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Extracellular Matrix and the Maintenance and Loss of Peripheral Immune Tolerance in Autoimmune Insulitis

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Abstract

There is a growing appreciation that the extracellular matrix (ECM) contributes to both the maintenance of immune tolerance in healthy tissues and to its loss at sites of autoimmunity. Here, we review recent literature on the role of ECM and particularly the glycosaminoglycans hyaluronan and heparan sulfate in the development of autoimmune, type 1 diabetes (T1D). Data from transplant models suggest that healthy islets are embedded within an intact ECM that supports beta-cell homeostasis and provides physical and immunoregulatory barriers against immune infiltration. However, studies of human insulitis as well as the non-obese diabetic (NOD) and DORmO mouse models of T1D indicate that autoimmune insulitis is associated with the degradation of basement membrane structures, the catabolism of the islet interstitium, and the accumulation of a hyaluronan-rich, pro-inflammatory ECM. Moreover, in these models of autoimmune diabetes, either the pharmacologic inhibition of heparan sulfate catabolism, the reduction of hyaluronan synthesis, or the targeting of the pathways that sense these ECM changes can all prevent beta-cell destruction. Together these data support an emerging paradigm that in healthy islets the local ECM contributes to both immune tolerance and beta-cell homeostasis while in chronic inflammation the islet ECM is permissive to immune infiltration and beta cell destruction. Therapies that support ECM-mediated “barrier tolerance” may have potential as adjunctive agents in combination regimens designed to prevent or treat autoimmunity.

Keywords

hyaluronan; heparan sulfate; diabetes; T1D; extracellular matrix

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Declaration of interest

PLB and NN are co-founders of Hyalos Therapeutics, a company developing novel small molecules to inhibit HA synthesis.

Introduction

The extracellular matrix (ECM) surrounds cells and tissues throughout the human body and it has long been known that these structures contribute to local homeostasis and function. However, there is a growing appreciation the ECM also provides barriers against immune infiltration in healthy tissues.

The contributions of the ECM to tissue homeostasis and peripheral tolerance are perhaps best understood for type 1 diabetes (T1D), an autoimmune disease characterized by lymphocyte-mediated destruction of insulin producing β-cells within the pancreatic islets (1). Most current models of the pathogenesis of T1D invoke the progressive, sequential loss of B-cell and T-cell tolerance to islet auto-antigens as well as a failure of immunoregulatory mechanisms that normally control potentially auto-reactive lymphocytes (1–3). Alongside mechanisms of central and peripheral tolerance that are presumably interrupted in this progression, there are suggestions that tissues may themselves provide barriers against immune-mediated destruction and that these are likewise lost in T1D. This can be inferred from histologic data from human T1D (4) as well experimental models of the disease, including the Non-Obese Diabetic (NOD) (5) and DORmO mouse models (Fig.1).

Here, we review recent literature on the role of ECM and particularly the glycosaminoglycans hyaluronan (HA) and heparan sulfate (HS) in the development of T1D. We first briefly review what is known about the ECM in healthy islets as well as data suggesting that it supports beta-cell homeostasis as well as providing physical and immunoregulatory barriers against immune infiltration. Next we review studies indicating that autoimmune insulitis is associated with the degradation of basement membrane structures, the catabolism of the islet interstitium, and the accumulation of a hyaluronan-rich, pro-inflammatory matrix and that these changes are permissive to immune infiltration and beta cell destruction. Finally we propose that agents that support ECM integrity may have therapeutic potential for prevention or possibly treatment of T1D.

The ECM in healthy pancreatic islets

In pancreatic islets, as in all tissues, cells exist within an ECM - a complex network of proteins, polysaccharides, and proteoglycans that constitute basement membrane (BM) and interstitial matrix structures. The detailed biochemistry and histologic distribution of islet ECM components are the subjects of several excellent recent studies and reviews (6–8).

