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Hyaluronan in Autoimmunity and Immune Dysregulation

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Abstract

The tissue microenvironment is believed to contribute to the pathogenesis of autoimmune diseases - a diverse set of conditions associated with sterile inflammation and immune-mediated destruction of tissues. However, the specific factors that contribute to local immune dysregulation in autoimmunity are poorly understood. One particular tissue component that has been frequently implicated is hyaluronan (HA), an extracellular matrix (ECM) polymer. HA is abundant in settings of chronic inflammation and contributes to immune activation, migration, and fibrosis. Here, we first describe what is known about the size, amount, and distribution of HA at sites of autoimmunity, with particular emphasis on the autoimmune diseases type 1 diabetes, multiple sclerosis, primary sclerosing cholangitis, and rheumatoid arthritis. Next, we examine the recent literature on HA and its receptors in adaptive immunity, specifically in regards to effector and regulatory T-cell biology. Finally, we address potential tools and strategies for targeting HA and its receptor CD44 in chronic inflammation and autoimmunity.

Keywords

hyaluronan; 4-methylumbelliferone; antigen presentation; immune diseases

THE TISSUE MICROENVIRONMENT IN AUTOIMMUNITY

Autoimmune diseases are chronic inflammatory states characterized by local immune dysregulation and antigen-specific immunity. While there are suggestions that autoimmunity in some instances may initially be triggered by infections [1,2], most sites of autoimmunity are nonetheless sterile. Therefore, endogenous inflammatory factors may be particularly important in the propagation and progression of autoimmunity. Most current models to explain the pathogenesis of autoimmunity invoke genetic and environmental factors together with the inflammatory context within which cryptic self-antigens are encountered [3–6].

The tissue microenvironment – a broad collection of cellular and non-cellular components which together form a multifaceted regulatory milieu – is known to play decisive roles in local immunity. In cancer immunology, the local cytokine milieu, the tissue extracellular

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matrix (ECM), and the mechanical properties of tissues are all implicated in cancer progression [7]. These effects are mediated via inflammatory signaling pathways, including effects on NF- κ B signaling [8], and impacts on Foxp3+ regulatory T-cell (Treg) number and function [9] which together favor tumor survival and drive abortive activation of immune cells. The result is local immune dysregulation and tumor escape from the host immune system. Similarly, the tissue microenvironment has been implicated in immune homeostasis by tissue resident memory T-cells [10].

There is a growing appreciation that the tissue microenvironment may play analogous roles in the pathophysiology of several autoimmune diseases. Not only are most autoimmune diseases tissue-specific, but within affected tissues the patterns of immune destruction are typically heterogeneous. The relapsing-remitting nature of many autoimmune disease has likewise been suggested to reflect temporal changes in the inflammatory milieu [11]. There are clear indications that *in vivo* conditions influence local Treg number and function [12].

These and other observations have fueled increasing interest in the tissue microenvironment in autoimmunity. Moreover, it has become easier to obtain disease human issues. In particular, the Juvenile Diabetes Research Foundation (JDRF) National Pancreatic Organ Donor (nPOD) program has revolutionized the study of human insulinitis in Type 1 Diabetes (T1D). nPOD provides access to well-characterized cadaveric human tissues from T1D subjects and controls as well as extensive logistical support. This is a game-changing resource that opens an unprecedented window into the pathogenesis of T1D. Analogous efforts towards building tissue repositories are being undertaken in other autoimmune diseases.

The tissue ECM and its contributions to local immune regulation in particular have become the focus of great interest. Recent studies have implicated heparan sulfate, laminin and basement membrane structures in the pathogenesis of T1D [13–16]. Similarly, catabolism of the ECM is believed to contribute to the pathogenesis of rheumatoid arthritis through exposure of antigenic targets and priming of local immunity [17]. Further components of the ECM are implicated in multiple autoimmune conditions.

Here, we focus on one particular component of the ECM - hyaluronan (HA) - and its contributions to autoimmunity and immune regulation. First we address what is known about HA in tissues under autoimmune attack. Next, we discuss the ways in which HA is known to impact immune regulation and adaptive immunity. Finally, we close by discussing therapeutic strategies that target HA and its receptor CD44 and how these might be used to treat inflammatory diseases, with emphasis on autoimmunity.

HYALURONAN

HA is an ECM glycosaminoglycan (GAG), which has many roles in normal tissue function and development. This includes providing support and anchorage for cells, facilitating cell-cell signaling, and facilitating cell movement and migration [18–23]. HA interacts with a complex network of ECM molecules that together exert decisive effects on the physical and immunologic properties of inflamed tissues [24–27]. In light of its central role in this

network, we have suggested previously that HA is a “keystone molecule” in the inflammatory milieu [27].

HA synthesis increases substantially at sites of acute inflammation, trauma and infection [29]. HA increases local edema [28] and contributes to an inflammatory cascade that drives leukocyte migration, proliferation and differentiation through effects on gene expression, cytokine production, and cell survival. These pathways and the impact of HA production on innate immunity are the subject of several excellent reviews [30].

HA is also present at sites of chronic inflammation. HA surrounds tumors [31], is increased at sites of chronic infection [32–34] and is abundant in chronic inflammatory diseases of diverse etiologies [23,35]. At these sites, HA undoubtedly impacts innate immunity. However, adaptive (antigen-specific) immune responses, which take time to develop, often make more central contributions to chronic disease processes. These include humoral (B-cell) and cellular (T-cell) immune responses against cancer and infection. In contrast to HA effects on innate immunity, the contributions of HA to adaptive immune responses is less well understood.

HA and Hyaladherins

The longevity and size of HA are determined in part by interactions with HA-binding proteins, called hyaladherins that protect HA from catabolism and turnover. These include inter- α -inhibitor (I α I) and TNF-stimulated gene-6 (TSG-6) [36]. Hyaladherins are thought to interact with HA by promoting the formation of macromolecular complexes that modulate leukocyte adhesion and activation, and thereby influence the inflammatory response [28,29,37]. Cross-linked HA ECM is capable of interacting with cell surface proteins, proteases, chemokines, and growth factors to impact processes involved in promotion of leukocyte adhesion, migration, activation, and retention.

The hyaladherin TSG-6 is reported to have immunomodulatory properties [37]. Under inflammatory conditions, covalent transfer of heavy chains (HCs) from I α I to HA catalyzed by the protein product of TSG-6, forms the HC-HA complex, a form of HA that promotes the adhesion of leukocytes to HA-rich matrices [37–39].

