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Neuroinflammatory biomarkers: From stroke diagnosis and prognosis to therapy☆

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ABSTRACT

Stroke is the third leading cause of death in industrialized countries and one of the largest causes of permanent disability worldwide. Therapeutic options to fight stroke are still limited and the only approved drug is tissue-plasminogen activator (tPA) and/or mechanical thrombectomy. Post-stroke inflammation is well known to contribute to the expansion of the ischemic lesion, whereas its resolution stimulates tissue repair and neuroregeneration processes. As inflammation highly influences susceptibility of stroke patients to overcome the disease, there is an increasing need to develop new diagnostic, prognostic and therapeutic strategies for post-stroke inflammation. This review provides a brief overview of the contribution of the inflammatory mechanisms to the pathophysiology of stroke. It specially focuses on the role of inflammatory biomarkers to help predicting stroke patients' outcome since some of those biomarkers might turn out to be targets to be therapeutically altered overcoming the urgent need for the identification of potent drugs to modulate stroke-associated inflammation. This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

1. Introduction

Stroke produces more than 9% of all deaths and is one of the leading causes of permanent disability worldwide. The most common cause of stroke is the occlusion of blood circulation by a thrombus (or embolism) although it can also be produced by the rupture of a vessel and subsequent bleeding in a certain region of the brain. Strokes can, therefore, be classified as ischemic or hemorrhagic. Ischemic strokes represent between 80% and 85% of all stroke cases, while hemorrhagic strokes are around 15%.

Despite many years of intensive research, therapeutic options for stroke patients remain very limited. Thrombolytic treatment with tissue Plasminogen Activator (tPA) is still the only Food and Drug Administration (FDA)-approved therapy for treating hyperacute ischemic strokes, together with mechanical thrombectomy, whereas no treatment is routinely used to overcome hemorrhagic strokes. tPA improves functional outcome and reduces neurological deficits in stroke patients. However, due to risks of hemorrhagic transformation, tPA can only be administrated within the first 4.5 h of ischemic stroke onset, reducing by up to 10% the number of patients that qualify for this treatment [1]. This therapeutic window has been slightly extended with the new endovascular approaches supported by several positive trials published this year (MR CLEAN, EXTEND-IA, ESCAPE, SWIFT PRIME, REVASCAT, etc.). However, most stroke patients only receive supportive care, which underscores the need of new therapeutic agents to fight stroke.

Although stroke is a highly complex disease, inflammation is known to be a major contributor to stroke pathophysiology [2]. During a stroke, cerebral brain injury evokes a massive upregulation of the inflammatory response. However, whether this response has beneficial or detrimental effects has long been the subject of controversy, with agreement still remaining elusive. Hence, the immune system is considered a promising tool to modulate brain damage during and after stroke. This review provides a brief overview of the pathophysiology of ischemic stroke, focusing attention on the underlying inflammatory process and its association with the discovery of stroke biomarkers and the possibility that these biomarkers might not only become diagnostic tools but may even turn out to be targets to be therapeutically blocked or stimulated in order to modulate the inflammatory response after the ischemic challenge.

2. Neuroinflammation in stroke: a dual role

During stroke, the lack of cerebral blood flow in the ischemic core causes a complete reduction of oxygen and glucose supply to cerebral neurons and other supporting cells. The absence of these vital fuels induces a series of biochemical and metabolic alterations that finally lead to massive cell death [3]. The area surrounding the dying core is

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known as the ischemic penumbra, a region with still viable cerebral tissue. Cells within this zone are functionally impaired but structurally intact, which makes that region potentially salvageable and suitable for therapeutic interventions. However, if oxygen and glucose supplies are not restored in this surrounding area, neuronal apoptosis processes are initiated, which results in a reduction of the salvageable tissue and an increase in the ischemic lesion.

Dying cells from the ischemic area release damage signals, commonly known as brain-released alarmins, which are recognized by the microglia [4]. Under immunological stimuli, these resident immune cells become activated and act as relevant players in the immune system's response together with peripheral leukocytes, which infiltrate the brain from circulation within a short period of time [5]. Both local and peripheral immune cells consequently produce an explosion of pro-inflammatory mediators surrounding the ischemic region, which further increase the permeability of the blood–brain barrier (BBB) and facilitate the infiltration of beneficial leukocytes to clear away the large amount of debris caused by cell death. To that end, pro-inflammatory cytokines act on endothelial cells, upregulating the expression of leukocyte adhesion molecules, and stimulating the synthesis of chemokines, which guide leukocytes to the site of injury. Here also the complement system has added importance as a player in inflammation, specifically in leukotaxis [6]. Several components derived from the cascade-like activation of the complement system act as opsonins (C3b, C4b, C5b), enhancing the inflammatory phagocytic response, while other components (C6, C7, C8, C9, C5b) form a membrane attack complex involved in cellular lysis. This cellular damage is mediated by the increased expression of complement cascade activators (C1q, C3) and receptors by dying neurons and glial cells during the acute phase of cerebral ischemia [7].