In brief, the BM that surrounds capillaries and encases each islet are mostly comprised of tightly interconnected networks of type IV collagen and laminin. These are interwoven with lesser amounts of heparan sulfate proteoglycans (HSPGs) such as perlecan, glycoproteins such as nidogens, and glycosaminoglycans such as hyaluronan (7–10). The islet BM differs somewhat between species, being continuous in mice and discontinuous in humans (11). In contrast to the BM matrix, the islet interstitial matrix present within the islet stroma is more diffuse and mostly contains collagens I, II, and IV, and fibrillin-2 (10,12). HSPGs are present as well, including HS polymers attached to the core proteins collagen type XVIII, versican, and syndecan-1 (8,13).

Together these ECM components provide critical structure and support to islet-resident cells. In particular, transplant studies indicate that the islet ECM communicates chemical and mechanical signals that mediate key aspects of islet physiology including survival (14–18), differentiation (19–22), proliferation (23–25),and insulin secretion (14,15,23,26,27). The ECM signals that support these cells are communicated in part via interactions between cell membrane receptors, such as integrins, and ECM polysaccharides and proteins, such as laminin, that contain the peptide signaling sequence arginine-glycine-aspartic acid (RGD) (28,29). In addition to providing structural support, the interstitial ECM modulates cellular behavior via the binding of growth factors to sulfated glycosaminoglycans such as HS (30,31) and it has been suggested that this may contribute to β-cell homeostasis (32). Capitalizing on these insights, ECM platforms are increasingly utilized to support islet transplantation efforts (33) as well as the development of stem-cell derived β-cells (34).

ECM catabolism and the loss of tissue integrity in autoimmune insulitis

In comparison to healthy islets, the ECM at sites of insulitis is altered in multiple ways. These include the loss of BM integrity, the catabolism of interstitial matrix, and the deposition of a pro-inflammatory matrix dominated by HA.

The transition between non-destructive peri-insulitis (Fig. 1B) and destructive insulitis (Fig. 1C) is accompanied by a breakdown in the islet BM, as demonstrated conclusively by Dr. Lydia Sorokin and her colleagues. In particular, there is a loss of laminin and perlecan staining within the BM in both humans with T1D (10) as well as in the NOD (9,10) and DORmO mouse models of the disease (Fig.2). This catabolism occurs in association with increased expression of cathepsins S, W, and C, and heparanase (9,10,35). In transplant studies, loss of islet BM integrity has major, adverse effects on islet function and viability (36) while inhibition of BM degradation can preserve islet function (37). Modeling studies suggest that the balance between degradation and repair of the BM may be a critical determinant in the progression to clinical diabetes (38). Perhaps consistent with the pathophysiology observed in T1D, disruption of the BM structures that maintain the blood-brain barrier is likewise seen in Multiple Sclerosis (MS) and in the experimental autoimmune encephalitis (EAE) model of that disease (39,40). Together, these data suggest that the islet BM functions as a physical barrier against leucocyte migration into islets and that degradation of this barrier is a critical step in progression to diabetes (Fig.3).

The subsequent infiltration of leukocytes into the islet stroma (Fig. 1C) is also associated with the catabolism of the interstitial matrix, in particular the loss of HS content, as demonstrated in beautiful work by Dr. Charmaine Simeonovic and her colleagues. They demonstrated that intra-islet HS is lost in both NOD mice(35) as well as in humans with T1D (13) in association with expression of heparanase by leukocytes (41). We observe similar findings in DORmO mice (Fig.2). This catabolism of HS has been reported to contribute to local destruction of β-cells via an increase in local reactive oxygen species that would otherwise be absorbed by HS, a conclusion based on the finding that HS protects islets from oxidative stress in vitro and the observation that much of the missing HS was intracellular (35). However, HS catabolism may contribute to β-cell destruction in additional ways, given HS's overlapping roles in innate immunity and as a depot for β-cell growth

factors (32,42,43). Moreover, glycosaminoglycan synthesis is complex and typically involves extracellular production before intracellular uptake such that the site of HS catabolism and function is uncertain.

