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The innate immune system after ischemic injury — lessons to be learned from the heart and brain

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Abstract

Innate immune cells are critically involved in ischemic complications of atherosclerosis. While new insight emerged on the origin and role of leukocytes in steady state, the knowledge about myeloid cell's sources, functions and fate after stroke is limited. In our review, we highlight open questions in this important area while examining potential parallels in the immune response after stroke and myocardial infarction. We stress the need to better understand systemic interactions between ischemic tissue, immunity and hematopoiesis, as turn over of leukocytes in inflammatory sites can be rapid, and cell production and supply may serve as future therapeutic targets to modulate inflammation in the vessel wall, the brain and heart.

Introduction

Stroke is the third most common cause of death in the USA, and the majority of strokes are due to thrombotic or embolic complications of atherosclerosis. One in four strokes are recurrent events[1], highlighting that both primary and secondary prevention are currently insufficient[2]. While it is increasingly agreed upon that innate immune cells importantly contribute to atherosclerosis and its ischemic complications, the role of leukocytes, their subsets, sources and fates after stroke are incompletely understood. Ischemic stroke and myocardial infarction (MI) have in common that the sustained tissue injury is sterile and that it is caused by a lack of oxygen. Distinct differences include the phenotype of injured cells and tissues, and the nature and timing of signals from ischemic brain and heart. Because both MI and ischemic stroke are caused by atherosclerosis, we suspect that there are many similarities. Therefore, the comparison of the immune response to these two most deadly complications of vascular disease may be useful. In our minireview, we highlight open questions regarding the systemic innate immune response after stroke, relating them to recent insight obtained after myocardial infarction (MI).

Local response in ischemic tissue and the role of monocyte subsets

Parabiosis experiments revealed that in the steady state, microglia primarily derive from local progenitors rather than from circulating leukocytes[3]. In response to stroke, microglia are rapidly activated and develop a pro-inflammatory phenotype[4]. Once brain tissue is

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compromised due to ischemia, the injury also triggers a systemic inflammatory response that contributes to lesion maturation and the removal of dead or dying cells[5, 6]. In patients[7, 8] and mice[9, 10] with stroke, acutely elevated blood counts of innate immune cells such as neutrophils and monocytes parallel data obtained after MI (reviewed in reference[11]). These blood leukocytes are recruited in large numbers to the ischemic brain, where they have a critical role in wound healing, but may also contribute to reperfusion injury[5, 12]. As seen in MI[11], the ischemic brain first recruits neutrophils and later monocytes[4]. However, in contrast to the ischemic heart, microglia substantially contribute to the cellular inflammatory response in the brain[4]. Limited data are available on the role of monocyte subsets in inflammation, healing and resolution of inflammation after ischemic brain injury. In the infarcted mouse heart[13], inflammatory Ly6C^{high} monocytes are recruited first via CCL2/CCR2 and dominate the first 3 days after injury. Ly6C^{high} monocytes are sources of inflammatory cytokines and pursue proteolytic and phagocytic removal of necrotic tissue. Likely, these cells give rise to M1 macrophages with similar pro-inflammatory functions. Starting on day 4 after MI, an inflammation resolution phenotype emerges, as Ly6C^{low} monocytes/macrophages are recruited via CX3CR1 to orchestrate tissue repair. These cells regulate angiogenesis and extracellular matrix production, but also continue phagocytosis of tissue debris. Blocking either monocytic phase impairs infarct healing and promotes heart failure in mice[13]. Several lines of evidence suggest analogous roles for monocyte and macrophage subsets after stroke. A parallel temporal pattern of inflammatory and pro-resolution macrophage phenotype occurs in murine brain after middle cerebral artery occlusion[14]. Contrasting MI data, a study[14] reports that Ly6C^{low} cells are not recruited separately via CX3CR1 but rather derive from CCR2^{+} Ly6C^{high} monocytes. Monocyte depletion increases hemorrhagic conversion of ischemic stroke, likely due to delayed myeloid cell repair functions[14]. On the other hand, deletion of CCR2 or its CCL2 ligand in mice results in smaller brain infarcts, together with decreased infiltration of monocyte/macrophages and pro-inflammatory cytokine production[15, 16]. These data point to potentially harmful as well as helpful functions of monocytes and macrophages after stroke, highlighting the necessity to better understand the role of subsets, timing, inflammation resolution and magnitude of innate immune responses in the injured brain.