Activated macrophages also secrete HA and hyaladherins, such as TSG-6 and versican [40,41]. The resulting HA–hyaladherin macromolecular complexes interact with a variety of cell-surface proteins, growth factors, chemokines, and proteases to modulate the adhesive properties and activation state of inflammatory cells [37–39]. The association of HA with serum-derived HA-associated protein (SHAP) occurs on the surface of inflamed endothelial cells during inflammation and supports leukocyte adhesion and rolling [42].

HA size

Along with its binding partners, the immunologic impact of HA is also influenced by its size. When initially synthesized, HA is predominantly high molecular weight hyaluronan (HMW-HA) polymers of between 2×10^5 to 2×10^6 Da [24]. This is subsequently broken down into shorter, HA polymers in a context-dependent manner. Inflamed tissues are associated with catabolic factors, including endogenous hyaluronidases, mechanical forces,

and oxidative stress [24], that degrade intact HA into a continuum of different-sized HA polymers, including low molecular weight HA (LMW-HA)(<120 kDa) and, ultimately, HA oligomers. As a result, longer polymers of HMW-HA typically predominate in most tissues under steady-state conditions while, shorter, LMW-HA polymers predominate at sites of active inflammation [43]. In light of these associations, HA size has been called a natural biosensor for the state of tissue integrity [44,45].

These changes in the size of HA are proposed to have functional consequences because of the differential interactions between HA polymers of different sizes and particular receptors.

HMW-HA, which predominates in healthy tissues, typically inhibits inflammation [30,31]. Consistent with this, administration of HMW-HA is anti-inflammatory in lung injury models [35,46,47], collagen-induced arthritis [24, 43,48,49], and a variety of other *in vivo* model systems [23,35,50,52].

LMW-HA, generated at sites of active tissue catabolism, is reported to promote the activation and maturation of dendritic cells (DC) [44], drive the release of pro-inflammatory cytokines such as IL-1 β , TNF-alpha, IL-6 and IL-12 by multiple cell types [50,53], drive chemokine expression and cell trafficking [54], and promote proliferation [55] and angiogenesis [37].

However, the pro-inflammatory nature of LMW-HA has recently been called into question. It was recently reported that endotoxin-free LMW-HA is non-inflammatory and that many of the aforementioned findings attributed to LMW-HA may reflect bacterial hyaluronidases (HA'ses) used to generate smaller fragments. While these findings await further validation, they call into question the role of HA fragments as a DAMP and suggest that our models for how LMW-HA impacts local inflammation may need to be revisited and potentially revised.

Smaller HA fragments, generated as a result of further HA catabolism, promote angiogenesis and regenerative tissue responses. These effects are attributed to signaling through the HA receptor RHAMM [56]. In addition, there are a number of other receptors reported to bind HA, including layilin, ICAM-1, and LYVE-1, but the size of HA that these receptors bind and their functions vis-à-vis adaptive immunity are poorly understood.

HA in Inflamed Tissues

HA can be organized into a variety of molecular architectures by forming cross-linked complexes with the above mentioned proteins, and can serve as ligands for leukocytes. Such interactions may trap the leukocytes and prevent eventual destruction of the tissue, as well as trap pro-inflammatory mediators [37]. These ECM molecules may initiate a cascade of events that promote inflammation by attracting inflammatory cells and promoting their activation [12].

In response to inflammatory mediators, tissue-resident cells generate a pro-inflammatory, HA-rich ECM. As a result, HA levels are greatly elevated in injured tissues, with HA synthase production increasing by as much as 80-fold [57]. Increases in HA are associated with chronic disease processes with unremitting inflammation, such as type 2 diabetes

(T2D) [58,59], liver cirrhosis, and asthma [60–62]. This accumulation of HA is part of a larger pattern of ECM deposition associated with persistent inflammation.

The result of this increased HA at sites of inflammation is the influx of inflammatory cells [63,64]. This is driven in part by HA effects on tissue water content and vascular permeability [65]. We recently reported that increased HA within islets under autoimmune attack also alters the mechanical properties of these tissues, making them softer and potentially changing the behavior of mechanoreceptive cell types as a consequence (Nagy et al., JBC in press). Fragments of HA also serve as chemoattractant that lead to the accumulation of leukocytes at sites of inflammation [66,67].

HA IN AUTOIMMUNITY

Autoimmunity is associated with tissue-specific, chronic inflammation. Autoimmune diseases arise when the immune system actively targets self-tissues for destruction. The pathogenesis of autoimmune diseases requires both the development of effector B- and/or T-cells reactive against self-antigens as well as the failure of regulatory mechanisms that normally reign these responses in. Autoimmunity can arise in nearly any tissue but among the most commonly are the thyroid (Graves' Disease), pancreatic islets (Type 1 Diabetes), central nervous system (multiple sclerosis) and joints (Rheumatoid Arthritis).

HA has been implicated in multiple autoimmune diseases including rheumatoid arthritis [68], lupus [69], Sjögren's syndrome [70], Hashimoto's thyroiditis [71]. However, the tissue ECM and HA in particular are perhaps best characterized in two autoimmune diseases, T1D and MS.

HA in Type 1 Diabetes

Type 1 Diabetes (T1D) is an autoimmune disease of the pancreatic β -cells. 30,000 Americans will develop T1D this year, most of them children and adolescents [72–74]. T1D is characterized by progressive, immune cell-mediated destruction of pancreatic β -cells [75,76] and the failure of regulatory mechanisms that normally prevent destructive insulinitis [6,77]. As a result, individuals with T1D lose the ability to produce insulin and require injections of exogenous insulin to survive. Many individuals with T1D go on to suffer complications of diabetes, including limb amputation, blindness, and kidney failure [6]. The local tissue environment is thought to contribute to immune regulation and the development of T1D [78–80], but precipitating factors are unclear.

In healthy islets, HA is a normal component of the peri-islet and intra-islet regions adjacent to microvessels [81] (Fig. 1A). Hyaladherins, including TSG-6 and Ia.I are likewise components of healthy, non-diabetic human and murine islets [13,25]. This ECM may support normal β -cell function and health, as suggested by a study using immortalized β -cells [82].

T1D is associated with substantial changes in the islet ECM and deposition of intra-islet HA. Early on during the disease process, there is breakdown in the peri-islet ECM in both human T1D as well as in mouse models of the disease [13,15,83,84]. There is also islet-specific

deposition of HA. Using human T1D tissue samples from cadaveric organ donors obtained through the JDRF nPOD program, we discovered that HA deposits were present in islets from recent-onset T1D donors but not in non-diabetic controls [24]. Further, we observed that HA deposits are both temporally and anatomically associated with autoimmune insulinitis in both T1D and in the DORMO mouse model of the disease [24,43]. Both the amount and distribution of HA closely tracked with the infiltration of CD3⁺ T-cells and the disappearance of insulin staining. HA was not increased within neighboring islets without active insulinitis [43]. These T1D-associated HA deposits were also associated with local alternations in hyaladherins, including reduced levels of intra-islet TSG-6 and IaI and increases in mRNA of a pro-inflammatory hyaladherin, versican [26]. We similarly observed decreases in the hyaladherins TSG6 and IaI during the progression to T1D in animal models of autoimmune diabetes, including DORMO and non-obese diabetic (NOD) mice [27].