The strongest support for an important, functional role for HS in local immune regulation is the finding that treatment with either exogenous HS or a small molecule inhibitor of heparanase, PI-88, prevented progression of established insulitis in NOD mice and prevented β-cell death (35). These treatments of course would also be expected to preserve BM integrity as well, given the abundance of HSPGs there, such that the *in vivo* site of action (BM versus interstitial matrix) is unclear. However, *in vitro*, HS protected β-cells themselves from oxidative damage. Consistent with a role for heparanase produced by infiltrating leukocytes in autoimmunity, we find that T-cells isolated from mice lacking heparanase ($HPSE^{-/-}$ mice) are substantially delayed in causing autoimmunity in EAE mice (unpublished results).

In addition to the loss of HS, other changes within the islet interstitial matrix have been reported as well including the loss of inter-alpha-inhibitor (IαI)(44) and tumor necrosis factor-stimulated gene-6 (TSG-6)(44,45), a pair of molecules with complex but generally anti-inflammatory properties (46,47).

In sum, these data suggest that, as with the loss of BM integrity, degradation of the islet interstitial matrix is another critical step in the progression to autoimmune diabetes (Fig.3).

Deposition of pro-inflammatory matrix at sites of autoimmune insulitis

In addition to degradation of the normal islet EMC, robust autoimmune insulitis (Fig. 1C, 1D) is also characterized by the deposition of a pro-inflammatory matrix dominated by HA, as originally shown by Dr. Tom Wight (48). In healthy tissues, HA provides structural support to skin, joints, and other tissues (49). At these sites, HA is typically bound to a diverse group of binding proteins, called hyaladherins, including TSG-6 and IαI (8), and these complexes are typically structurally stable and pro-tolerogenic (50,51). However, at sites of inflammation, both HA production and catabolism are greatly upregulated (49) leading to the accumulation of HA (Fig.2). These promote leukocyte migration and activation within inflamed tissues (49,52). HA deposits are present at sites of autoimmune insulitis in both human T1D (48) as well as the NOD (53) and DORmO (44) mouse models of the disease and most of this HA consists of small fragments (54), consistent with the previously noted depletion of local hyaladherins that protect HA from catabolism. Similar HA deposits are present at sites of autoimmune attack in RA (55), MS (56), and other autoimmune diseases (57,58).

The strongest support for an important, functional role for HS in local immune regulation is the finding that treatment with 4-methylumbelliferone (4-MU), an inhibitor of HA synthesis (59), prevented progression of established insulitis in both DORmO and NOD mice (44,60). This effect was associated with the suspension of cytolytic killing of β-cells and the expansion of CD4+Foxp3+ regulatory T-cells. However, when 4-MU treatment was stopped and HA synthesis resumed, diabetes rapidly ensued (44). Consistent with these results,

treatment with recombinant hyaluronidase prevents diabetes in NOD mice (61). Treatment with an antibody clone (IM7) that causes cell surface shedding of CD44, the primary HA receptor, likewise prevents diabetes in the NOD model (61). Moreover, this same suite of treatments (4-MU, hyaluronidase, and anti-CD44 antibodies) are effective treatments in mouse models of Rheumatoid Arthritis (62,63) and MS (56,64,65) as well.

The ECM influences Treg number and function. HA triggers CD44-mediated AKT and ERK1/2 signaling that suppresses Treg expansion (44,64). Consistent with this, immunized $CD44^{-/-}$ mice have increased FoxP3+ Treg (64,66,67). However, 4-MU has only modest effects on Treg numbers in naïve mice and uninflamed tissues (unpublished data) while un-immunized $CD44^{-/-}$ mice have normal Treg numbers (68), suggesting that HA may only suppress Treg expansion in the setting of inflammation. Indeed, culture plates coated with HA actually promote Treg survival (69,70). These seemingly contradictory findings (that HA could both inhibit Treg expansion as well as promote Treg survival) are consistent with established roles for AKT and ERK1/2 in both the inhibition of Treg expansion as well as promotion of Treg survival (71,72). Together these data suggest that HA limits Treg expansion at sites of active inflammation but preserves the capacity for immune tolerance at a later time when HA is cleared.

