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Threat Matrix

Low-Molecular-Weight Hyaluronan (HA) as a Danger Signal

Jonathan D. Powell
Maureen R. Horton

Johns Hopkins University School of Medicine

Abstract

Whether or not T cell receptor engagement leads to full activation or tolerance is determined by the context in which the antigen is encountered. Antigen presented by activated APCs in the presence of costimulation leads to full T cell activation, while antigen presented by resting APCs leads to tolerance. Pathogen-associated molecular patterns in the form of toll-like receptor ligands play a critical role in activating APCs and promoting T cell activation. In this review we hypothesize that low-molecular-weight species of the extracellular matrix polymer hyaluronan also performs this function by acting as an endogenous danger signal.

Key Words

T cells
Hyaluronan
Danger theory
TLR
Costimulation

Introduction

The fine specificity of TCR recognition plays a crucial role in the ability of the adaptive immune response to respond to infections. However, this step (termed Signal 1), which heralds recognition, can lead to either full T cell activation or T cell tolerance *(1)*. What is critical in terms of whether activation or tolerance prevails is the context in which the antigen is recognized. That is, whether or not the antigen is presented in the presence or absence of costimulation (termed Signal 2) *(2)*. In such a model, the decision as to whether the end result of antigen recognition will lead to tolerance or immunity is determined by the activation status of the antigen presenting cell (APC). Simply, activated APCs will express costimulatory molecules and thus induce immunogenic T cell responses. Alternatively, resting APCs, which lack costimulatory molecules on their surface, will present antigen to T cells in a fashion that will promote tolerance. Indeed, dendritic cells, the most potent APCs, can act as potent inducers of tolerance.

Within this paradigm the nature of the antigen in terms of whether it is derived from *self* or *non-self* is not the critical determinant of whether T cell recognition will lead to activation or tolerance. What is important are molecules that have the ability to activate APCs. Based on these observations, Janeway proposed that the immune system responds to pathogen-associated molecular patterns (PAMPS) that are inherent to the make-up of

Jonathan D. Powell
The Bunting-Blaustein Cancer Research Building
1650 Orleans Street, Suite 443
Baltimore, MD 21231
E-mail: Poweljo@jhmi.edu

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infectious agents and serve to distinguish them from host molecules *(3)*. These PAMPs are recognized by pattern-recognition receptors (PRRs) that are part of the innate immune system *(4)*. Medzhitov and Janeway discovered such receptors in the form of toll-like receptors (TLRs) *(5)*. As predicted, the TLRs, of which there are approx 10 different types, recognize bacterial products such as LPS and flagellin, yeast products such as zymosan, and double-stranded RNA that are integral to the life cycle of many viruses. Furthermore, PRR engagement leads to the activation of APCs, including dendritic cells, inducing them to present their antigens in the context of costimulation. This theory explains the ability of the immune system to recognize and to appropriately respond to "infectious non-self." For example, a vaccine of foreign antigens given in the absence of an adjuvant often leads to little or no immune response or even tolerance. However, when given with adjuvant, whose components activate TLR receptors, such a vaccine leads to immunity.

Although the above model explains the ability of the immune system to fight infections, it does not directly explain the ability of immune responses to be generated against transplanted organs and tumors. In an effort to explain these phenomena, Fuchs and Matzinger proposed that the immune system is activated when it recognizes antigen in the presence of danger signals *(6–8)*. In their model, such signals could be derived from either the host or infectious agents. TLR engagement by bacterial products could induce danger signals as could necrotic cell death independent of infection. Alternatively, apoptotic cell death, which is a nonpathologic process, would not induce danger signals. Likewise, infectious agents that do not induce danger signals would not initiate vigorous immune responses. The precise nature of these host-derived signals is emerging. For example, Interferon alpha appears to play an important role in indicating danger and thus promoting immune activation *(9)*. Most recently, it has been shown that insoluble uric acid crystals can act as danger signals. Cellular-derived uric acid, which is a byproduct of nucleotide metabolism, is released upon necrotic cell death *(10)*. By fractionating the cytoplasm of necrotic cell extracts, Shi et al. determined that one molecule responsible for activating dendritic cells in their system was uric acid crystals. In as much as the production of uric acid and its release is independent of any infectious agents, these data underscore the ability of host-derived molecules to activate immune responses under stressed conditions.