HA accumulation in T1D is not limited to islets. HA accumulation also occurs in lymph nodes and spleens in both human and mouse models of T1D [24]. In normal human pancreatic lymph node and spleen tissues, sparse HA staining is seen in B-cell follicular germinal centers and in reticular networks along niches of immune cells in the T-cell compartment [48]. In T1D, prominent changes in the HA-rich ECM occur in specific regions of B- and T-cell activation. Specifically, HA is abundant in the B-cell follicular germinal centers in T1D, which show an intense punctate HA staining that is distributed over the germinal center areas. Prominent accumulations of HA also occur in the T-cell compartment, where HA deposits form large, amorphous aggregates along the reticular network, which appear enlarged and thickened [48]. The finding of accumulation of HA in specific regions of B- and T-cell activation in pancreatic lymph node and spleen in T1D expands the possibilities of HA involvement in T1D pathogenesis beyond that of insulinitis [48].

Interventions that promote the integrity of HA can influence the incidence of autoimmune diabetes in animal models. TSG-6 administration to NOD mice enhances the generation of regulatory T cells, inhibits the activation of both antigen-presenting cells and T cells, and prevents the progression of destructive insulinitis [85]. In addition, a resistance to diabetes is induced in the NOD mouse by the systemic blockade of the HA receptor, CD44, or after administration of the HA-degrading enzyme, hyaluronidase [86].

To further define the contributions of HA to insulinitis, we treated animal models of T1D with 4-methylumbelliferone (4-MU), a pharmacologic inhibitor of HA synthesis [87]. We found that 4-MU prevented diabetes and preserved insulin content within islets in DORMO mice, despite ongoing, robust lymphocytic infiltrates [43]. These effects were stable while mice were on continuous treatment for over a year. This treatment was initiated when the mice were 8 weeks of age, at which time insulinitis is typically well established in DORMO mice [43]. This is a strong, protective phenotype in a model in which the incidence of autoimmune diabetes is typically 100% [88]. Furthermore, a short 1-week course of 4-MU treatment was sufficient to prevent diabetes progression in the canonical mouse model of T1D, the NOD mouse [43]. We conclude from these data that HA production is necessary for destructive insulinitis and that 4-MU treatment can forestall progression to diabetes in multiple mouse models of T1D.

HA in Multiple Sclerosis

Another autoimmune disease associated with increased HA at the site of immune attack is MS. MS, an autoimmune disease of the central nervous system (CNS), characterized by infiltration of inflammatory cells into the CNS and localized destruction of myelin, the insulating layer surrounding axons [89–91]. MS affects approximately 400k individuals in the US [92] and the disease has a major impact on their quality of life and longevity [93].

The pathology of MS is characterized by loss of oligodendrocytes, the myelin producing cells in the CNS, and localized destruction of myelin, accompanied by axonal degeneration and infiltration of inflammatory cells into the CNS [89–91]. In most patients, the disease starts with a course that is characterized by episodes of neurological symptoms followed by full or partial recovery (relapsing-remitting MS, RR-MS). The pathogenesis of this stage is mainly associated with an active immune response, which includes activation of microglia (CNS resident macrophages), infiltration of lymphocyte and myeloid cells into the CNS, and phagocytosis of myelin by microglia-derived and infiltrating macrophages. However, over a variable period of time, the disease develops into a progressive form, with neurological deterioration progressing at a consistent rate (secondary progressive MS, SP-MS). Features of this stage include mainly neuronal and axonal degeneration, cortical demyelination and cognitive impairment [90].

Along with infiltrating macrophages and lymphocytes, astrocytes have been implicated in the pathogenesis of MS [90]. The most abundant cell type in the brain, astrocytes perform an array of physiological functions, including maintenance of CNS homeostasis, regulation of blood-brain barrier integrity, modulation of blood flow, and support of synaptic transmission [94]. In addition, upon activation by danger signals, astrocytes actively participate in the CNS innate immune response and are the main source of inflammatory mediators, including several complement components, cytokines and chemokines [95,96,97].

HA deposition is associated with the development of MS. In the healthy CNS, low levels of HA are produced by astrocytes, the main non-neuronal cell type. This HA is deposited as ECM complexes in the spaces between myelinated axons and between myelin sheaths and astrocyte processes [98] (Fig. 1B). In fact, HA is one of the predominating components of the ECM in the CNS [99]. However, upon injury or inflammation, levels of HA increase in damaged areas and are associated with the reactive response of astrocytes [100,101]. HA is highly abundant in demyelinated lesions in MS and in its animal model, experimental autoimmune encephalomyelitis (EAE) [98,101–103]. In early EAE lesions, accumulation of HA is thought to be due to production of HA by activated CD4⁺ T-cells and microglia [102]. In later stages of CNS lesions, though, HA production is thought to be by reactive astrocytes [102]. Higher turnover of HA, due to increased expression of hyaluronidases, is thought to result in preferential accumulation of LMW breakdown products of HA in MS lesions [104]. These LMW fragments might provide a feed-forward loop to astrocytes, which have been shown to respond to LMW-HA by proliferation, sustaining their reactive response [101]. The HA in MS lesions has been implicated in promoting T-cell, microglial and macrophage activation [105,106]. In addition HA has been implicated in supporting the local immune response leading to the destruction of myelin by facilitating entry of inflammatory cells into

the CNS parenchyma [107]. Moreover, HA is also known to contribute to EAE by inhibiting oligodendrocyte maturation and preventing repair of damaged myelin [102,108].

In keeping with a pathologic role for HA in autoimmune demyelination, we and others have recently shown that inhibition of HA production by 4-MU treatment potently reduces disease severity and incidence in EAE [107,109]. We demonstrated that this therapeutic effect is not only a result of the polarization of the T-cell response away from a pathogenic Th1 response, but also the reduction of infiltration of these cells into the CNS. In addition, we found that 4-MU treatment reduces astrogliosis *in vitro* and *in vivo*, at sites which were associated with infiltration of inflammatory cells [107].

