altered areas and enhances the alteration of the tissues. According to Z. Dembič [16], the increase of autoreactivity depends not on barrier violation, but on presence of tissue disintegration signal (or lack of integrity signals), which alter behavior of antigen-presenting cells. Probably all autoantigens are prone for immunological surveillance, so the contribution of disclosure in autoimmunity response may be not so tremendous. The following facts in proof of this statement were shown (and not recently!).

In the absence of the immune response sensitive immunodiagnostic procedures (like ELISA, RIA etc.) reveal impactful amounts of the autoantigens, which were formerly regarded as covered, for example, myelin basic protein. This does not necessarily provoke encephalomyelitis [17]. It was shown, that the permeability of BBB for macromolecules is restricted predominantly for the blood to CNS direction and to a many times lesser extent for the CNS to blood [18]. Nowadays the BBB is not considered to be the border between immune system and CNS. The local immune subsystems are found within and also behind BBB and consist of local antigen presenting cells (astrocytes) and effectors (intrathecal lymphocytes) [19]. In the previous century Pio del Rio-Hortega insisted on macrophageal origin of microglial cells [20] and today it is shown, that microglial cells are very close to macrophages. They express main histocompatibility complex (MHC) I and also can express MHC II antigens in case of cytokine or viral stimulation. Microglial cells produce cytokines and can present CNS autoantigens to local effectors (intrathecal lymphocytes). Its noteworthy,
that the activation of BBB endotheliocytes by microglial cells promotes the expression of cell adhesion molecules and increase the permeability of BBB for lymphocytes [21]. The described local immune mechanisms in the BBB area usually have low activity, but its enhancement can lead to the autoimmune alteration of the CNS, which can be clinically relevant, for example, in multiple sclerosis. Immune and phagocytic behavior of microglial cells is sensitive to many factors, for example to thyroid hormones [22].

In recent years these classical data about routine brain-immune interaction were supported by newly discovered brain lymphatic vessels [23] and description of so called glymphatic system of brain, as well as communication between cerebrospinal and intracerebral interstitial fluids and key role of astrocyte aquaporins in brain glymphodynamics [24]. Lymph drainage from brain to cervical lymph nodes has been recently proven [25]. Glymphatic system function is altered and jeopardized both in hemorrhagic and ischemic cerebral circulatory disorders. Of course, all these data considerably modified the concept of BBB, compared to its classical version.

The dysfunction of the BBB is specific for the severe neurologic diseases, such as multiple sclerosis, brain tumors, hemorrhagic and ischemic stroke. At present, most researchers focus on the study of the neurons during acute damage to the central nervous system, while relatively little attention is paid to the changes of the BBB. The areas of the brain, which were subjected to ischemia and subsequent reperfusion, usually have massive microcirculatory disorders with relevant clinical implications [26]. In acute cerebral ischemia capillarostasis, diapedesis, BBB disruption are found both in the affected basin and at a considerable distance from the zone of vascular catastrophe due to hypoxic alteration of cerebral structures [14]. The early damage of BBB may be also the cause, but not the result of brain parenchyma cells alteration [27]. O’Connell G.C. et al. [28] studied 16 candidate genes which may be predictive for the BBB disruption. ITGA3 gene encodes the adhesion molecule
integrin alpha-3. The overexpression of AKAP7 gene, which is co-expressed with ITGA3, showed the highest prognostic value as a biomarker for BBB alteration after the stroke.

Disruption of the BBB in ischemic stroke contributes to the tissue disintegration and leakage of brain autoantigens, including the myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein (MOG). The leakage of brain autoantigens is followed by the activation of the immune system and migration of immunocytes to the CNS, which promote the local inflammation. MOG-reactive splenocytes secrete neurotoxic Th1-cytokines, TNF-α, IFN-γ and exacerbate brain damage in ischemic stroke. The MOG-reactive splenocytes can promote neuronal death or injury directly or indirectly through the augmentation of the BBB permeability and enhancing of transendothelial transport and infiltration of immune cells into the brain [12].

Many authors believe that the immune cells activation promotes the production of the antibodies to the myelin basic protein. The growth of the serum levels of antibodies to myelin basic protein at the end of acute period of ischemic stroke correlates with severity of the post-stroke period. By contrast the patients with better clinical outcome have the decreased level of the organ-specific antibodies at the end of the acute period of stroke [29]. However, the question of whether the more active autoimmune response to myelin basic protein contributes to worse recovery of neurological functions, or an inability to recover the lost function provokes an autoimmune response to increase neuroplasticity during continuous recovery remains open [30]. Recently it has been demonstrated that brain ischemia induces T-cell responses, which are specific to the neuroantigens and enhance brain injury [31].