In this article we present evidence to support the hypothesis that the low-molecular-weight species of the extracellular matrix component hyaluronan (HA) acts as a host-derived danger signal.

HA as a Component of the Extracellular Matrix (ECM)

HA, a negatively charged high-molecular-weight (HMW) glycosaminoglycan, is ubiquitously distributed in the ECM *(11,12)*. It is found in the basement membrane of normal lungs, joints, and vitreous fluid and makes up about 70% of the proteoglycan content of the lungs *(13)*. It is primarily produced by fibroblasts and to a lesser degree by smooth muscle cells and appears to function in water homeostasis, plasma protein distribution and transportation, joint lubrication, and matrix structure *(11,12)*. In vivo, at sites of inflammation, the HMW HA (size 1×10^6 KDa) can be depolymerized to lower-molecular-weight (LMW) (size 2×10^5 KDa) fragments via oxygen radicals and enzymatic degradation by hyaluronidase, β-glucuronidase, and hex-

osaminidase. Likewise, inflammatory cytokines, such as tumor necrosis factor-α, can stimulate pulmonary fibroblasts to produce increased amounts of HA fragments *(14)*. Furthermore, increased concentrations of HA have been found in bronchoalveolar fluid from patients with sarcoidosis and idiopathic pulmonary fibrosis as well as in the joints of patients with rheumatoid arthritis *(15,16)*.

In its high-molecular-weight form, HA is biologically inert in terms of its ability to activate immune cells. On the other hand, in the setting of inflammation or tissue destruction, HA is broken down into its lower-molecular-weight components. Unlike the intact ECM, these lower-molecular-weight species possess the ability to activate the innate immune response *(17)*. Fragment size is important in signaling, as fragments larger than 500 kDa or smaller than six sugars do not signal *(18)*. These breakdown products of HMW HA have the ability to induce a diverse array of inflammatory mediators. We have shown that a wide array of inflammatory genes is upregulated by low-molecular-weight HA fragments in mouse macrophages. HA fragments stimulate macrophage expression of members of the chemokine family [macrophage inflammatory peptide-1α (MIP-1α), macrophage inflammatory peptide-1β (MIP-1β), KC, RANTES, macrophage chemoattractant protein-1 (MCP-1), and interferon-induced Protein-10 (IP-10)], cytokines (IL-8, IL-12, and TNF-α), as well as the matrix-modifying enzymes [murine metalloelastase (MME), inducible nitric oxide synthase (iNOS) and plasminogen activator inhibitor-1] *(18–25)*. HA fragments inhibit constitutively expressed urokinase in addition to synergizing with interferon-γ (IFN-γ) to induce chemokines such as monokine induced by IFN-γ (MIG) and IP-10 *(20,22)*. Several of these chemokines, such as MIP-1α, MIP-1β, KC, and RANTES function as leukocyte chemoattractants and are implicated in inflammation and fibrosis.

In addition to activating tissue macrophages (and dendritic cells, see below), HA also has the ability to induce cytokine expression in epithelial cells *(26–29)*. Not just a passive barrier, the epithelial layer is active in host defense by releasing cytoprotective mucus, defensins, and a host of lipid mediators, prostaglandins, and leukotrienes *(30)*. The airway epithelium participates in inflammatory responses in part through the generation of numerous cytokines and chemokines that mediate recruitment and activation of inflammatory cells. The epithelium is involved in local cytokine networks allowing first response to noxious agents, such as smoke, as well as cross talk with structural cells to help provide an effective inflammatory response *(30)*. Furthermore, upon stimulation/insult, bronchial epithelium can generate a wide variety of cytokines (IL-6, TNF-α, IL-1), chemokines (IL-8, IP-10, MCP-1), and growth factors (TGF-β, GM-CSF, CSF-1) *(30–32)*. Data are now emerging that HA has the ability to activate this epithelial-derived protective response. It has been shown that HA can induce IL-8 expression in epithelial cells *(29)*. Our own group has been able to show that LMW HA can induce IL-8 and IP-10 expression in lung epithelial cells (Powell and Horton, unpublished). From a mechanistic point of view, as the epithelial barrier is breached and tissue destruction occurs, HMW HA is broken down to LMW HA, which in turn activates epithelial cells to promote inflammation. Thus, when protective barriers are breached (such as the epithelium), HA becomes not only a target of inflammation, but a participant in promoting the immune response. Table 1 summarizes the diverse spectrum of the HA-induced response in terms of the cells that can be activated by LMW HA and the wide range of mediators released in response to LMW HA stimulation.

