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LOCATION OF ICAM-1 CLEAVAGE SITES BY MMP-13 IN THE VASCULAR ENDOTHELIUM OF ENOS DEFICIENT MICE

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During atherosclerosis, attachment of circulating mononuclear cells to the vessel wall is mediated by adhesion molecules, including intracellular cell adhesion molecule 1 (ICAM-1). In aortas of apoE-null mice, ICAM-1 was found located in lesion-prone sites of the aorta, and the importance of this inflammatory molecule was evidenced in atherosclerotic mice deficient in ICAM-1, which resulted protected from atherosclerotic lesions.

The precise role of matrix metalloproteinases (MMPs) in the early steps of atherosclerosis is under debate. In this regard, the effect of MMPs in the shedding of adhesion molecules was investigated *in vitro*, although no data were reported in the context of atherosclerosis. Lack of endothelial NOS (eNOS, NOS3) increases leukocyte-endothelial adhesion, smooth muscle cell migration, platelet aggregation, and atherosclerosis in mice. However, the mechanisms by which NOS3 could prevent atherosclerosis still remain unclear. Here we found that in atherosclerotic NOS3/apoE-deficient mice, an increased monocyte adhesion, and a significant reduction of MMP-13 expression were detected when compared to apoE-deficient animals. In addition, we found that MMP-13 cleaves ICAM-1 both *in vivo* and *in vitro*.

Mass spectrometry analysis revealed two cleavage sites at positions E61 and G98, close to the ICAM-1 extracellular N-terminal domain. The relevance of MMP-13 and ICAM-1 on cellular adhesion was found in COS-7 cells expressing ectopic ICAM-1, in which RAW 264.7 cell adhesion was inhibited by the presence of active MMP-13. Our findings may help to explain at the molecular level the protective effect of endothelial NO in atherosclerosis.