Along with effects on Treg, HA-CD44 interactions also influence other lymphocyte populations in ways that may impact autoimmunity. This includes well-established effects on costimulation, polarization towards a Th1 phenotype, and trafficking (49). This biology may occur in draining lymph nodes as well as sites of insulitis given that HA accumulates in pancreatic lymph nodes in T1D as well as in islets themselves (48).

HA/CD44 interactions may also directly impact β-cells adversely. In a fascinating study, it was reported that NOD mice deficient for CD44 were protected from autoimmune diabetes. In transfer experiments this protection was associated with the absence of CD44 in recipient mice rather than on donor lymphocytes. Moreover, islets cultured in vitro with HA demonstrated enhanced apoptosis (73). The mechanisms behind these observations are unclear. Nonetheless, they raise the intriguing possibility that the HA-rich matrix present at sites of autoimmune insulitis may contribute directly to β-cell demise in T1D.

Together these data suggest that deposition of a HA-rich matrix contributes to the loss of immune tolerance in pancreatic islets by creating a pro-inflammatory milieu that drives immune dysregulation (Fig.3).

ECM barrier effects in peripheral immune tolerance

The data presented here suggest that intact ECM may promote β-cell homeostasis as well as provide barriers against immune infiltration. These mechanisms of barrier tolerance may complement previously characterized mechanisms of central and peripheral tolerance (Fig. 4).

While this is perhaps an appealing model, numerous questions remain. The contributions of lymphocytes versus other leukocyte subsets to ECM catabolism and deposition are unclear, as are the temporal aspects of these processes and their relationship to other local

inflammatory events. In NOD mice, autoimmune insulitis begins not long after the islet remodeling that occurs after weaning (74) while in humans viral infections precede T1D in some cases (75). It may be that the local inflammation associated with such events causes critical lapses in barrier tolerance that help make the local ECM permissive to autoimmunity. Alternatively, activated T-cells themselves can catabolize the local ECM as well as priming other cell populations to secrete a pro-inflammatory matrix (76,77). Sorting out the “chicken and egg” question regarding the timing of these events and the responsible cells and molecules is likely to be important to understanding barrier tolerance and why it fails.

Other questions remain as well. How important are different ECM components to barrier tolerance? Are the stages of insulitis progression functionally distinct or are they interdependent? How does the ECM in draining lymph nodes contribute to immune activation? There are some evidence that similar ECM structures provide barrier tolerance in CNS tissue and that these likewise are disrupted in MS (78,79) but it would be important to know whether the same phenomenon are present in other tissues as well. Clearly there remains much to learn about the ECM at sites of autoimmunity and its contributions to barrier tolerance.

Targeting the ECM to Promote Immune Tolerance in T1D – The Potential Path Forward

The observations discussed here may be relevant to autoimmunity prevention. There is great interest in identifying mechanisms of peripheral tolerance that can be targeted therapeutically in T1D. Since screening for auto-antibodies can identify individuals at risk of T1D after the initial loss of tolerance but before clinical diabetes, the hope is that by re-establishing or reinforcing immunoregulatory checkpoints, further disease progression can be forestalled (80). Despite great progress, however, effective and benign prophylactic regimens have not been identified (81,82). An emerging strategy is to develop immunomodulatory combination therapies with complimentary synergistic mechanisms (83). The data reviewed here suggest that it may be possible to target the ECM to promote local immune tolerance in ways that are fundamentally different from existing T- or B-cell directed therapies. Moreover, several of the agents in question, particularly PI-88 and 4-MU are either in clinical trials currently or have an extensive clinical track record in humans (84,85).

However, here again multiple questions remain. Is targeting the ECM a safe strategy in adolescents and children who are still growing and extensively remodeling their tissues? What are the consequences of inhibiting ECM synthesis or breakdown in humans? Would protection from T1D require indefinite targeting of these pathways or would more targeted therapy be sufficient?