Table 1. HA Promotes Inflammation by Activating a Diversity of Cell Types

Cell Type	Effect	Size of HA
Macrophages *(18,20–24,44,73)*	Increased: MIP-1 α, MIP-1β, MCP-1, RANTES, KC, IP-10, iNOS, MMP12, PAI-1, TNR-α, IL-1β, IL-12, IGF-1 Decreased: urokinase	200 kDa
Dendritic cells *(17,74)*	Increased iNOS, IL-1β, TNF-α, IL-12	Tetra-hexasaccharides
Endothelial cells *(75–77)*	Increased: NO,IL-8, MCP-2, KC, cell adhesion, proliferation, neovasculatization	3–25 disaccharides
Neointimal arterial cells *(78)*	Decreased proliferation	4–16 saccharides
BV-2 microglial cells *(79)*	Increased iNOS	
Fibroblasts *(80)*	Increased: MMP9, MMP13	
Renal tubular epithelial *(26–28)*	Increased: COX-2, MCP-1, VCAM-1	
Lung epithelial-A549 cells *(29)*	Increased IL-8	
Peritoneal mesothelial cells *(81)*	Increased: MCP-1, IL-8	
Kupffer *(82)*	Increased iNOS	200 kDa
Murine 3LL tumor cells *(80)*	Increased MMP9, MMP13 and urokinase	
Chondrosarcoma *(83)*	Increased urokinase and urokinase receptor	
Murine mammary carcinoma *(84)*	Decreased tumor growth Increased apoptosis	2500 kDa
Human colon carcinoma *(84)*	Decreased tumor growth Increased apoptosis	2500 kDa

The Role of LMW HA in Promoting Acquired Immunity

Upon infection, pathogen-derived molecules activate the innate immune response. Activated neutrophils, macrophages, and NK cells are recruited to the infected tissue and release various cytokines, chemokines, reactive oxygen species, and degradative enzymes. In addition, this activation leads to the upregulation of hyaluronan synthase genes as well as hyaluronidases, which serve to degrade high-molecular-weight HA into small fragments *(11,12,33,34).*

Resting-tissue-specific dendritic cells (DCs) are constantly sampling the environment through the process of macro-pinocytosis *(35).* In the resting state such DCs present host-derived antigens in the absence of costimulation. The presentation of antigen by resting DCs can be a potent inducer of T cell tolerance. Upon activation, the DC upregulates its expression of costimulatory molecules such as B7.1 and B7.2 and migrates to the draining lymph node where it activates antigen-specific T cells and promotes the acquired immune response. In this model, the outcome of whether or not TCR recognition of antigen will lead to activation or tolerance is very much dependent upon the activation status of the DC.

Pathogen-derived molecules such as LPS, zymosan, double-stranded RNA, and CpG-rich DNA play important roles in the activa-

tion of DCs and hence the initiation of acquired immune responses *(36)*. LMW HA also has the ability to activate DCs. Incubating resting immature DCs with LMW HA results in the upregulation of Class II, B7.1, B7.2, and CD40 *(17)*. Not surprisingly, the HA-stimulated DCs promote vigorous T cell activation in response to antigen *(37)*. In addition, HA has the ability to promote CD154-induced mobility and clustering of DCs *(38)*. Using an HA inhibitory peptide, Mummert et al. have been able to demonstrate the ability of HA to promote maturation and migration of activated Langherhan cells *(39)*. Inflammation in the skin leads to the maturation and migration of Langherhan cells to the draining lymph nodes. By blocking HA with an inhibitory peptide, this response was mitigated. Such observations implicate the generation of LMW HA in the skin as playing a role in DC maturation and migration in vivo.

