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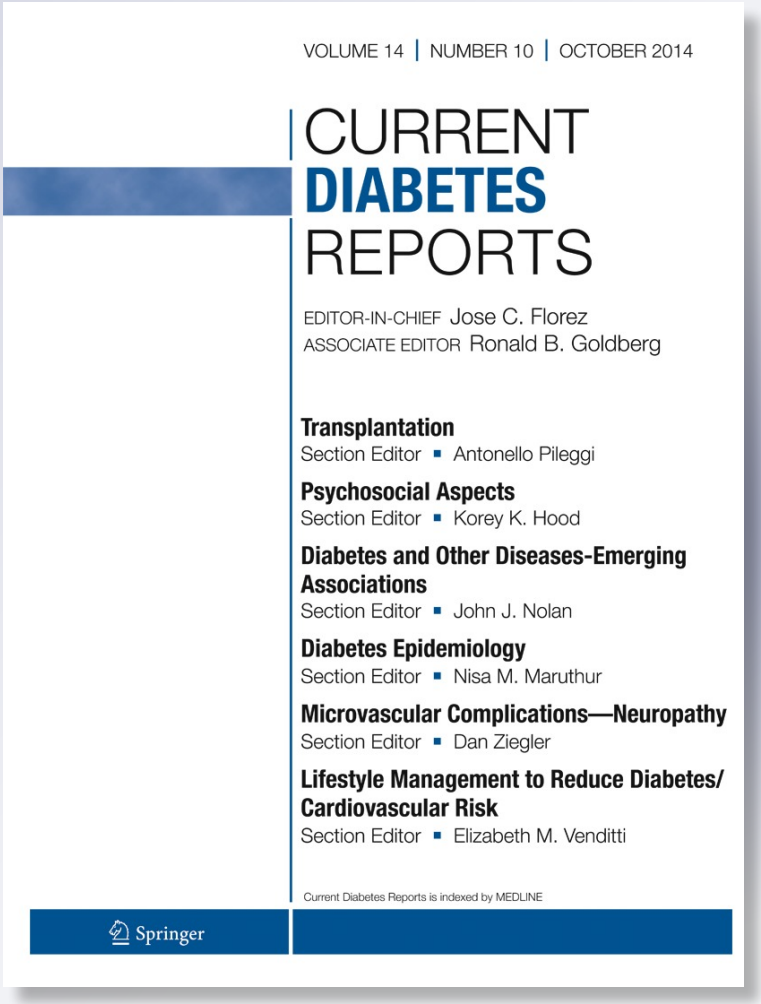
Current Diabetes Reports

ISSN 1534-4827
Volume 14
Number 12

Curr Diab Rep (2014) 14:1-11
DOI 10.1007/s11892-014-0552-7



Springer

Curr Diab Rep (2014) 14:552
DOI 10.1007/s11892-014-0552-7

PATHOGENESIS OF TYPE 1 DIABETES (A PUGLIESE, SECTION EDITOR)

Extracellular Matrix Components in the Pathogenesis of Type 1 Diabetes

Marika Bogdani · Eva Korpos · Charmaine J. Simeonovic · Christopher R. Parish · Lydia Sorokin · Thomas N. Wight

Abstract Type 1 diabetes (T1D) results from progressive immune cell-mediated destruction of pancreatic β cells. As immune cells migrate into the islets, they pass through the extracellular matrix (ECM). This ECM is composed of different macromolecules localized to different compartments within and surrounding islets; however, the involvement of this ECM in the development of human T1D is not well understood. Here, we summarize our recent findings from human and mouse studies illustrating how specific components of the islet ECM that constitute basement membranes and interstitial matrix of the islets, and surprisingly, the intracellular composition of islet β cells themselves, are significantly altered during the pathogenesis of T1D. Our focus is on the ECM molecules laminins, collagens, heparan sulfate/heparan sulfate proteoglycans, and hyaluronan, as well as on the enzymes that degrade these ECM components. We propose that islet and lymphoid tissue ECM composition and organization are critical to promoting immune cell activation, islet invasion, and destruction of islet β cells in T1D.

Keywords Extracellular matrix · Hyaluronan · Hyaladherins · Laminin · Heparan sulfate · Cathepsins · Heparanase · Islet · Islet infiltration · Diabetes · Immune regulation

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This article is part of the Topical Collection on *Pathogenesis of Type 1 Diabetes*

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Introduction

The extracellular matrix (ECM) is the noncellular component of all tissues and organs. It is composed of various proteins and polysaccharides which interact through entanglement and cross linking to form well-defined three-dimensional structures, providing cells with not only biomechanical support needed to maintain tissue organization and integrity but also receptor-mediated intracellular signals that are essential for tissue development and homeostasis [1••, 2••, 3, 4]. Within any given tissue, there is a complementary set of specific ECM components that govern normal tissue function. ECM can exist in the form of specialized structures within tissues, such as in basement membranes, or as part of the space between cells of the stroma of tissues as interstitial matrix. Any disturbance in the composition and/or organization in these components can alter tissue architecture and promote loss of normal tissue function. Such changes occur in tissues

Published online: 26 October 2014

undergoing inflammation, and in fact, the integrity of the ECM may control events promoting the inflammatory response [5].