Similar to HA, CD44 is expressed at low levels in the healthy CNS, mostly by astrocytes, and is induced upon injury, being strongly expressed by reactive astrocytes [110]. In chronic MS lesions, high expression of CD44 has been shown to be exclusively found on reactive astrocytes [102]. Although the sparse lymphocytes found in chronic MS lesions do not express CD44 [102], its expression is significantly upregulated on circulating T cells in MS patients experiencing a relapse [111]. Similarly, CD44 expressing lymphocytes are present in early EAE lesions, whereas CD44 is upregulated on astrocytes at later lesions stages [102]. As mentioned above, 4-MU treatment inhibits leukocyte trafficking to inflamed CNS tissue [107]. This is in line with observations that CD44 contributes to lymphocyte adhesion and diapedesis through the blood-brain barrier [112,113]. Reports on the implications of CD44 deficiency for neuroinflammation in EAE are conflicting, however. While two reports demonstrate that CD44 deletion ameliorates EAE and there are indications that CD44 is particularly important for the entry of pathogenic T cells into the CNS parenchyma [114,115], another report shows that CD44 deficiency worsens EAE [116]. These discrepancies could be accounted for by differences in genetic background and differential residual expression of CD44 variant isoforms in the animals used. In addition to studies using genetic models, it has been shown, though, that blocking CD44 using a monoclonal antibody prevents EAE by reducing T cell infiltration into the CNS [113], demonstrating the importance of CD44 for this process.

Additionally, considering that reactive astrocytes both express CD44 and produce high levels of HA, this HA might provide a feed-forward loop, sustaining the reactive astrogliosis response in MS lesions [117,118]. Through their production of HA, reactive astrocytes as such might play a role in the infiltration of pathogenic T cells into the CNS. This is in line with recent studies that illustrate a role for astrocyte activation in mediating MS and EAE progression and the recruitment of T cells to the CNS in EAE [97,119].

With regard to specific CD44 isoforms, one report has demonstrated that v3, v7 and v10 are elevated in MS lesions, whereas v4, v5, v6 and v9 are not detectable in either healthy or MS brain tissue [120]. CD44-v3 is found sporadically on infiltrating lymphocytes in early lesions, but mostly by phagocytic macrophages in chronic lesions. CD44-v7 is expressed predominantly by endothelial cells and few perivascular lymphocytes. CD44-v10 is expressed primarily by astrocytes in early MS lesions, as well as endothelial cells and perivascular lymphocytes, and phagocytic macrophages in chronic lesions. It was further shown that v7 and v10 expression on both T cells and antigen presenting cells impacts

disease in EAE, being required for the migration of pathogenic T cells, as well as regulatory T cells, into the CNS [120].

Interestingly, CD44 is expressed by oligodendrocytes and their progenitors (OPC) [102,121] and it has been shown that the receptor is required for migration of OPC to demyelinating lesions [121]. In addition, in a potential therapeutic setting, it has been shown that CD44 expression is required for the migration of systemically administered neural progenitor cells, which can give rise to neurons, oligodendrocytes and astrocytes, across the CNS endothelium and their invasion of the CNS parenchyma [122,123]. Although contributing to inflammation, HA deposits might therefore also provide a trigger for the initiation of regenerative mechanisms in demyelination. On the other hand, however, as discussed above, there are indications that the abundance of HA in chronic MS lesions impairs the remyelinating capacity of OPC, preventing efficient repair of myelin [102,108].

HA in Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints, and is associated with increased leukocyte infiltration into the synovial tissue [124]. This robust immune response often results in damage to the bones and cartilage of the affected joints. RA has a 0.24% global prevalence, ranking it as the 42nd highest contributor to global disability, severely affecting the mobility and livelihood of those affected [125].

RA is characterized by leukocyte infiltration into the synovial compartment and subsequent hypertrophy of the synovial lining as well as activation of bone-remodeling osteoclasts [124]. Due to a combination of prearthritic factors such as genetic components and erroneous post-translational protein modification, the immune system loses tolerance to self, causing innate and adaptive immune cells to infiltrate and attack the synovial region of the joint. Initial stages are characterized by the activation of antigen-presenting cells and fibroblast-like synoviocytes, the incorrect modification of proteins post-translation, and an increase in serum autoantibodies; this is thought to be due to the activation of B-cells in response to citrullinated self-proteins [124]. As the disease progresses, however, B- and T-cells begin to attack the joints, the synovial lining is activated, and leukocytes infiltrate the synovial compartment of the joint. This infiltrate is composed of macrophages, T-cells (mostly CD4+, with some CD8+), B-cells, and neutrophils. Data suggests that by blocking TNF α , which is known to stimulate osteoclast precursors to move from the bone marrow into the periphery and facilitate the differentiation into mature, bone-resorbing cells, disease-activity assessments are improved [126,127].

HA deposits in the synovial compartment have been correlated with RA. Hayes et al. found 3.5 times more CD44 expression in synovial tissue from RA patients than from osteoarthritis patients and 10.7 times more than in synovial tissue from patients with joint trauma but no chronic arthritis. These studies indicate that CD44 is up-regulated in the synovial cells of RA patients and that the level of CD44 expressed in synovial tissue is correlated to the severity of inflammation [128]. Additionally, in a normal synovial compartment, HA is located diffusely in loose connective tissue of the synovial villus and in the blood vessel-associated perivascular connective tissue. During the tissue destruction associated with RA, however, further increased amounts of free hyaluronic acid gather in the synovial fluid of the

joint [129]. In RA patients, complexes of serum-derived hyaluronan-associated proteins (the heavy chains of inter- α -trypsin inhibitor family molecules) and HA have been shown to accumulate in the inflamed synovial region, correlating to the progression of the disease [129,130]. These deposits of HMW-HA are associated with the connective tissue next to the synovial lining and connective tissue in regions with neo- and hypervascularization; the deposits of HA were located in the same regions as in the synovium of a normal joint, simply with significantly increased amounts [129]. In type B synoviocytes isolated from RA patients, researchers have noted the up-regulation of HAS1 in response to IL β (through inducing the translocation of the NF- κ B into the nucleus) and TGF- β . In healthy patients, however, IL β had no effect on the regulation of HAS1 [131]. The stimulation of these type B synoviocytes in RA patients likely factors into the accumulation of HA in the synovium, but it is still unknown what exactly about HAS1 regulates the inflammatory response [131].