HA promotes the elaboration of various chemokines and cytokines that can promote acquired immune responses. For example, in both macrophages and DCs, HA upregulates IL-12 *(17,24)*. IL-12 plays a critical role in generating Th1 helper T cells. Along these lines, HA also initiates the transcription and elaboration of the C-C chemokines MIP1α, MIP1β, and RANTES *(18)*. The C-C chemokines are small proteins that share similar structure and are a part of the chemokine superfamily and were initially defined functionally by their ability to act as chemoattractants for monocytes, eosinophils, basophils, and lymphocytes *(40)*. More recently, these chemokines were shown not only to act as chemoattractants in the inflammatory response, but also are involved in enhancing and directing T cell–mediated responses *(41,42)*. MIP-1α, for example, appears to preferentially attract CD4+ T cells and act to promote the production of IL-2 and Th1 type cytokines *(43)*.

Through the induction of various cytokines and chemokines HA can influence the nature of the inflammatory response. On the other hand, the presence of certain cytokines and chemokines in the inflammatory milieu can also regulate the response to HA. We have found that IL-10 and IFN-γ independently inhibit HA-induced expression of MIP-1α, MIP-1β, and KC at both the mRNA and protein levels *(23)*. Whereas IL-10 inhibited most of the HA-induced chemokines tested, IFN-γ selectively inhibited only MIP-1α, MIP-1β, and KC *(23)*. This inhibition did not require prestimulation and occurred even when the cytokines were added up to 3 h after stimulation with HA. For MIP-1α, the inhibition by IFN-γ occurred at the level of transcription, whereas IL-10 predominantly decreased the stability of MIP-1α mRNA. IFN-γ and IL-10 equally inhibited macrophage expression of MIP-1β mRNA at the level of transcription, but MIP-1β mRNA stability was decreased to a greater extent by IL-10. These data identify a previously unrecognized role for IL-10 and IFN-γ as regulators of ECM-induced macrophage expression of inflammatory chemokines. Since IL-10 is associated with inhibiting Th1 type responses the ability of IL-10 to block HA induced chemokine production is not surprising. The ability of the model Th1 cytokine IFN-γ to inhibit HA-induced chemokine production is not precisely clear. Perhaps, this represents a negative feedback loop designed to prevent over-exhuberrant immune responses.

While IFN-γ inhibits HA-induced MIP-1α, MIP-1β, and KC, HA synergizes with IFN-γ to produce the CXC chemokines IP-10 and MIG *(20,44)*. As its name implies, MIG was originally described as a gene specifically upregulated by IFN-γ *(45–47)*. Upon binding to its receptor CXCR3, on both CD4+ and CD8+ T cells, MIG promotes chemotaxis of these cells. Also, MIG has been implicated in

Table 2. Comparison of TLR ligand and HA-Induced Activation

	Conventional TLR Ligands	HA
Source	Bacteria, yeast, viruses (exogenous)	Broken down extracellular matrix (endogenous)
Signaling	Often MyD88 dependent, activates NF-κB	MyD88 dependent, activates NF-κB
Effect on macrophages	Increases expression of cytokines, chemokines, MMPs and iNOS	Increases expression of cytokines, chemokines, MMPs and iNOS
Effect on DCs	Promotes maturation and activation	Promotes maturation and activation
Effect on T cells	Enhances T cell activation via its effect on DCs	Enhances T cell activation via its effect on DCs

contributing to the host inflammatory response against infection and tumor immunity *(48,49)*. MIG has been shown to be upregulated in a number of autoimmune disorders as well as the development of atherosclerosis *(50–53)*. The generation of MIG null mice has further elucidated the role of this chemokine in vivo. It has been found that MIG plays an important role in skewing toward a Th1 immune response, contributes to the optimal generation of humoral immune responses to intracellular bacterial infections, and activates dendritic cells *(41)*. The accumulating data suggest that MIG plays a key role in the adaptive immune response by recruiting T cells and enhancing their interactions with B cells and dendritic cells *(41)*.