Aseptic inflammation that occurs in the CNS in ischemic stroke alters the brain tissue and exposes CNS antigens. The experimental study showed the upregulation of autoreactive CD4+ T-cells, CD8+ T-cells and CD19+ B-cells at 4 days after the stroke onset. The mice with large infarct volume showed early lymph nodes (but not the spleen) T- and B-cell
autoreactivity for the subunit of the NMDA receptor – NR2A. If the volume of the brain infarct was low, than MAP-2 and myelin derived peptides autoreactivity was found to be elevated. And these autoimmune reactions were present during 10 days after the stroke onset. Thus, the ischemic stroke induces secondary autoimmune response to neuronal antigens and long-term inflammation [30]. Wang Z.K. et al. [32] also showed the role of invariant natural killer T-cells in brain alteration and brain edema in the model of focal permanent cerebral ischemia. Propagation of CD8+ T-cell-mediated and NK-cell-mediated immunity in acute brain ischemia can be promoted by astrocytic interleukin-15 [33]. The future research of T-cells activity in acute stroke may be the key for the new strategies for the treatment of the long-term degenerative consequences of stroke [34].

The experiments showed the expression of the MHC antigens and cell adhesion molecules by the BBB endothelial cells. The loss of the BBB integrity leads to the endothelial and glial cells activation and following inflammatory process. During the inflammation, the complex interaction of cytokines and adhesion molecules provokes attraction and invasion of leukocytes, which increases the damage to the brain tissue [12, 14, 35].

It should be noted, that the immune response is related to the processes of blood clotting and fibrinolysis. This dependence is largely due to the presence of active compounds possessing the properties of procoagulants, anticoagulants, activators of fibrinolysis in T- and B-cells. Proinflammatory cytokines, affecting the endothelial cells and macrophages, contribute to an increase of production and secretion of procoagulants, as well as a decrease in the formation of anticoagulants. The acute focal ischemia of the brain turns the monocytes (macrophages) into a hyperactive state, which increases the IL-1α synthesis. The degree and duration of the increase of IL-1α level are of prognostic importance for the course and outcome of the stroke [29].
Within hours after a stroke, microvacuoles, eosinophilic cytoplasm, pyknotic nuclei in neurons and the first signs of the BBB alteration can be found. The following can be divided into three stages. In stage I leukocytes begin to penetrate into the damaged area. In phase II macrophages come through the BBB and the astrocytes activity remains, in phase III a pseudocyst is formed [7]. The damage to the BBB after the onset of the stroke also has two phases: the first one starts within a few hours and the second begins in 24-48 hours after the stroke onset. Matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, are involved in both early and late phases of the BBB damage, but early damage is largely due to MMP-2 activity. Accordingly, an increase of MMP-9, which is considered to be one of the main factors of BBB disintegration in ischemic stroke, starts in 4 hours after the stroke onset and lasts up to 4 days. At this time starts the degradation of the dense connective proteins (occludin, claudine-5, adhesion molecules, etc.), which naturally constitute the actin-myosin skeleton and are distributed in the form of short filaments and diffuse monomers between endothelial cells. However, in ischemia the actin filaments polarize into linear stress fibers, which lead to complete destruction of the BBB and subsequent swelling of the brain [36].

MMP-9 is mainly secreted by neutrophils, which infiltrate BBB, and endothelium of microvessels [27]. Polymorphonuclear granulocytes are the first subset of leukocytes, that appear in ischemic brain tissue, thus they were previously suspected to damaged neurons. However, recently it was found that during the acute phase of ischemic damage this type of cells is not found in the parenchyma of the brain. At this time, they remain trapped within the neurovascular units and leptomeningeal spaces, thus the neutrophil-mediated neuronal death may not require the presence of neutrophils near the target brain cells. The observation of the absence of active migration of polymorphonuclear neutrophils through the BBB during the early stage of reperfusion is not limited to the experimental studies, but is also confirmed by postmortem studies in stroke patients [7]. The chronic cerebral ischemia is accompanied by
increase of granular leukocytes with azurophilic granules, containing elastase, myeloperoxidase, cathepsin G and acid hydrolases. The movement of inflammatory cells into the perivascular space is promoted by the disruption of the connective proteins in the BBB. Involvement of perivascular areas activates resident macrophages and mast cells and thus promotes the release of proinflammatory cytokines, vasoactive mediators and the infiltration of leukocytes [37]. Experimental stroke models show that circulating leukocytes penetrate the brain by diapedesis and accumulate in the injury area within a few hours after a stroke. CD4+ T-cells are activated when the peptide antigens are present on the surface of MHC II cells, including dendritic cells, macrophages and B-cells. CD8+ T-cells react to antigenic peptides of the MHC I, which are present on most types of cells [30].