High molecular weight (HMW) HA has been shown to provide physical lubrication for slow joint movement, and be physiologically protective, suppressing fibronectin fragment-mediated cartilage destruction. In a T-cell mediated injury model, HMW HA seems to stimulate anti-inflammatory effects that protect liver cells through the reduction of pro-inflammatory cytokines tumor necrosis factor-alpha, interferon gamma, macrophage inflammatory protein 2, and interleukin 4, an effect not seen in treatment with LMW HA [132]. HMW HA polymers in RA patients depolymerize, generating these HA fragments that have instead been suggested to signal through TLR4, TLR2, and CD44 to promote inflammatory responses from inflammatory cells [130,133]. Both molecular weight distribution and concentration of hyaluronan were noted to be lower in RA patient synovial fluid, supporting this data on HA breakdown (HMW-HA to LMW-HA) [134,135]. This may explain part of the pathway by which HA plays a role in the development of autoimmunity in RA patients.

Increased CD44 expression was found in adjuvant-induced arthritic rats on the synovial lymphocytes, macrophages, and lining cells during different developmental stages, and the synovial fluid T lymphocytes of rheumatoid human patients (compared to the peripheral blood lymphocytes from the same individuals), suggesting a role for this receptor in RA inflammation [136,137]. Due to studies such as these, there have been numerous efforts in RA treatment via targeting of the HA receptor. Hutas et al. showed that anti-CD44 antibody treatments in a murine rheumatoid arthritis model prompt platelet deposition on granulocytes, decreasing the number of circulating granulocytes. When mice are treated with anticoagulants, though, it decreased this depletion of granulocytes and the effect of anti-CD44 antibody on relieving swelling in the joint. This suggests that the anti-inflammatory effect of this antibody may stem from its ability to affect the quantity of granulocytes in circulation [138]. Sarraj et al. found that mice with CD44 and CD62L knockouts had leukocytes that adhered less firmly to the endothelium of the synovial vessel walls of mice with induced rheumatoid arthritis, a decrease in adhesion correlated with the movement of granulocytes into the synovium and the degree of inflammation [139]. Nedvetzki et al. found that collagen-immunized mice injected with anti-CD44 mAbs had less arthritic symptoms (paw swelling and clinical scores) and an inflammatory response that subsided within 2 days of its initiation [140]. Upon further study, they noted that the anti-CD44 mAbs interfered with arthritogenic splenocyte activity, mitigating development of arthritis; without co-

injection of anti-CD44 mAbs (only PBS), injection of splenocytes from arthritic mice into SCID mice caused paw swelling and limb inflammation, with 90% of the mice displaying proximal interphalangeal joint erosion after 10 days as compared to the 10% in mice injected with anti-CD44 mAbs [140]. In exploring the effects of the KM81 anti-CD44 mAb, the researchers found that its Fab' fragments have an inhibitory effect on collagen-induced arthritis, implying that the antibody does not regulate CD44 cell surface expression or Fc-dependent pathways and instead inhibits the function of CD44, prompting insight on the mechanism of anti-CD44 mAbs [140].

In treating this disease, researchers have found that HA injections have shown beneficial effect. Exogenous HA can stimulate the production of endogenous HA and regulate the growth and function of chondrocytes through cross-linking their CD44 receptors. Watanabe et al. found that HA suppresses LPS-induced RANKL expression, whose pathogenic expression stimulates the persistence of bone-resorbing osteoclasts in human rheumatoid arthritis synovial fibroblasts [141]. Through clinical trials in humans, a significant improvement in symptoms was found from week 5 to week 9 after injection of HA in humans [142].

HA in Thyroiditis

Autoimmune thyroiditis describes diseases associated with a direct immune response towards self-thyroid tissue. A range of autoantibodies are formed towards thyroid epitopes such as TSHr (thyrotropin receptor), thyroid peroxidase, and thyroglobulin [143,144]. Due to the evidence that HA contributes to activation of the adaptive response in other autoimmune disorders, it is likely that there is a similar effect here. It has been shown specifically, however, that blocking of CD44 on autoreactive T-cells in experimental autoimmune thyroiditis (EAT) does not prevent their extravasation into thyroid tissue, which directly contradicts similarly seen effects in T1D, EAE, and CIA [145]. Studies into whether or not HA participates in the induction of EAT have not, as of this point, been heavily pursued.

HA appears to be strongly associated with the sequelae of adaptive immune activation in autoimmune thyroiditis. In humans, activating antibodies targeted toward thyroid tissue that form during autoimmune thyroiditis can lead to rapid local deposition of HA, leading to an enlarging of the thyroid (goiter). It is believed that both thyrocytes themselves as well as fibroblasts contribute to this deposition [146]. The deposition has been associated with a direct activation of the TSHr by activating antibodies, leading to HA deposition mediated by multiple HAS isoforms [147,148].

Furthermore, the activating antibodies that induce HA deposition in the thyroid can also bind to similar receptors expressed on fibroblasts in the ocular cavity and induce HA deposition [149]. This leads to expansion of the tissue which physically pushes on the eyes until they protrude, a condition called exophthalmos. Synergistic effects of FGF have been seen to induce HA deposition alongside cytokine production during Grave's Disease, again suggesting its importance in thyroiditis associated orbitopathy [150]. Together, these studies suggest that HA may contribute both to induction as well as pathogenicity of autoimmune thyroiditis an associated orbitopathy.

HA in Primary Sclerosing Cholangitis

Primary Sclerosing Cholangitis (PSC) is a chronic inflammatory disease of the biliary ducts in the liver resulting in destruction and fibrosis [151,152]. Disease progression, although variable, is almost universal in all patients, resulting in a loss of intrahepatic bile ducts, cirrhosis and eventually liver failure [153,154]. PSC affects about 7 per 100,000 people in the US population with approximately 6,000 receiving liver transplants for this condition over the past 20 years (UNOS data) and 20% developing cholangiocarcinoma (cancer in the bile ducts) [155]. There is increasing evidence that immune mechanisms play an essential role in the pathogenesis of PSC [156]. The disease is often associated with the presence of autoantibodies and occurs in concert with other autoimmune diseases, particularly ulcerative colitis [157]. However, many trials of pharmacological agents have failed to demonstrate a beneficial effect in treating this condition, including immunosuppressive regimens [154,158–160]. Currently, there are no approved treatment options for PSC which alter disease progression.

PSC is associated with increased serum levels of HA [161]. Serum HA levels have been found to perform as well as other predictive scoring systems for PSC [162,163]. Conversely, inhibition of HA synthesis has been shown to prevent progression of autoimmunity in several animal models of PSC. The agent used in the aforementioned studies to inhibit HA synthesis and prevent autoimmune disease is 4-methylumbelliferone (hymecromone).