Table 2 compares the properties of HA and PAMPS. As is the case with PAMPS, LMW HA has the ability to promote the activation and maturation of resting DCs and promote the activation of acquired immune responses. Furthermore, HA-induced cytokine and chemokine production attracts and activates T cells and potentially promotes Th1 mediated responses.

HA Receptors and Signaling

HA binds to proteins of the LINK superfamily all of which contain a 100 residue polypeptide that specifically interacts with HA *(54)*. For the most part, LINK proteins are components of connective tissue. An exception, CD44, is expressed as an integral cell surface protein on neutrophils, T cells, macrophages, and DCs *(55)*. The binding of HA to surface CD44 has been shown to promote cell migration, clustering, and adhesion. In addition to CD44, a novel HA binding receptor, lymphatic vessel endothelial HA receptor (LYVE-1), is expressed on lymphatic endothelium and is thought to play a role in the transport of HA from tissue to lymph as well as presenting HA to CD44+ leukocytes and thus enhancing their transmigration into lymph *(56)*. A third cell surface HA receptor, receptor for hyaluronan mediated motility (RHAMM), has also been described *(57)*. RHAMM has been implicated in promoting transformation, migration, and metastatic spread of a number of cancer types *(58)*. Recently, RHAMM has been shown to interact with actin and regulate cell mitosis through spindle poles *(59)*.

While the role of CD44 in leukocyte trafficking is well established, the role of CD44 in mediating HA-induced signal transduction has not precisely been defined. For example, our own group is able to demonstrate HA-induced activation in macrophages derived from WT and CD44 knockout mice (unpub-

lished data). Recently, Simon's group has proposed that LMW HA signals via TLR-4 *(37).* This hypothesis is based on the observation that bone marrow–derived DCs from C3H/HeJ and C57BL/10ScCr mice carrying mutant TLR-4 alleles were non-responsive to LMW HA as measured by TNF-α secretion and the ability to promote T cell proliferation. In addition, anti-TLR-4 antibodies blocked the HA-induced response of DCs derived from human monocytes. A critical signaling component of TLR is MYD88. Our group has data demonstrating that macrophages from MYD88 KO mice fail to respond to LMW HA (unpublished findings). However, we find that LMW HA-induced activation of macrophages and bone marrow–derived DCs from TLR-4 mutant and KO mice is equivalent to Wt controls as measured by chemokine gene expression. The reason for these contrasting findings is not precisely clear and may be related to the activation status of the APCs or the different parameters measured. Alternatively, although for both groups HMW HA does not induce activation, we use HA of around 200 kDa while Simon's group uses HA oligosaccharides. Nonetheless, the fact that pathogen-derived molecules and a degraded form of "self" both utilize TLR is of great interest and further supports the role of LMW HA as a "danger signal."

While the precise receptors involved in HA-induced signaling are currently being defined, it is clear that NF-κB plays an important role in this process *(60,61).* HA stimulation results in NF-κB activation and pharmacologic inhibition of this activation prevents HA-induced iNOS, MIG, and MIP-1α *(19,44).* Furthermore, we have demonstrated that IFN-γ synergizes with HA to induce MIG via NF-κB p50/p65 heterodimer binding to the –129 and –154 sites on the MIG promoter *(44).* The signaling pathways leading to HA-induced NF-κB activation are currently being defined. HA-induced signaling results in the activation of PKC and ras *(61).* Inhibition of either of these pathways blocked NF-κB activation of reporter constructs in T-24 cell lines. Interestingly, in epithelial cells, HA-induced IL-8 production is independent of NF-κB signaling and is upregulated by MAP-kinase activity. Alternatively, HA-induced IP-10 production in epithelial cells requires NF-κB but not the MAP-kinase pathway (Horton and Powell, unpublished). Finally, we have also been able to show that HA-induced NF-κB activation involves MYD88, IRAK, and TRAF6. Such findings are consistent with the notion that HA signals via a TLR (Horton and Powell, unpublished).

