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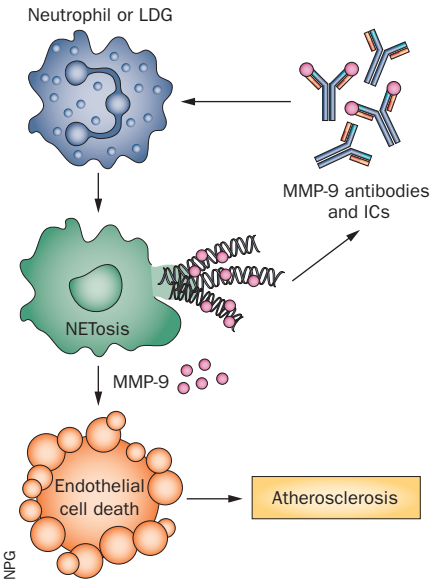
CONNECTIVE TISSUE DISEASES

A NET of peril for endothelial cells in SLE?

Neutrophil extracellular trap (NET) formation—a process known as NETosis—is increased in systemic lupus erythematosus (SLE), particularly in a distinct subset of proinflammatory, low-density granulocytes (LDGs) isolated from peripheral blood mononuclear cell fractions of patients with SLE. LDG NETs have previously been found to promote endothelial cell apoptosis, but just how do NETs contribute to vascular damage in SLE? A new study by Mariana Kaplan's group suggests a mechanism by which matrix metalloproteinase (MMP)-9 associated with NETs triggers endothelial dysfunction by activating endothelial MMP-2, and suggests that MMP-9 also acts as an autoantigen in SLE.

"We decided to focus on MMPs because it is known that these enzymes are associated with vascular damage and their serum levels are increased in SLE sera," says Kaplan about this latest work, which builds on the group's previous studies of LDG NETs. "As MMPs are present in neutrophil granules and granular contents are usually externalized during NET formation, we examined if they were implicated in endothelial damage in SLE."



Assessing the expression of MMPs in NETs, the investigators found that active MMP-9 is externalized in NETs from SLE LDGs at higher levels than in NETs from normal-density SLE neutrophils or LPS-stimulated neutrophils from healthy individuals. Zymography analysis demonstrated the activity of not only MMP-9 but also MMP-25, another neutrophil MMP, in the NETs, to a greater extent in SLE LDGs than in LPS-stimulated control neutrophils.

Incubation of human umbilical vein endothelial cells (HUVECs) with NETs from SLE LDGs led to morphological changes indicative of endothelial cytotoxicity, including the loss of the HUVECs' characteristic 'cobblestone' appearance. Immunofluorescence illustrated the accumulation of NET-derived MMP-9 in the plasma membrane of the HUVECs. This MMP-9 was shown to co-localize with LL-37, a neutrophil antimicrobial protein previously shown to be externalized in NETs, thus confirming that the MMP-9 was NET-bound.

MMP-9 induced endothelial cell death and vascular dysfunction via activation of pro-MMP-2 released from endothelial cells, suggesting a mechanism for endothelial cell damage. Notably, NET-induced vascular dysfunction and apotosis was abrogated by specific inhibition of MMP-9 and by broad inhibition of MMPs. Furthermore, neutralization or depletion of MMP-9 in NETs decreased the activation of endothelial MMP-2 that occurred following incubation with NETs. As Kaplan highlights, "Inhibition of this MMP-2 activation specifically restored endothelial function and improved vascular viability."

Kaplan's team detected MMP-9 in samples of sera both from patients with SLE and from healthy individuals, although at much higher levels in the former group. They also identified MMP-9 present in immune complexes (ICs) in sera from patients with high titres of anti-double-stranded DNA antibodies. These ICs were found to induce NET formation in control neutrophils through reactive oxygen species and NADPH oxidase-dependent pathways.

Anti-MMP-9 IgGs were detected in SLE sera and in sera from patients with rheumatoid arthritis (a condition that is also associated with increased cardiovascular risk and NET formation) at elevated levels in comparison with sera from healthy individuals. Like the MMP-9-containing ICs, anti-MMP-9 antibodies were able to enhance NETosis in control neutrophils. Moreover, anti-MMP-9 antibodies bound to MMP-9 on LDG NETs and enhanced their activity.

Together, the results of the study point to a model for the role of NET-bound MMP-9 in endothelial dysfunction. As Kaplan summarizes: "We found that SLE LDGs externalize increased amounts of active MMP-9 and that this enzyme activates MMP-2 present in endothelial cells and specifically induces endothelial dysfunction and apoptosis. Furthermore, immunogenic complexes containing MMP-9, as well as anti-MMP-9 antibodies, induce NET formation, thereby perpetuating a vicious cycle characterized by enhanced NET formation and accelerated vascular damage."

MMP inhibition has been explored as a potential therapeutic approach in vascular damage, and the findings put new focus on the role of NET-derived MMP-9. Kaplan emphasizes that in-depth characterization of the molecular mechanisms by which aberrant NET formation induces endothelial damage in SLE is key, and plans intensive investigations to test whether inhibition of NET formation in animal models of lupus and in human systems could mitigate end-organ damage.

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Original article Carmona-Rivera, C. *et al.* Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. *Ann. Rheum. Dis.* doi:10.1136/annrheumdis-2013-204837