EFFECTS OF THROMBIN CLEAVAGE OF OSTEOPONTIN ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND ROLE OF EXTRACELLULAR PROTEASOME IN REGULATING ITS CHEMOTACTIC ACTIVITY WITH IMPLICATIONS IN MULTIPLE SCLEROSIS

1)Ferrara B.. 2)Dianzani C.. 3)Bellavista E.. 4)Liepe J.. 5)Gigliotti CL. 6)Clemente N.. 7)Boggio E.. 8)Comi C.. 9)Verderio C.. 10)Riganti L.. 11)Keller C.. 12)Chiocchetti A.. 13)Dianzani U.. 14)Mishto M..

Dipartimento di Scienza e Tecnologia del Farmaco

Osteopontin (OPN) is a proinflammatory cytokine that plays a pathogenetic role in multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). OPN exerts its role by recruiting autoreactive T cells into the central nervous system. Secreted OPN (OPN-FL) is cleaved by thrombin generating N- and C-terminal fragments, which exert different biological activities. Another protease present in different types of biological fluids, including blood, is the extracellular 20S proteasome. In several autoimmune diseases, included MS, plasma extracellular proteasome levels are increased and mark cell damage and immunological activity.

A first aim of this work was to evaluate the effects of OPN fragments on human cells and EAE by focusing on processes involved in the relapse/remission pattern of MS. A second aim was to investigate if OPN can be processed by the extracellular proteasome modulating the OPN activity.

Firstly, OPN effects have been evaluated in vitro on apoptosis and cytokine secretion of T cells, and migration/adhesion of lymphocytes and endothelial cells, by using recombinant forms of OPN-FL, OPN-N, and OPN-C produced in an eukaryotic system. The three OPN forms displayed a similar inhibition of T cell Activation-Induced Cell death-AICD (20% inhibition), which plays a key role in switching off the immune response. Effects of OPN treatment on T cell cytokine secretion showed upmodulation of IL-17 induced by OPN-N and downmodulation of IL-10 induced by OPN-C. A similar coordinated effect of the OPN fragments was detected on lymphocyte adhesion to human umbilical vein endothelial cells (HUVEC) and migration. Both these processes were supported by OPN-FL, but OPN-C induced only adhesion (80% stimulation), whereas OPN-N induced only migration (70% stimulation). On HUVEC tubulogenesis, both OPN-N and OPN-C displayed a higher effect than OPN-FL. Also in vivo experiments showed that thrombin-mediated cleavage of OPN plays a key role in OPN function, since OPN-FL was much more effective in inducing EAE relapses than OPN-FLmut, a point-mutated OPN resistant to thrombin-mediated cleavage. Use of the recombinant OPN-C and OPN-N revealed that induction of the relapse was ascribable to OPN-C.

Secondly, it has been investigated if the extracellular proteasome modulates the OPN activity on cell migration. In HUVECs and monocytes, the treatment with 20S proteasome significantly hampered the chemotactic activity of OPN-N, whereas it increased the chemotactic activity of OPN-FL and, especially, OPN-C on HUVEC and lymphocytes. This suggests that proteasome-mediated degradation of OPN-FL and OPN-C generates novel OPN chemotactic fragments. To identify these active fragments, proteasome digestion products of OPN-C have been analyzed by mass spectrometry. This analysis detected 6 main fragments, which were then synthetized and analyzed for their chemotactic activity. Four of these peptides exerted a strong chemotactic

activity towards HUVEC and lymphocytes in a dose-dependent manner. Since the levels of both the extracellular proteasome and OPN are increased in MS patients, these data suggests that these peptides may play a role in MS.

Finally, experiments showed that two peptides derived from the proteasome-mediated digestion displayed chemotactic activity also toward several tumor cell lines, which suggests that they may play a role in tumor metastatic dissemination, since the levels of both OPN and extracellular proteasome are often high in the tumor mass.

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