While further work clearly remains to be done and numerous questions remain, the ECM is clearly an exciting frontier in our understanding of immune tolerance and T1D.

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Abbreviations:

HA	Hyaluronan
HS	Heparan Sulfate
ECM	Extracellular Matrix
T1D	Type 1 Diabetes
BM	Basement Membrane

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Highlights:

- Islet extracellular matrix provides homeostatic support to pancreatic islets as well as physical and immunologic barriers against immune infiltration.
- Degradation of islet basement membrane and interstitial heparan sulfate are required for islet immune infiltration and insulitis
- Increased hyaluronan deposition and fragmentation promotes pro-inflammatory lymphocyte responses and islet destruction
- Treatment preserving or restoring ECM halts disease progression and promotes regulatory T cells

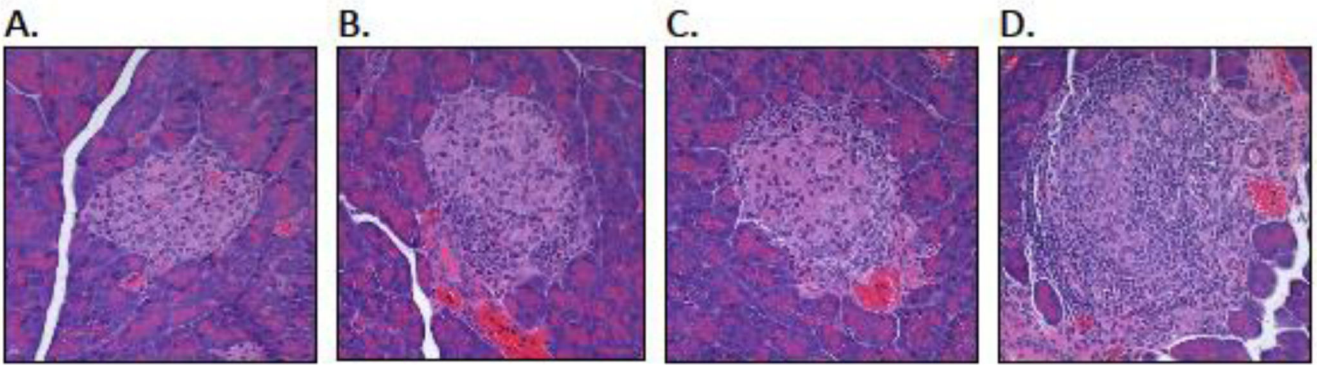

Figure 1: Autoimmune insulitis in the DORmO mouse model of T1D is associated with the progressive, sequential loss of local tissue barriers against autoimmunity.

H&E staining of islets from DORmO mice at various ages and stages of progressive insulitis: (**A**) no infiltration (3–4 weeks of age), (**B**) peri-insulitis (4–5 weeks), (**C**) insulitis (8 weeks), and (**D**) islet destruction (12 weeks).