Pre-existing chronic inflammation exaggerates local cellular response

In atherosclerotic plaques, leukocytes contribute decisively to growth, inflammation, instability and rupture. In patients, ischemia often results from artery-occluding atherosclerotic plaque and thus occurs in a setting of chronic inflammation that generated the vulnerable, ruptured plaque in the first place. The immune response to ischemic heart and brain in the setting of atherosclerosis may be fundamentally different — we hypothesize exaggerated — when compared to external wounding after trauma in an otherwise healthy person. Excessive levels of circulating inflammatory monocytes hamper resolution of inflammation, impair infarct healing and cause heart failure in ApoE$^{-/-}$ mice after coronary ligation[17]. Thus, pre-existing chronic inflammation alters critical signals compared to a healthy steady state, and the raised systemic immune activity associated with atherosclerosis may impair the resolution of inflammation after ischemic brain injury. If stroke results from atherosclerosis, inflammation resolution in the ischemic brain may be disturbed, and harmful functions of inflammatory leukocytes may impair outcome. This hypothesis should be tested experimentally. Of note, increased leukocyte and monocyte counts in the blood correlate with post-MI heart failure progression in clinical studies[18], and the blood level of the CD14^{high}CD16^{-} monocyte subset is associated with poor outcome and increased mortality in patients with stroke[19].

Rapid cell turnover in ischemic tissue motivates study of leukocyte supply

In sites of acute inflammation, the turn over of innate immune cells, especially neutrophils, monocytes and macrophages, may be surprisingly rapid. Fate-mapping studies determined that even 5 days after ischemic myocardial injury, myeloid cells are recruited in high numbers to the healing tissue, accounting for their relatively short infarct residence time of only 20 hours[20]. Similar numbers are not available for stroke; however, the leukocyte turnover may also be rapid. It will be important to understand local cell kinetics in the brain acutely after stroke, as high leukocyte turnover and ongoing recruitment necessitate increased leukocyte supply and production, all of which could be targeted therapeutically.

The splenic monocyte reservoir after MI and stroke

An interesting feature shared by ischemic brain and heart injury is a transient decrease in spleen size, likely reflecting the organ's release of leukocytes[21, 22]. Studies in animals splenectomized prior to ischemic insult of the brain or heart showed a decreased infiltration of innate immune cells into inflamed tissues[20, 23]. More recently, a lower number of splenic monocytes was described in an autopsy study of patients with acute MI[24]. A decrease in spleen size has been documented in patients with acute stroke[25]. Contrary to the systemically increased levels of innate immune cells, post-stroke inflammation is accompanied by a severe loss of lymphocytes in blood and spleen. Lymphopenia after stroke most likely results from lymphocyte apoptosis, which may contribute to the reduction in spleen's size[9] and possibly to compromised immunity.

Accelerated hematopoiesis increases leukocyte production

Because the heightened demand for leukocytes in the ischemic lesion quickly exhausts ready-made cells in the body's reservoirs (i.e. blood, bone marrow and spleen), the hematopoietic system likely increases cell production. In mice with MI, we observed transfer of leukocyte progenitors from the bone marrow to the spleen, and splenic monocyte production[20, 26] (Figure). Despite the health burden caused by stroke and MI, surprisingly little is known about how the bone marrow compartment reacts to ischemic injury, and how it is alerted after MI or stroke. The widespread systemic inflammatory response in both patients[7] and animal models[27] argues for an activation of the hematopoietic system after stroke. The recently reported increased hematopoietic stem and progenitor cell activation in mice and in patients with MI[26] is likely generalizable although its impact on the brain, heart and vasculature are probably organ and tissue specific. Future studies will investigate hematopoietic stem cell behavior and their progeny's fate after ischemic brain injury, and examine the pathways that regulate bone marrow activity after stroke.

Danger signals alerting immune and hematopoietic system after stroke and MI

Long-range signals transfer information from the site of injury to the site of innate immune cell production. These could be transmitted in the bloodstream in form of intracellular danger signals released from dying cells in the wound, acting as receptor ligands on progenitor cells or on the hematopoietic niche (Figure). Alternatively, signals could be delivered via extravascular routes through the fibers of the sympathetic nervous system. Noradrenaline, released from sympathetic nerves in the bone marrow after MI, binds to β_3 adrenergic receptors expressed on mesenchymal stromal cells, providing the microenvironmental cues in hematopoietic tissue after myocardial infarction[26]. Identifying and inhibiting those signals may help to curtail leukocyte overproduction and -supply, as both are likely rate-limiting inflammation in the brain, the heart and the artery wall.

Increased leukocyte production may cause recurrent stroke and MI

The cells that are produced in response to ischemia not only travel to the injury site but may be diverted to atherosclerotic lesions in the arterial wall. Similar cell types, especially neutrophils and inflammatory monocytes, are early responders after MI and stroke as well as major instigators of inflammatory atherosclerosis. In parallel to the high re-occurrence rates in patients with ischemic complications of atherosclerosis, we observed that MI as well as stroke accelerates atherosclerosis progression in ApoE$^{-/-}$ mice[26]. If we improve our understanding of the putative crosstalk between the immune and hematopoietic systems with injured brain and heart (Figure), we may expand our clinical ability to prevent recurrent ischemia. The development of new therapeutic strategies modulating the hematopoietic system with translatable pharmacological interventions may present a crucial step towards secondary prevention of stroke and MI. On this path, it will likely be helpful to compare the systemic immune response after MI and stroke, and to determine the prevailing parallels and differences.