Nonetheless, there is a growing body of evidence reporting that infiltrating immune cells also impair the ischemic brain. Infiltrated leukocytes produce inflammatory cytotoxic mediators that prolong the inflammatory response, increase brain damage and contribute to edema formation and hemorrhagic transformation, secondary complications that commonly influence stroke outcome [2]. The presence of these inflammatory mediators during the acute phase of stroke can, thus, be a threat to neuronal cell survival and repair.

Because of this inflammatory collateral damage, many attempts have been made to improve stroke outcome by using strategies that suppress the immunologic response after stroke. Regulatory T cells (T_{reg}) and B cells (B_{reg}) are two subpopulations of lymphocytes that act as important neuroprotective modulators of the immune response under many pathological conditions such as cerebral ischemia [8]. T_{reg} and B_{reg} have been characterized as stroke-limiting protective cells that preserve the immune homeostasis by counteracting the production of pro-inflammatory mediators and modulating the activation of effector lymphocytes and microglia in the ischemic region. However, despite the advances in the understanding of the function of T_{reg} and B_{reg}, further research should be conducted in order to assess whether their modulation might have application as a novel therapeutic approach for this devastating disease.

Furthermore, it is widely acknowledged that in severe strokes the extension of brain lesion highly correlates with the strength of the neuroinflammatory reaction. In extreme cases, the massive burst of circulating pro-inflammatory mediators is frequently excessive, which causes the ineffectiveness of the immune system to respond to secondary pathological stimuli elsewhere [9]. The disproportionate overactivation of the peripheral immune cells culminates in the exhaustion of mature leukocytes, a fact that causes the need to recruit immature leukocytes instead. This subpopulation is, however, unable to respond appropriately to brain injury, which leads to the deregulation of the immunological signaling pathways. This is the case for monocytes, where the recruitment and expansion of their immature subpopulation causes lymphocytopenia, a condition that significantly contributes to post-stroke immunosuppression [4].

In addition, the excessive concentration of pro-inflammatory mediators can promote the release of glucocorticoids and catecholamines by the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. This results in the stimulation of anti-inflammatory pathways, through the release of interleukin (IL)-4, IL-10 and transforming growth factor (TGF)-β, and the inhibition of pro-inflammatory mechanisms, which include the decreased expression of IL-1β, tumor necrosis factor (TNF)-α, several chemokines and several proteins involved in antigen-presentation processes [10]. The rapid and inappropriate activation of these mechanisms also contributes to stroke-related immunosuppression, which enhances the risk of infection after cerebral ischemia. In line with this, 23% to 65% of stroke patients develop infections, predominantly respiratory, urinary and catheter-related infections, after stroke onset [11]. Moreover, patients that develop post-stroke infections are thought to suffer worse outcomes with an increased mortality risk.

Beyond these detrimental consequences and side effects, the immune system also works to resolve this post-ischemic inflammation at later stages. The production of anti-inflammatory mediators and the removal of the remaining inflammatory molecules are major points in the mechanisms of inflammation suppression, which takes place after the initial burst of inflammation. During this period, the infiltrating pro-inflammatory macrophages (M1 macrophages) turn into anti-inflammatory (M2 macrophages) when stimulated by IL-4, IL-10 and TGF-β, among others [12]. These M2 macrophages antagonize the inflammatory response through the clearance of residual necrotic debris and the release of neuroprotective factors, including insulin-like growth factor and fibroblast growth factor, both participating in the recovery of ischemic brain injury by promoting neuroregeneration [13]. However, the mechanisms through which pro-inflammatory macrophages shift to an anti-inflammatory state during the recovery phase of stroke are not fully understood.

Due to this dual function of the immune system (Fig. 1), the inflammatory response after ischemia requires close control during stroke progression and resolution: permitting leukocyte infiltration to clear cell debris in the acute phase, avoiding harmful effects produced by either the accumulation of these circulating cells into the brain and their suppression at the peripheral level, and promoting their regenerative benefits some days later. Adjustments at this level seem promising as new therapies for stroke.

3. Brain–blood relationship

As the brain is an extremely high-demand system and neurons need tight regulation of the extracellular microenvironment, the integrity of the BBB is essential. Under physiological conditions, the BBB permits the maintenance of ion concentrations within very narrow ranges, allows the entry of nutritional supplies to brain cells and avoids the inflow of neurotoxic and harmful molecules [14]. Moreover, the BBB regulates and restricts the access of immune cells and immune mediators to the brain compartment, which provides privileged brain protection against peripheral insults. Under physiological conditions, leukocyte recruitment across the BBB is responsible for the maintenance of the immunologic privilege of the central nervous system (CNS). However, under pathological conditions, there is a reduction of the BBB tightness and an increase in the barrier leakiness (process referred as *BBB disruption*), which increases the BBB permeability, alters the natural immunological isolation of the brain and allows massive immune cell infiltration to aid the clearing of debris and repair of damage. In addition, this disruption of the BBB also contributes to a massive and uncontrolled exchange of molecules between the brain and the peripheral circulatory system and vice versa. The release of brain specific molecules into the peripheral circulation alerts the immune system to the presence of a damaged brain area and contributes to increasing the inflammatory response to the ischemic challenge.