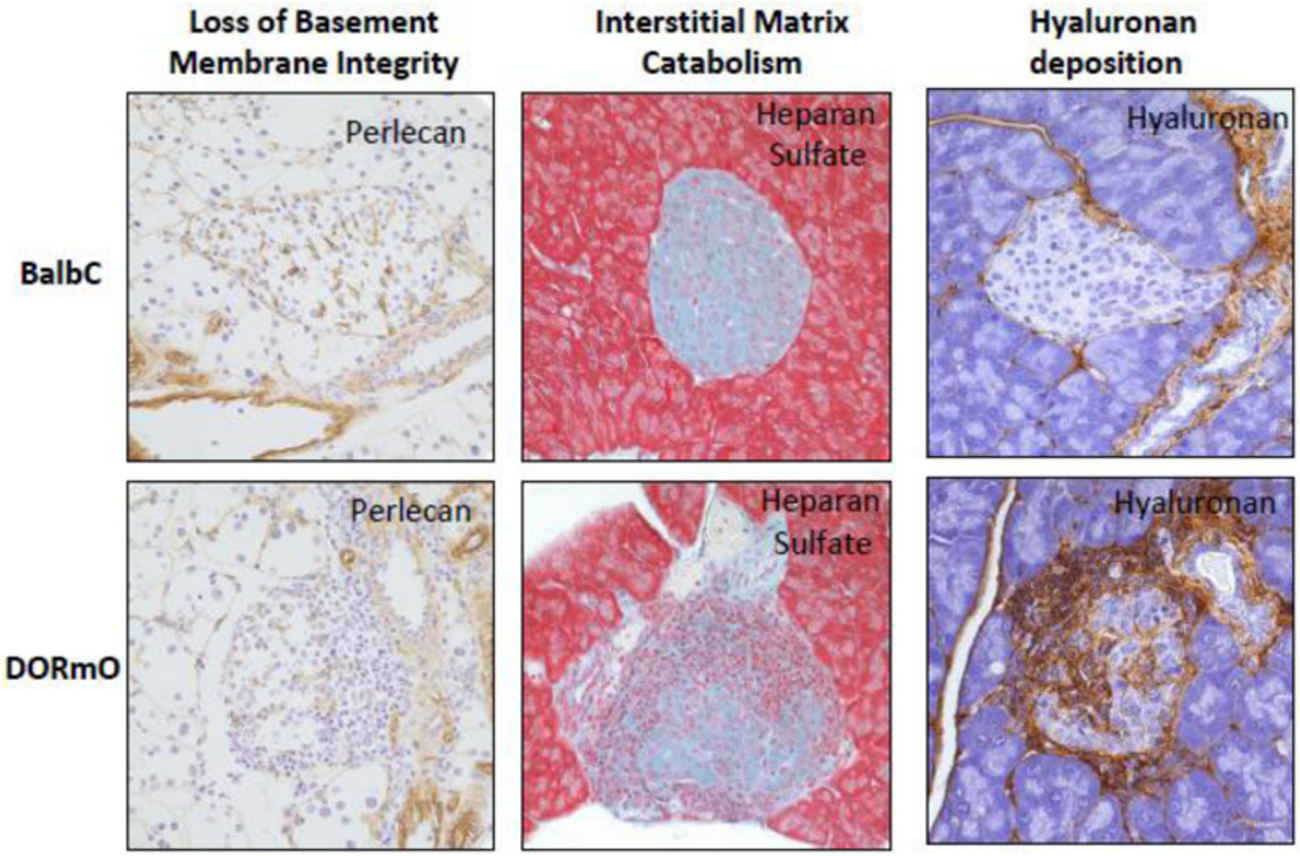

Figure 2: Autoimmune insulitis is associated with the degradation of islet basement membranes, the catabolism of the interstitial matrix and the deposition of a pro-inflammatory matrix dominated by hyaluronan.

Histologic staining of the islet ECM molecules in healthy BalbC mice and in the DORmO mouse model of T1D. In DORmO mice, basement membrane integrity is lost, as evidenced by the break-down in perlecan staining (left). Interstitial matrix likewise undergoes catabolism, as evidenced by the loss of heparan sulfate structures (center). Finally, there occurs the deposition of a pro-inflammatory matrix dominated by hyaluronan (right).