Clinical Implications

Elevated levels of LMW HA have been observed in a number of autoimmune disorders including rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, and dermatomyositis *(62–65).* In addition, LMW HA has been found in the lungs of patients with emphysema and *in situ* in rejecting kidney allografts *(66,67).* We and others have been interested in examining the role of LMW HA in a mouse model of pulmonary fibrosis. To this end, the bleomycin model of lung injury in animals, which approximates the pathology seen in fibrotic lung disorders such as idiopathic pulmonary fibrosis, has been studied *(68).* Intratracheal administration of bleomycin causes an initial alveolitis followed by a fibroproliferative phase with dysregulated matrix remodeling and ultimately fibrosis *(68).* There is increased turnover, production, and accumulation of LMW HA fragments in bleomycin-injured lungs *(16).* Additionally, recently it has been shown that there is increased accumulation of HA fragments and inflammation in CD44 null mice treated with

bleomycin *(69)*. These observations suggest that the CD44 receptor is critical for the clearance of HA fragments from sites of injury and that the lack of clearance leads to enhanced inflammation. Such findings suggest that, normally, HA is rapidly removed from the site of inflammation. When LMW HA concentrations increase due to dysregulated production and/or dysregulated clearance, these fragments can promote autoimmune disease. Along these lines, Wang et al. have shown that injecting mice with HA can promote experimental arthritis in mice *(70)*. Such mice display an increase in HA bound synovium infiltrating lymphocytes, particularly CD4+ T cells. Wang et al. propose that inappropriate levels of HA and other glycosaminoglycans can promote immunity and inhibit self-tolerance by changing the dynamics of the tissue microenvironment.

Based on the observation that HA is increased during inflammation, a number of trials have been performed to validate the use of circulating HA as a non-invasive marker of disease. For example, it has been shown that increased levels of serum HA in children complaining of joint pain could distinguish between JRA patients and patients without rheumatic diseases *(63)*. Furthermore, HMW HA has been utilized therapeutically as a means of decreasing inflammation and tissue destruction. In this regard, it has been shown that the intra-articular injection of HWM HA has some clinical efficacy in decreasing the inflammation in patients with RA *(71)*. Also, exogenous HA has been administered to try to improve respiratory function in patients with emphysema *(72)*. While the precise mechanism whereby HMW HA protects tissues is still unknown, we would propose that the biologically inert HMW HA in part acts by blocking the ability of LMW HA to activate the innate immune response. In addition, it may be that HMW HA has the ability to block TLR signaling.

The ability of LMW HA to promote inflammation makes it a potential therapeutic target in terms of treating autoimmune diseases. This ability might be potentially exploited in terms of vaccine development as well. The ability of HA to activate DCs, promote IL-12 production as well as the elaboration of numerous cytokines and chemokines suggests that LMW HA might act as a potent adjuvant.

A Model of LMW HA as a Danger Signal

In health, biologically inert HMW HA plays an important role in maintaining tissue integrity and water and solute homeostasis in the extracllelular space. Upon noxious insult, in the form of infection, ischemia, environmental exposure, for example, HMW HA is broken down into LMW species. This is facilitated by the increased production of HA, the generation of reactive oxygen species, and the elaboration of hylauronidases. The now biologically active LMW HA promotes the release of reactive oxygen species, cytokines, chemokines, and destructive enzymes by a variety of cells ranging from fibroblasts and epithelial cells to macrophages and dendritic cells. The LMW HA also facilitates the recruitment of CD44+ leukocytes. In this manner LMW HA acts to amplify the inflammatory response.

However, concomitant with the ability of LMW HA to enhance inflammation, HA can also directly activate DCs. Inasmuch as DCs play a critical role in the initiation of the acquired immune response, LMW HA thus has the ability to influence the decision between T cell tolerance and activation. HA induces DCs to mature and migrate to the lymph node. Furthermore, HA-stimulated DCs upregulate costimulatory molecules and are potent activators of T cells.