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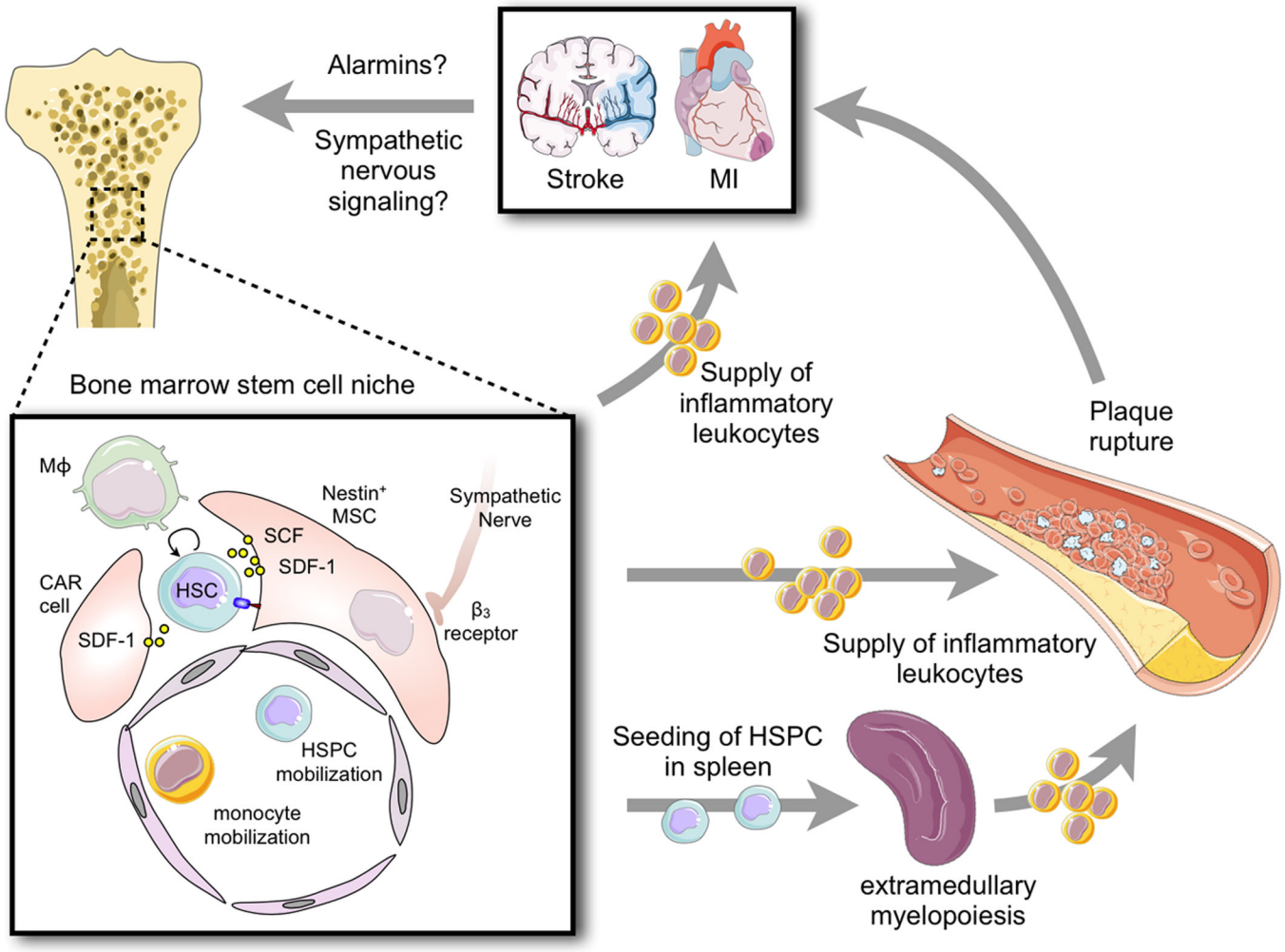

Figure 1. Putative organ networks after myocardial infarction and stroke

The cartoon illustrates events after ischemic injury of either the brain or the heart that lead to accelerated disease progression in atherosclerotic plaque (some events are experimentally proven in mice, others are still hypothetical). The enlarged inset depicts processes in the bone marrow microenvironment after MI. Here, niche cells provide signals that regulate hematopoietic stem cell activity, retention and leukocyte production. After MI, increased sympathetic nervous signaling releases noradrenaline in the bone marrow niche, which binds to β_3 adrenoreceptors on niche cells. These withdraw the soluble factor SDF-1 which results in increased hematopoietic progenitor cell activity and emigration to extramedullary sites. Similar processes may be active after stroke. Increased production of leukocytes then feeds an expanded pool of circulating monocytes which are recruited to the injured brain or myocardial infarct, but also to atherosclerotic plaque in higher numbers, accelerating plaque growth and vulnerability. This feedback loop may cause the high clinical reoccurrence rates of MI and stroke. HSC: hematopoietic stem cell. HSPC: hematopoietic stem and progenitor cells. CAR: CXCL12 abundant reticular cell, Mφ: macrophage.