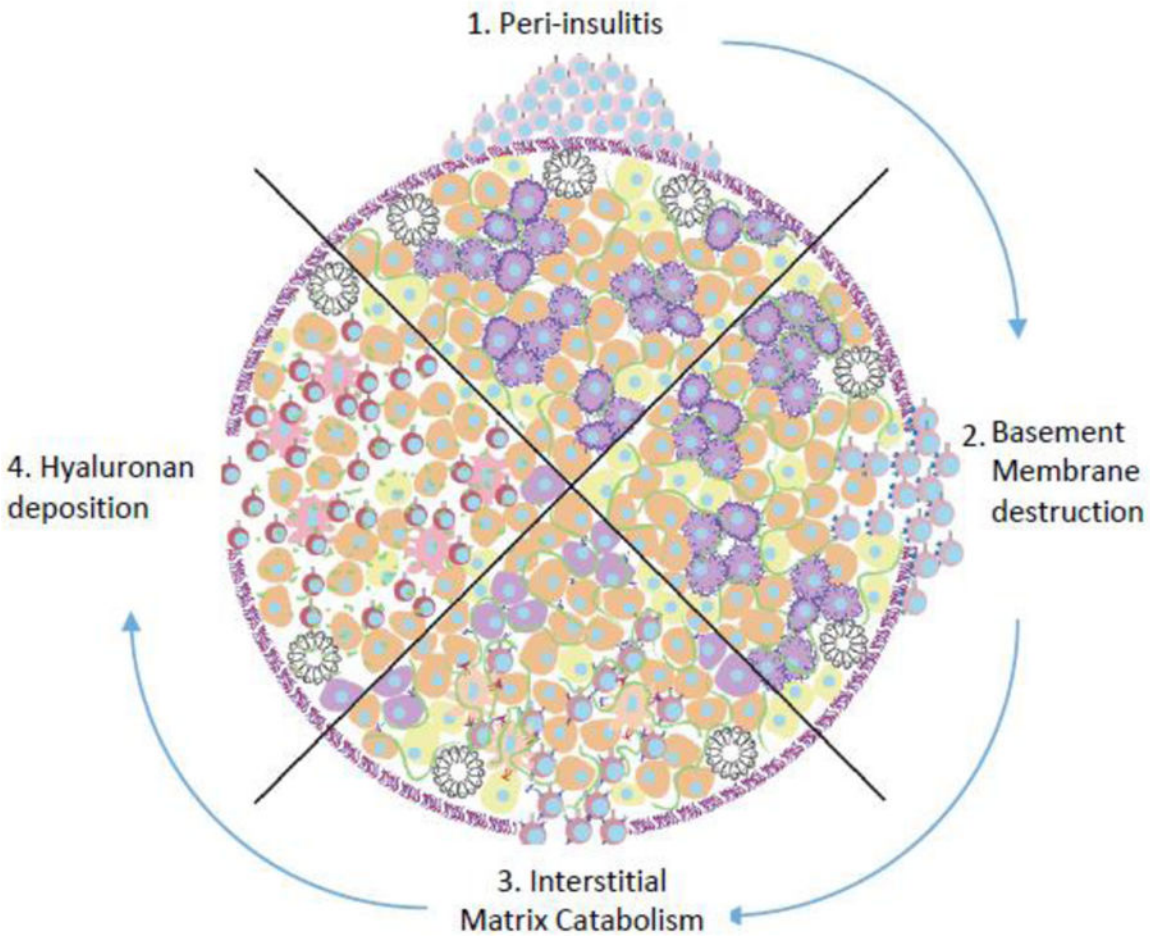

Figure 3: A schematic of the sequential loss of ECM barriers against in autoimmune insulitis.

In healthy islets, the ECM provides physical and immunoregulatory barriers against immune infiltration. However, in autoimmune diabetes, effector T cells evade central and peripheral tolerance and accumulate around islets (top). These auto-reactive cells require a break in the laminin rich basement membrane for entry (right). Degradation of islet HS leads to beta cell death (bottom). Deposition of HA allows for further infiltration, effector cell activation, and inhibition of Treg expansion (left). Along with effects on infiltrating leukocytes, this remodeling of the islet ECM also adversely impacts β-cell health.