HA as Biomarker

Though other autoimmune diseases have not necessarily had HA tied to pathogenesis, its prevalent use as biomarker in sterile inflammation suggests its importance. Inflammatory bowel disease has been associated with increased luminal HA and its binding proteins, where HA fragments have been thought to promote local inflammation [164–166]. Further implications have been seen for HA as a biomarker of autoimmune inflammation, such as in autoimmune hepatitis [167] and scleroderma [168,169]. Though a definitive link of HA to the pathogenesis of these diseases has not been identified, the presence of HA and its binding proteins are strong indicators of disease. Based on the pathogenic activity of HA found in other autoimmune diseases, its activity is likely shared in these as well.

HA AND IMMUNE CELLS

The primary effector cells that mediate autoimmunity in T1D, MS, and other autoimmune diseases are lymphocytes, in particular T-cells [5,170]. These cells are involved in immune responses that result in death of self-tissues targeted in autoimmunity (Fig. 2,3), including insulin-producing β -cells and myelin producing Schwann cells. However, many autoimmune diseases are also associated with humoral immune responses that are thought to contribute, through poorly defined mechanisms, to the incidence of many different autoimmune diseases [171]. Indeed, the presence of auto-antibodies against particular self-antigens can be used to predict the development of several autoimmune diseases, including T1D [6].

In the following sections, we will review what is known about the interactions between HA and lymphocytes that contribute to adaptive immune responses and the incidence of autoimmunity.

HA, B-cells, and humoral immunity

HA influence on B-cell activity is supported by the fact that B-cells express CD44, as well as observations that activated, but not un-activated, B-cells directly bind HA [172]. LMW-HA, through interactions with CD44, promotes B-cell chemotaxis and cytokine expression in an asthma model [173]. CD44 costimulation on B-cells is reported to play a role in maturation and subsequent antibody production [174]. Furthermore, B-cells and B-cell-derived tumors also express RHAMM [175,176] and ICAM-1 [177] but the functional role of these receptors on healthy B-cells is undefined. We recently reported that HA deposits are present in lymph node germinal centers in both humans and mice with autoimmune diabetes but again, the functional contribution of this finding is unknown. In general, the contribution of HA and its receptors to humoral immunity is poorly characterized and is a topic ripe for investigation.

HA, T-cells, and cellular immunity

There has been extensive study of HA in T-cell biology and cellular immunity. Activated T-cells upregulate CD44 surface expression [178] and this, through interactions with HA, has been shown to influence T cells in a state dependent manner, allowing fine tuning of lymphocyte function and phenotype. HA thereby influences T-cell survival, activation, polarization and expansion.

CD44 interactions with HA influence cell survival. Unlike in tumor cells, where Yasuda et al., reported that CD44 stimulation down regulates Fas expression and Fas-mediated apoptosis [179], HA effects on CD44-transfected T-cell lines was Fas independent [180].

HA and CD44 binding can potentiate activation of T-cells [181]. This is believed to be mediated via p56(lck) activation and phosphorylation of ZAP-70, in a manner where CD44 acts in complex with CD3 and CD4 [182–184]. The costimulatory effect of CD44 ligation in these studies is most pronounced at subthreshold levels of TCR or BCR stimulation [185]. It has been suggested that CD44 facilitates TCR complex capping [186] and thereby brings protein kinases into apposition to promote phosphorylation and downstream signaling events [184]. CD44 is reported to interact with the TCR-complex tyrosine kinases Lck and Fyn in this manner [187,188]. CD44 has been implicated in signaling cascades that are downstream of either TCR or BCR signaling, including the PI3K/AKT, MAPK/ERK, Ras, and BMP pathways [189,190].

Another way that HA contributes to activation of B- and T-cells is through effects on antigen presentation. LMW-HA believed to promote the maturation of dendritic cells (DC) through effects on TLR signaling, thereby making DC more efficient at antigen presentation [191,192]. HA localizes to the immune synapse [193,53] and promotes cell-cell interactions [35]. HA accumulation in these specific immune cell regions modulates antigen presentation and enhances immune cell proliferation, migration, and adhesion [194], which then results in potentiation of downstream immune responses.

In addition to potentiating the strength of antigen-specific signals, CD44 signaling can exert qualitative effects on T-cell function and phenotypes. Studies with CD44^{-/-} mice indicate that CD44 costimulation promotes differentiation towards the TH1/TH17 lymphocyte subsets and away from TH2 helper T-cell responses [195,196]. CD44 has been implicated in the persistence of immune memory of Th1 cells but not Th2 [197].

HA has been shown to play a role in lymphocyte homing, by effecting recruitment at the endothelium, extravasation, and motility [198]. Expression of CD44 drives homing of immune cells via binding to selectins present on the walls of blood vessels [199]. CD44-mediated stimulation induces pyk2 and lck mediated actin polymerization used in cell motility [198].

HA and Regulatory T-cells

In healthy individuals, autoimmunity is suppressed by populations of regulatory T cells, including Treg [200]. These are CD4⁺ T cells that express the transcription factor FoxP3⁺ and regulate the behavior of effector T cells and other leukocytes. The absence or depletion of Treg leads to multi-systemic autoimmunity in mice and humans [77,201–203] whereas their adoptive transfer can resolve or prevent autoimmunity in animal models [88,204]. The ratio of activated effector T-cells relative to Treg is thought to be a central determinant in the loss or maintenance of immune tolerance at sites of inflammation [205].

Despite the evidence implicating Treg in autoimmunity, intrinsic defects in Treg number or function has proved elusive in human autoimmune diseases, including MS and T1D. Many studies have also reported normal Treg numbers in the peripheral circulation of individuals with these diseases [12,206,207]. Similarly, the *ex vivo* function of Treg is often comparable to normal controls [208–212].

One potential explanation is that autoimmunity is associated with impaired Treg function *in vivo* but not *in vitro*. Indeed, Treg are present in pancreatic islets and CSF fluid of individuals with T1D and MS [88,213] but these evidently do not suppress inflammation *in situ* [88,214,215]. Similarly, expanded Treg in mouse models of diabetes have defects *in vivo* but not *in vitro* [88,216]. Moreover, there is evidence implicating impaired Treg stability *in vivo* in both T1D [217] and MS [208,218].

We and others have reported roles for HMW-HA in supporting Treg homeostasis. Mizrahy and colleagues were the first to demonstrate an association between CD44 and Foxp3 expression [219]. We subsequently reported that HMW-HA enhanced the function and viability of Tregs [53,220,221]. Subsequently, we and others reported that HMW-HA promotes Treg functionality via increased survival. Foxp3 expression and production of IL-10 [50,221–223]. Along similar lines, HMW-HA, in the context of a TCR signal, induces conventional T-cells to produce IL-10 and behave like type 1 regulatory cells (TR1) [50,222].