Janeway predicted and proved the role for PAMPS in activating the acquired immune

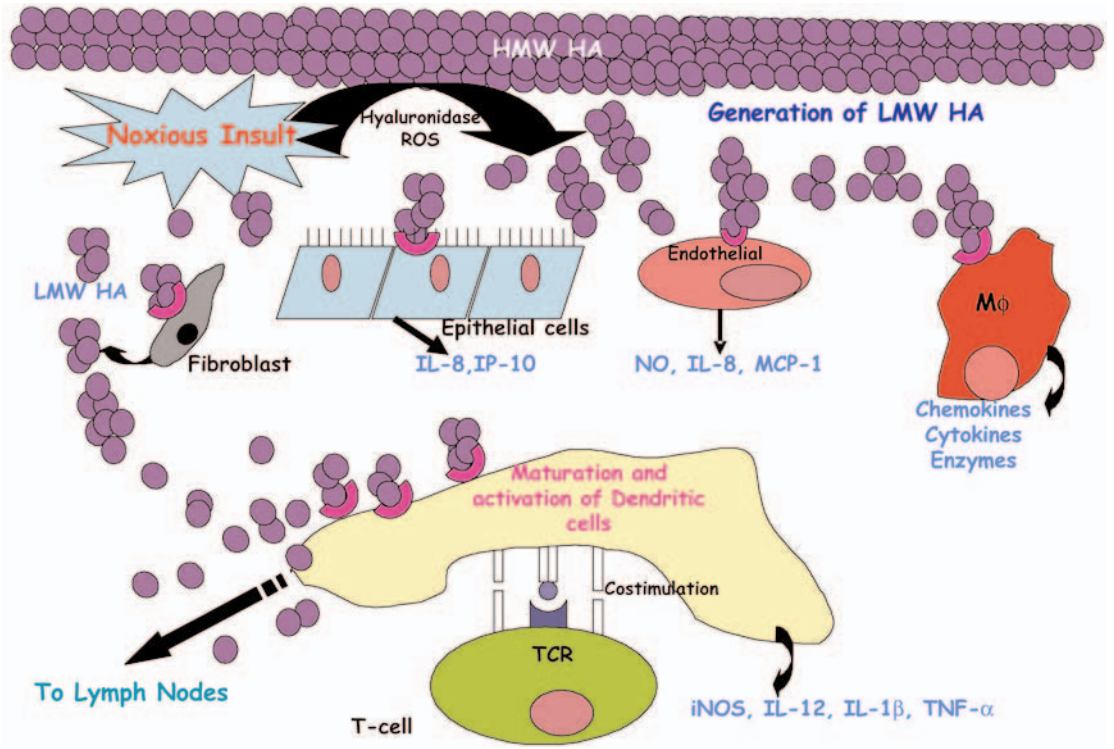

Fig. 1. HA as a danger signal: In health, high-molecular-weight (HMW) HA supports tissue integrity and water and solute homeostasis. Upon a noxious insult such as an infection, ischemia, or environmental toxin, the generation of reactive oxygen species and hyaluronidases act to break down HA into lower-molecular-weight (LMW) forms. The LMW HA activates epithelial cells, endothelial cells macrophages, and fibroblasts to secrete chemokines, cytokines, and degradative enzymes, all which serve to enhance inflammation. In addition, the LMW HA activates dendritic cells resulting in their maturation, upregulation of costimulatory molecules, and migration to the lymph nodes. The activated DCs in turn present antigen to T cells thus leading to a vigorous T cell response. Of note, LMW HA might also travel to the lymph nodes where it can also promote immunity. =HA, =HA receptor.

response. In this regard, it is of interest that, like most PAMPS, LMW HA activates DCs in an MYD88 dependent fashion (Table 2). Thus, we would propose that LMW HA acts as a self-derived danger signal. When the integrity of the ECM is breached and inflammation ensues, the generation of LMW HA activates DCs and hence the adaptive immune response. As shown in Fig. 1, this occurs *in situ;* however, the transport of LMW HA from the site of inflammation to draining lymph nodes by specific receptors (LYVE) suggests that HA may also act as a long-range alarm alerting cells of the lymph nodes to trouble in the tissues. As the insult is dealt with and inflammation resolves, the LMW HA is removed and new matrix is generated as the tissue heals. Alternatively, if LMW HA is inappropriately produced or inefficiently cleared, this persistence of danger may act to promote autoimmune disease or chronic inflammation leading to fibrosis.

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