Modulation of *in vitro* nitric oxide production in murine macrophages by immobilized and soluble glycosaminoglycans.

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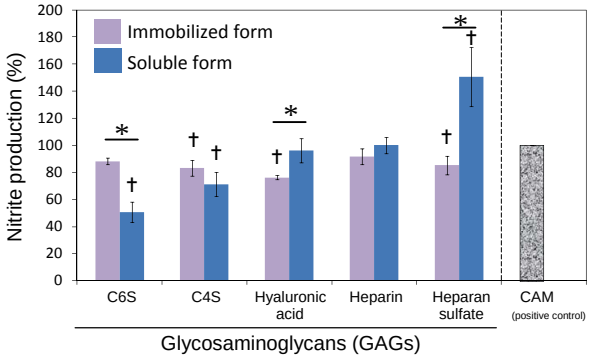
Statement of Purpose: Macrophages (MØ), pivotal effector cells of the immune response, are able to switch their functional phenotypes into either pro-inflammatory or anti-inflammatory subsets in response to microenvironmental cues, e.g. cytokines and extracellular matrix.[1] The former subset, also known as M1 or classically activated MØ (CAM), produces free radicals such as nitric oxide (NO) that are toxic to microbes, but can also cause collateral tissue damage at high doses. Recent studies indicate that glycosaminoglycans (GAGs), linear polysaccharides ubiquitously expressed in the body in varying forms, may play a role in immune regulation.[2] The purpose of this study was to investigate how NO production in CAM is modified by exposure to various GAGs, presented either in the immobilized or soluble forms.

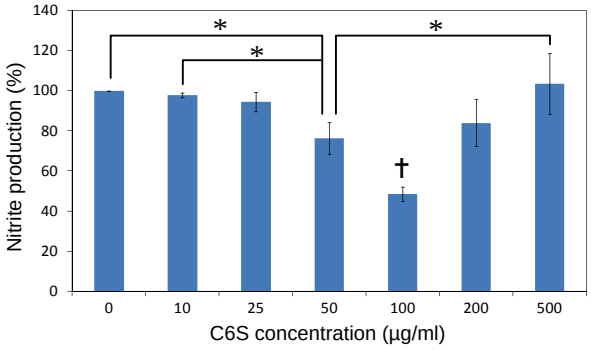
Methods: Bone marrow cells isolated from hind limbs of BALB/c mice were maintained in Iscove's Modified Dulbecco's Medium (IMDM) containing 20% heat-inactivated fetal bovine serum and recombinant macrophage-colony stimulating factor (M-CSF) for 7 days. The resulting monocyte/macrophage population (>97% purity) was then dislodged and seeded onto 24-well plates pre-coated with gelatin that allows the subsequent covalent immobilization of various GAGs, namely chondroitin-6-sulfate (C6S), chondroitin-4-sulfate (C4S), hyaluronic acid, heparin and heparan sulfate. At 2 h post-seeding, cells were stimulated to CAM by adding bacterial lipopolysaccharide and interferon-γ (LPS/IFN-γ) into the medium. In parallel experiments, cells were allowed to attach on standard 24-well plates (i.e. without GAG coatings) for 2 h before exposure to LPS/IFN-γ, in the absence or presence of various soluble GAGs. Supernatants were assayed at 24 h post-treatment for nitrite, a stable metabolite of NO, using a Griess assay.

Results: In the absence of LPS/IFN-γ, MØ treated with GAGs secreted minimal amount of NO after 24 h of culture, indicating that GAGs studied here do not initiate a pro-inflammatory response. Upon activation with LPS/IFN-γ, a considerably increase in NO levels was observed in MØ cultured on standard 24-well plates. Of the different immobilized GAGs tested, C4S, hyaluronic acid and heparan sulfate resulted in small but statistically significant reduction in IFN-γ-induced NO production in MØ at 24 h post-treatment (p<0.05). Conversely, differential modulation of NO production appeared more obvious when GAGs were presented to cells as soluble molecules: (1) heparan sulfate dramatically enhanced NO production up to 151±22% (p<0.05); (2) NO levels in the cultures remained unchanged in the presence of heparin or hyaluronic acid; and (3) both C6S and C4S significantly reduced NO production in CAM to 51±8% and 71±9% (p<0.05), respectively (Figure 1). Further investigations showed that soluble C6S exerted a biphasic effect on NO production in CAM, in which it suppressed its production



*(Note: † p<0.05 vs. CAM; * p<0.05; n = 3-5 from three independent experiments).*

Figure 1. Effects of immobilized vs. soluble GAGs on nitrite production in CAM.



*(Note: † p<0.05 vs. all other groups; * p<0.05; n = 6 from three independent experiments).*

Figure 2. Nitrite production in CAM in response to different concentrations of soluble C6S.

in a dose-dependent manner at lower concentrations (10-100 μg/ml), followed by a reversion to control values at higher concentrations (Figure 2). On the other hand, no significant changes in NO production were observed in CAM cultured on C6S-immobilzed surfaces with different coating concentrations, suggesting the impact of the mode of C6S presentation on NO production in CAM.

Conclusions:

Our results indicate differential roles of various GAGs in modulating NO production during a pro-inflammatory response, which may contribute to their immunoregulatory ability. The effects are, at least in part, depends on their mode of presentation to cells, i.e. immobilized vs. soluble. Specifically, soluble C6S was able to suppress NO production in CAM in a biphasic, dose-dependent manner. Studies are currently in progress to evaluate the effects of soluble C6S on the expression of other inflammatory mediators in CAM and the possible underlying mechanism(s).

References:

1. Stout RD. J. Immunol. 2005:175: 342-349.
2. Campo GM. J. Cell. Biochem. 2009: 106:83-92.