We subsequently reported that 4-methylumbelliferone (4-MU), an inhibitor of HA synthesis, promoted Treg numbers. 4-MU treatment of mice following immunization was associated with greater FoxP3⁺ Treg and reduced Th1. Consistent with this, oral 4-MU treatment halted

autoimmunity in mouse models of Th1-polarized autoimmune diseases including T1D [43], MS [107], and RA [33]. Conversely, we do not find that 4-MU treatment is of particular benefit in models of diseases dominated by Th2 cells, including murine models of asthma (unpublished data).

The two strands of data seem to contradict the previous findings. How can HA both inhibit Treg expansion and promote Treg homeostasis? One way to reconcile this contradiction is to see them as relevant to different phases of an immune response. HA may inhibit the induction of Treg at sites of active inflammation associated with antigen presentation but promote Treg phenotypic stability and survival in the absence of antigenic priming. This would be perhaps consistent with a need to promote immune suppression within uninjured or healing tissues but not necessarily have increased lymphocyte numbers. Mechanistically, the discriminatory factor between these paths could be the presence or absence of TCR signals; HA in the presence of antigenic signals might promote T-cell activation and expansion while preventing Treg induction while HA in the absence of TCR activation does not promote activation but instead promotes homeostasis.

Other innate immune signals have similar activation-specific effects on Treg expansion and function. TLR agonists like LPS are reported to promote Treg expansion but impair Treg function [224–226]. In addition, the effect of HA on Treg might depend on the balance of HMW and LMW polymers at sites of inflammation. High turnover of LMW-HA fragments at sites of inflammation, in conjunction with inhibition of de-novo synthesis of HA by 4-MU might switch the balance towards an HMW-HA rich environment, favoring homeostasis and function of Treg.

TARGETING HA THERAPEUTICALLY

Given the substantial evidence linking HA to the progression of autoimmune disease in animal models, there has been substantial interest in targeting HA production and signaling therapeutically (Table 1).

4-methylumbelliferone

Since more and more studies highlight the role of HA in inflammation, autoimmunity, and cancer, there has been great interest in identifying pharmacologic tools to inhibit HA synthesis. A strategy to prevent the proinflammatory activity of LMW-HA is to limit HA synthesis using 4-MU. 4-MU functions as a competitive substrate for UDP-glucuronyltransferase (UGT), an enzyme involved in HA synthesis [227]. HA is produced from the precursors UDP-N-acetyl-glucosamine (UDP-GlcNAc) and UDP-glucuronic acid (UDP-GlcUA) by the three HA synthases HAS1, HAS2 and HAS3. HA strands are generated by the alternating transfer of an UDP-residue to N-acetylglucosamine and glucuronic acid via the UDP-glucuronyltransferase (UGT). HA synthesis is limited by the availability of UDP-GlcNAc and UDP-GlcUA [228]. In the presence of 4-MU, it covalently binds through its hydroxyl group at position 4 to glucuronic acid via the UGT. As a consequence, the concentration of UDP-glucuronic acid declines in the cytosol and HA synthesis is reduced [229]. This therewith reduces 4-MU the UDP-GlcUA content inside the cells. 4-MU inhibits HA synthesis by depleting the HAS enzyme UDP-GlcUA, which is

consumed by 4-MU glucuronidation. There is another 4-MU mechanism known, but it is unclear how exactly it works. Here 4-MU reduces the expression of HAS [230], UDP glucose pyrophosphorylase and dehydrogenase mRNA [231].

A few studies have investigated the impact of 4-MU on HA synthesis in autoimmunity and inflammation. 4-MU has been used *in vitro* to inhibit HA synthesis by several human pathogens and to study their interactions with different kinds of human cells [232,233]. *In vivo* studies have shown that 4-MU treatment prevented lung injury and reduced inflammatory cytokine levels in mouse models of staphylococcal- [234] and lipopolysaccharide-mediated acute lung injury [235]. 4-MU also has protective effects on non-infectious inflammation, including airway inflammation [236] and renal ischemia and reperfusion [237]. In addition, *in vitro* and *in vivo* 4-MU inhibits in multiple cancer cell types the proliferation, migration, and invasion [238,239]. 4-MU also restores normoglycemia and promotes insulin sensitivity in obese, diabetic mice via increased production of adiponectin [240]. 4-MU has also been reported to improve disease in some autoimmune mouse models. 4-MU treatment was beneficial in the collagen-induced arthritis model [241]. We and others have reported that 4-MU treatment limits the progression of EAE [107,109] and autoimmune diabetes in both the DORMO and NOD mouse models [43]. We show that this therapeutic effect is not only a result of the polarization of the T cell response away from a pathogenic Th1 response, but also the reduction of infiltration of these cells into sites of autoimmune attack. Additionally, because 4-MU treatment lifts the inhibition of Foxp3⁺ Treg induction and function by LMW-HA, this inhibition of the pathogenic response is aided by an increase of Treg numbers [43,107,109].

We have reported that 4-MU treatment prevented cell-cell interactions required for antigen presentation [35] and others have described inhibitory effects on T-cell proliferation [238]. There are also indications that 4-MU treatment may make some models of inflammation worse. 4-MU treatment was associated with worse atherosclerosis in ApoE-deficient mice fed a high-fat diet [242].

4-MU has been shown to inhibit HA production in multiple cell lines and tissue types both *in vitro* and *in vivo*, and has received much attention as a potential therapeutic in inflammation, autoimmunity and cancer [229], unrelated to its clinical use for bile duct disorders [243].

Anti-CD44 antibodies

Monoclonal antibodies targeting CD44 have been shown to be beneficial in several animal models of autoimmunity, including the NOD mouse model of autoimmune diabetes and the Collagen-induced arthritis model of rheumatoid arthritis [113]. These effects may result from effects on lymphocyte trafficking or apoptosis rather than effects on the local ECM milieu.

Monoclonal antibodies targeted towards CD44 have been used in a variety of animal autoimmune models to reduce disease severity. The most commonly used antibody is the IM7 clone, whose binding activity to leukocytes is present independent of the expressed CD44 variant. Weiss et al have used this antibody to markedly reduce the development of

autoimmune diabetes in the Non-Obese diabetic (NOD) mouse model [86]. Due to anti-CD44's protective effect during diabetogenic splenocyte adoptive transfers, the group concluded that CD44 positive leukocytes were a viable target to treat autoimmune insulinitis. Other groups have further used monoclonal antibodies to CD44 in alternative autoimmune models. Verdrengh et al have used the IM7 antibody clone to inhibit development of collagen induced autoimmune arthritis, also suggesting CD44 positive lymphocytes and monocytes as the target [244]. Nedvetski et al have shown similar results, but expanded treatment to the alternate anti-CD44 clone KM81, which saw a lessened effect compared to the IM7 clone [140]. Antibodies to CD44 have also been used to inhibit development of EAE. Brocke et al suggest that via administration of IM7 anti-CD44, trafficking of encephalitogenic T cells to the CNS can be inhibited, limiting the progression of disease [113]. From these studies, it appears that targeting the CD44 receptor with monoclonal antibodies is a viable method to treat autoimmunity.

