ImmunoTools *special* Award 2013

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Role of visfatin on inflammatory atherosclerotic vulnerability for ischemic stroke

Background: Ischemic stroke is one of the leading causes of morbidity and mortality in developed and developing nations. From the last decade, incidence of ischemic stroke has increased, and may even reach acute coronary syndromes within the next two decades. The definition of ischemic stroke has been recently updated as a focal neurologic deficit of acute onset in the presence of cerebral infarction (detected by diffusion-magnetic resonance imaging [MRI]). Cardiac arrhythmias (such as atrial fibrillation) or diseases favouring intravascular coagulation (including cancer) are conditions often associated with ischemic stroke. However, the rupture of a carotid plaque, with subsequent thrombotic occlusion of the vessel lumen and possible embolism remains the most commonly pathophysiological cause of an ischemic stroke. Neutrophil recruitment towards carotid plaques and infarcted cerebral tissue plays a crucial role in carotid plaque vulnerability and ischemic brain injury. Systemic and “intraplaque” vulnerability as well as cerebral tissue resistance to ischemic injury are orchestrated by several soluble mediators, including hormones, cytokines and chemokines. CXC chemokines (i.e. CXCL8 or its murine equivalent CXCL2) have been shown as the most important mediators of neutrophil recruitment and activation at inflammatory sites in cardiovascular diseases. The recently discovered adipocytokine visfatin might modulate recruitment and activation of leucocytes by increasing carotid plaque vulnerability and post-infarction cerebral damage.

Hypotheses: Among different inflammatory mediators, visfatin could interfere with CXCL8-mediated activities on inflammatory cells, governing cardiovascular vulnerability in humans and mice. Treatments with specific visfatin or CXC chemokine inhibitors might reduce leukocyte recruitment and activation within carotid plaques and infarcted brain, thus improving outcomes of ischemic stroke.

Specific aims:

1. To investigate in patients asymptomatic and symptomatic for ischemic stroke the possible correlation between the levels of visfatin in serum and in different portions of carotid plaques and CXCL8-induced leukocyte infiltration and release of inflammatory products (such as neutrophil elastase, metalloproteinases and myeloperoxidase).
2. To investigate the possible role of visfatin on CXC chemokine-mediated proinflammatory activities in 2 different in vivo mouse models: a) shear stress-induced carotid plaque vulnerability; b) leukocyte-mediated neuronal injury in transient focal cerebral ischemia.

3. To explore if treatments with visfatin inhibitor (FK866) or CXC chemokine antagonist (Evasin-3) modulates leukocyte-mediated carotid plaque vulnerability and neuronal injury in the 2 mouse models mentioned above.
4. To investigate in vitro the influence of visfatin on CXC and CC chemokine-induced pro-inflammatory functions in human and mouse primary neutrophils and monocytes.

Experimental Design and Methods: Human and mouse carotid intraplaque vulnerability parameters will be assessed by histology and Real Time RT-PCR. The levels of serum mediators will be measured by ELISA. For in vivo experiments, we will use the well-described mouse models of "cast" placement on common carotid (plaque vulnerability) and transient focal cerebral ischemia. Extent of brain necrosis, leukocyte infiltration will be defined by histology. The expression of visfatin, CXCL8 (or its murine equivalent CXCL2), neutrophil elastase, metalloproteinase-9, as well as myeloperoxidase will be assessed by histology and Real Time RT-PCR. In vitro experiments will be performed by RT-PCR, ELISA, FACS analysis and Boyden chamber chemotaxis assay towards recombinant CC and CXC chemokines. MMP-9 activity will be assessed by zymography

ImmunoTools antibodies and recombinant CC and CXC mouse and human chemokines will be crucial in our project for being used in the in vitro experiments with human and mouse neutrophils and monocytes (Aim 4). In particular, FITC - conjugated anti-human CD1a, CD11a, CD14, PE - conjugated anti-human CD11b, CD11c, FITC - conjugated anti-mouse Gr-1 or PE - conjugated anti-mouse CD11b (as well as Ab Control IgG) will be used to assess if pre-incubation with visfatin might affect the expression of these molecules on neutrophil and monocyte surface when these cells are exposed to recombinant CC and CXC chemokines (again from ImmunoTools). These CC and CXC recombinant human and mouse chemokines will be used as appropriate stimuli to test neutrophil and monocyte chemotaxis and neutrophil degranulation (Myeloperoxidase, MMP-9 and elastase release) in vitro.

Expected Relevance: The proposed experiments should provide new insights on the role of visfatin and its pharmacological inhibition on CXC and CC chemokine-mediated carotid vulnerability and related ischemic stroke in humans and mice.

ImmunoTools *special* AWARD for **Fabrizio Montecucco** includes 25 reagents

FITC - conjugated anti-human CD1a, CD11a, CD14, Control-IgG1, Control-IgG2a, Control-IgG2b,

PE - conjugated anti-human CD11b, CD11c, Control-IgG1, Control-IgG2a, Control-IgG2b,

recombinant human cytokines rh GRO-alpha,

FITC - conjugated anti-mouse Gr-1, isotype control IgG2b

PE - conjugated anti-mouse CD11b, isotype control IgG2b

recombinant mouse cytokines rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm MCP1 / CCL2, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm RANTES / CCL5

DETAILS