The cytokines promote the leukocyte infiltration of the ischemic core, activation of microglial cells, inducible form of cyclooxygenase-2 and nitric oxide synthase in ischemic stroke [38]. The brain alteration elevates the serum levels of heat shock proteins-70 – endogenous ligands for the toll-like receptor-4 (TLR4), which is the key receptors of innate immunity. Toll-like receptors (TLRs) initiate the synthesis of pro-inflammatory cytokines by activating the nuclear factor and are capable of triggering the immune response in both infectious and non-infectious diseases [39]. By the structure, TLRs belong to the IL-1 receptor family. IL-1 is a mediator of microglial neuroimmune functions, it is secreted locally in response to cerebral ischemia.

All TLRs are integral transmembrane proteins with the similar structure. At rest, unactivated TLRs are present on the cell membrane in the monomeric state. Most receptors form homodimers, while TLR2 forms heterodimers with TLR1 or TLR6 depending on the ligand. When activated by exogenous or endogenous ligands, the receptors dimerize, and it leads to subsequent signal transmission inside the cell and activation of cytokine synthesis. TLR2 and TLR4 are involved in ischemia-reperfusion injury. TLR4 mediates immune
response to systemic bacterial infection and brain alteration. TLR2 expression rapidly increases after the stroke onset, long before the activation of microglia. Activation of TLR4 is accompanied by the expression of cytokines and other signal peptides, including MMP-9 and tumor necrosis factor-α (TNF-α). In ischemic stroke the effector receptors of TNF-α (type 1 TNF-receptor, Fas-receptor, etc.) regulate apoptosis in neurons and nonneural cells involving caspase-dependent and caspase-independent pathways [40–42]. Thus, inflammation is regarded as the key mechanism of ischemia-reperfusion injury, and the anti-inflammatory treatment is promising in acute stroke [43–46]. In particular, lymphocytes are regarded as the target for the neuroprotection in acute ischemia [47]. Li P. et al. [48] showed that C-C chemokine receptor type 5 is a critical molecule for T cells-mediated BBB protection and it is potential target to optimize acute ischemic stroke therapy. Nalamolu K.R. et al. [49] showed, that attenuation of TLR2- and TLR4-mediated inflammation after the stroke promotes ischemic brain damage.

The infarct core becomes a source of MMPs, various proteins and molecules, that initiate an autoimmune response accompanied by production of immunoglobulins with abnormal temperature solubility and immune complexes with cryoproperties. The cerebrospinal fluid level of nerve growth factor autoantibodies in patients with ischemic stroke is 180-190% as compared with the reference values. The decrease of the nerve growth factor level diminishes neurotrophic support. As for the S100-β protein, on the 1st day after the stroke onset the concentration of S100-β protein antibodies is also increased by 25-50% [29]. It should be noted, all these processes are not a sequence of events, but a complex network of intertwining cascades [6].

Altered brain cells can promote activation of immune system. Numerous intracellular components which vacate destroyed cells can activate toll-like receptors on various cells, followed by upregulation of proinflammatory molecules and presentation of antigens by
dendritic cells. Escalation of cell death reduces the anti-inflammatory effect of neurons and neurotransmitters on microglia [37]. Some researchers suggest that autoimmune reactions promote chronic inflammation and can be a risk factor of dementia. They are also associated with the phenomenon of “anamnestic recall”, which results from autoreactive T-cells activation and comprises the temporary reoccurrence of stroke symptoms that have been regressed in this patient, usually in case of systemic infection [13].

In conclusion, most researchers confirm the relationship between the severity of immunobiochemical changes and clinical outcome of ischemic stroke. Despite the large number of the studies of immune-inflammatory status in ischemic stroke patients, many questions remain open, first of all, concerning the role of stroke-induced immune activation in the neuroreparation. A study of immune response and inflammatory reaction in pathogenesis of ischemic stroke and its influence on the clinical outcome are also important for the new approaches to diagnostics and searching for the new molecular therapeutic targets.

**Highlights:**

- The ischemic stroke is accompanied by aseptic inflammation, that exposes costimulatory molecules of immune system and neuronal antigens.

- The increase of autoreactivity in ischemic stroke depends predominantly not on BBB violation, but on presence of tissue disintegration signal, activation of innate immunity and probably alteration of glymphodynamics.

- The stroke-induced immune activation may promote reparation phenomena in brain.

**Disclosures**

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**Author contributions**

NVT and APT were responsible for the first draft, which was critically reviewed, further developed and approved by all authors. VAY and AVR performed the literature search, collected and extracted the data, performed the quality assessment of included published papers. IVL, AGV, LPC edited and revised the manuscript. All authors contributed to data interpretation, critically reviewed all manuscript versions and approved the final version.

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Exchange with macromolecules between brain and systemic circulation in health

485x374mm (72 x 72 DPI)
Exchange with macromolecules between brain and systemic circulation in stroke

506x380mm (72 x 72 DPI)