Conclusion

In light of these data, we propose a model whereby the inappropriate accumulation of HA at sites of chronic inflammation creates an environment permissive to autoimmunity by restricting Treg differentiation (Fig. 3). Moreover, we postulate that clearance of HA upon the resolution of inflammation (or 4-MU treatment) may reestablish a regulatory checkpoint by allowing Treg differentiation and, thereby, increasing the ratio of FOXP3⁺ Tregs to T effector cells. This ratio is known to be critical in enforcing peripheral tolerance [202], and Tregs are known to play a critical role in protection from autoimmune diabetes in both human T1D [77] and mouse models of the disease [245] This model may help explain how tissue factors influence local Treg numbers and function in autoimmune insulinitis [78,212].

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Abbreviations

HA	hyaluronan
ECM	extracellular matrix
GAG	glycosaminoglycan
T1D	type 1 diabetes
T2D	type 2 diabetes
MS	multiple sclerosis
RA	rheumatoid arthritis
HAS	HA synthase
LMW	low molecular weight

HMW	high molecular weight
SHAP	serum-derived HA-associated protein
DAMP	damage associated molecular pattern

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Figure 1. Hyaluronan is prominent in mouse models of autoimmune diabetes and multiple sclerosis

A. pancreatic islet sections from healthy mice (left) and mice with autoimmune insulinitis (right) stained for HA (brown). **B.** HA (red) is enriched at sites of inflammation in the CNS, hallmarkd by activation of glial fibrillary acidic protein (GFAP, green) expressing astrocytes, in the mouse model of MS, EAE.

Figure 2. Hyaluronan has been implicated in the facilitation of autoimmunity by promoting leukocyte activity

(A) Oligosaccharides of HA (green) have been suggested to act as ligands to the TLR group of receptors, promoting maturation in these APC. (B) HA has been suggested to facilitate the stimulation of lymphocytes by APCs within lymphoid organs. (C) HA receptors are used by leukocytes to egress from vessels into surrounding tissue by binding to HA on the luminal side of endothelial cells. The effector activity of lymphocytes has been implicated as a target for HA to promote autoimmune activity. (D) HA is found in close proximity to Beta cells within diabetogenic pancreas, and HA is strongly associated with lymphocyte presence in these individuals. (E) HA has been found in proximity to lymphocytes and destroyed myelin in models of multiple sclerosis. (F) In models of rheumatoid arthritis, HA is found in the synovial fluid of affected joints, and has been implicated in facilitating immune destruction of the tissue

Figure 3. Hyaluronan at tertiary sites of inflammation (pancreatic islet shown here) and secondary lymphoid tissue promote inflammatory Th1 T cells while suppressing the expansion of antiinflammatory Treg

Sterile and infectious inflammatory insults serve to promote both tertiary tissue HA deposition by stromal cells as well as HA deposition by antigen presenting cells within secondary lymphoid tissue. Within the lymph nodes, robust HA coats on APC serve to promote induction of Th1 CD4⁺ T Cells while suppressing Treg. Together, this recruits inflammatory cells to the sites of autoimmunity and promotes destruction of self-tissue. Of note, in the absence of antigenic signals, HA promotes Treg homeostasis, suggesting that TCR signals and active antigen presentation are critical to the impact of HA on local T-cell populations.

Table 1

Autoimmune diseases treated with HA targeting therapies.

Disease	Study Model		Therapy	Treatment (Dose)	Reference
Rheumatoid Arthritis	Collagen Induced Arthritis (CIA) in DAB/1J mouse		4-Methylumbelliferone (4-MU)	Oral .5mg/gm–3 mg/gm body weight administered daily in 300 ul of 5% arabic gum from days 23–42 (disease develops by day 23)	Yoshioka, 2013
	Collagen Induced Arthritis (CIA) in DBA/1 mouse		Anti-CD44 antibodies	Post-disease onset intraperitoneal injections of 150ug anti-CD44 every other day for up to 10 days	Nedvetski, 1999
	Collagen Induced Arthritis (CIA) in DBA/1 mouse		Anti-CD44 antibodies	.5mg per mouse, 2x/week for 6–7 weeks following disease induction	Verdrengh, 1995
	Rat Antigen Induced Arthritis (AIA) in Lewis rat		High Molecular Weight Hyaluronan (HMW-HA)	.5mg HA (1.7×10^6 Da) injected intraarticularly in 50 ul PBS 1x/week post disease induction	Roth, 2005
Multiple Sclerosis	1	Experimental Autoimmune Encephalomyelitis (EAE) with proteolipid protein (PLP) in SJL/J mice	4-Methylumbelliferone (4-MU)	5% mix in ground chow 1 day prior to EAE induction	Mueller, 2014
	2	Myelin Oligodendrocyte Glycoprotein (MOG) in C57Bl/6 mice			
	3	MOG in Dark Agouti (DA) Rats			
	EAE induced with Myelin Basic Protein (MBP) reactive T cells in C57Bl/6 and (PL × SJL)F1 mice		Anti-CD44 antibodies	250 ug anti-CD44 injected i.p. every other day up to 16 days post EAE induction	Brocke, 1999
	EAE with Myelin Oligodendrocyte Glycoprotein (MOG) in C57Bl/6 mouse		4-Methylumbelliferone (4-MU)	5% mix in ground chow	Kuipers 2016
Type I Diabetes	Non-Obese Diabetic (NOD) mouse and DOrMO (DO. 11xRIPmOVA) mouse		4-Methylumbelliferone (4-MU)	5% mix in ground chow	Nagy, 2015
	Non-Obese Diabetic (NOD) mouse		Anti-CD44 or Hyaluronidase	150 ug anti-CD44 prior to transfer and every other day for 4 weeks 20 units Bovine Testicular hyaluronidase every other day post adoptive	Weiss, 2000

Disease	Study Model	Therapy	Treatment (Dose) transfer up to four weeks	Reference
Systemic Lupus Erythematosus	Human anti-DNA mAB in BALB/c mouse	hCDR1 peptide	50 ug/mouse subcutaneous at time of induction	Sela, 2005