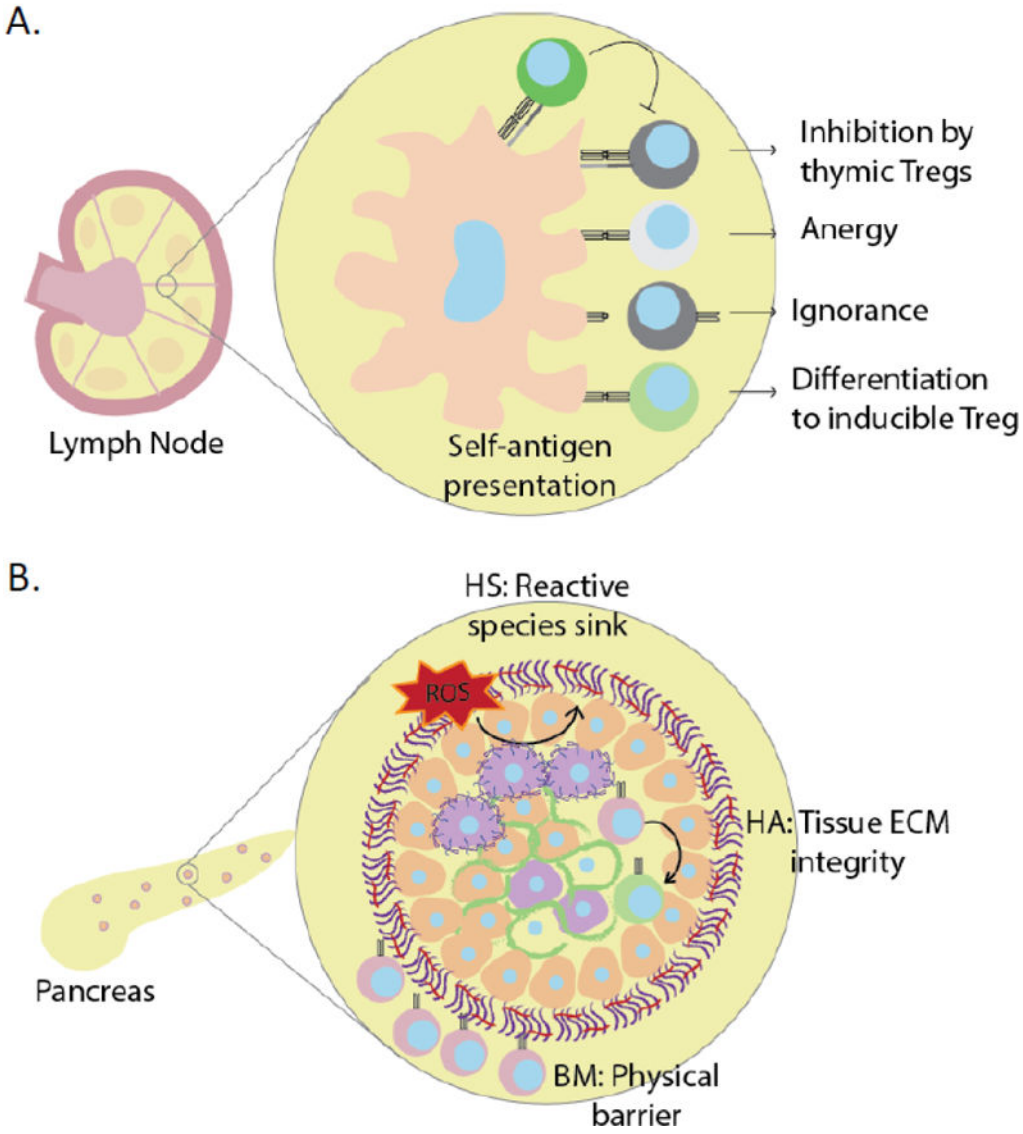

Figure 4: Peripheral and barrier tolerance mechanisms.

(A) Peripheral tolerance of autoreactive effector T cells is maintained via inhibition by thymic Tregs, induction of anergy, antigenic ignorance, or differentiation to inducible Tregs. (B) The intact islet ECM provides physical and immunologic barriers ("barrier tolerance") that contribute to immune tolerance. These include a basement membrane that prevents immune infiltration, protective barriers against reactive oxygen species as with HS, and immunomodulatory barriers like the stabilization and sequestration of HA that limit immune activation.