Type 1 diabetes (T1D) results from an immune cell-mediated destruction of the pancreatic β cells, which takes place in a permissive inflammatory environment. While our understanding of this inflammatory environment is incomplete, the ECM must serve as a conduit or substrate for the invading cells leading to the destruction of the cells within the islet.

The focus of this review will be to highlight the work from three different research groups in different parts of the world (Muenster, Germany; Canberra, Australia; and Seattle, USA) that have been working with the JDRF Network for Pancreatic Organ Donors (nPOD) [6] to determine if specific changes occur in ECM components and in compartments in islets and lymphoid tissues during the development of T1D. Each group has focused on different ECM components and how they are changed in the pathogenesis of this disease.

The review is divided into three sections. The first section from Drs. Korpos and Sorokin concentrates on the importance of basement membrane integrity as a barrier to lymphoid and myeloid cell invasion in islets in T1D and how specific enzymes that target the breakdown of ECM proteins play a key role in cellular invasion and destruction of the islet. The second section from Drs. Simeonovic and Parish focuses on heparan sulfate proteoglycans (HSPGs), an important ECM component of the basement membrane barrier and the novel observation that heparan sulfate (HS) accumulates intracellularly in β cells in normal islets, but decreases during islet isolation and T1D pathogenesis. Mechanisms regulating HS loss and the importance of inhibiting the depletion of β cell HS in the prevention of T1D are discussed. The third section from Drs. Bogdani and Wight describes studies that have revealed a previously unrecognized component of the ECM in islets and lymphoid tissues, hyaluronan, and the changes seen in this component as T1D develops. Hyaluronan is known to impact events associated with immunity and inflammation, highlighting its potential importance in the pathogenesis of T1D.

Basement Membranes and Interstitial Matrix of the Pancreas

While the cellular structure of the pancreas is well described, its ECM has only recently gained attention and has been shown to contribute to pancreas development [7, 8••], homeostasis [9••, 10, 11], and to pathological processes such as inflammation/diabetes [12, 13, 14••, 15••]. Broadly, the ECM is divided into basement membranes (BMs), tight networks of specialized glycoproteins that act to separate tissue compartments but that also direct cellular processes, and the looser interstitial matrix (IM) typical of the stroma of most organs [16, 17••, 18]. In the pancreas, BMs predominate, occurring around each acinar cell of the exocrine pancreas, surrounding blood vessels and ducts, and encasing each pancreatic islet (Figs. 1 and 2). The IM, which confers tensile strength and elasticity to tissues mainly due to the presence of fibrillar collagens, is limited in the pancreas and occurs as a thin layer immediately subjacent and external to the peri-islet BM and surrounding large ducts and blood vessels (Figs. 1 and 2). In tissues where there is an extensive stroma such as the skin, hyaluronan (HA) is abundant in the IM [19]; however, in the pancreas, it is likely to be limited and associated with hyaladherins (HA-binding molecules) such as versican, inter-alpha-inhibitor (IαI), and tumor necrosis factor-stimulated gene-6 (TSG-6) [20••].

Laminin and collagen type IV networks are the major components of all BMs, both self-assemble into suprastructures that are interconnected by the HSPGs [21] and by the nidogens [22–25]. However, each of these BM molecules occurs in several isoforms that can combine to form BMs that differ in their biochemical composition and function. Of all BM components, the laminins are considered to be the biologically active components, as they bind to cell surface integrin and non-integrin receptors and transduce signals to the cell that impact on proliferation, migration, and differentiation. Collagen type IV, by contrast, is important for structural integrity [26]. The laminins are heterotrimers composed of α, β, and γ chains and named according to their chain composition (e.g., laminin 111 is α1, β1, γ1); today, 18 different isoforms are known with differential expression patterns and functions [27]. The highly negatively charged nature of the HSPGs resulting from their sulfated HS glycosaminoglycan chains promotes charged interactions with molecules such as cytokines and growth factors, which can thereby also impact cell behavior [28].

In the mouse peri-islet BM collagen type IV, agrin, perlecan, and nidogen-1 and nidogen-2 have been reported to occur together with several laminin isoforms, in particular laminins 211/221 and 411/421, but not those characterized by laminin α1, α3, or α5 chains [13, 15••, 29]. In the human peri-islet BM, while all major BM components are the same as in mouse, laminin 411/421 is missing and is replaced by laminin 511/521 [10, 11, 13, 15••, 29]. However, endothelial BMs of blood vessels within pancreatic islets are rich in laminin α4 and α5 both in mouse [15••] and human [11, 15••], and have been shown in mouse to be essential for β cell adhesion, proliferation, and insulin secretion [9]. In vitro studies have confirmed that laminin 511 also contributes to maintenance of human β cell phenotype [30]. In both mouse and human, the IM underlying the peri-islet BM is composed of the fibrillar collagen types I and III, collagen type VI, fibronectin, fibrillin-2, and matrilin-2 [15••, 31].

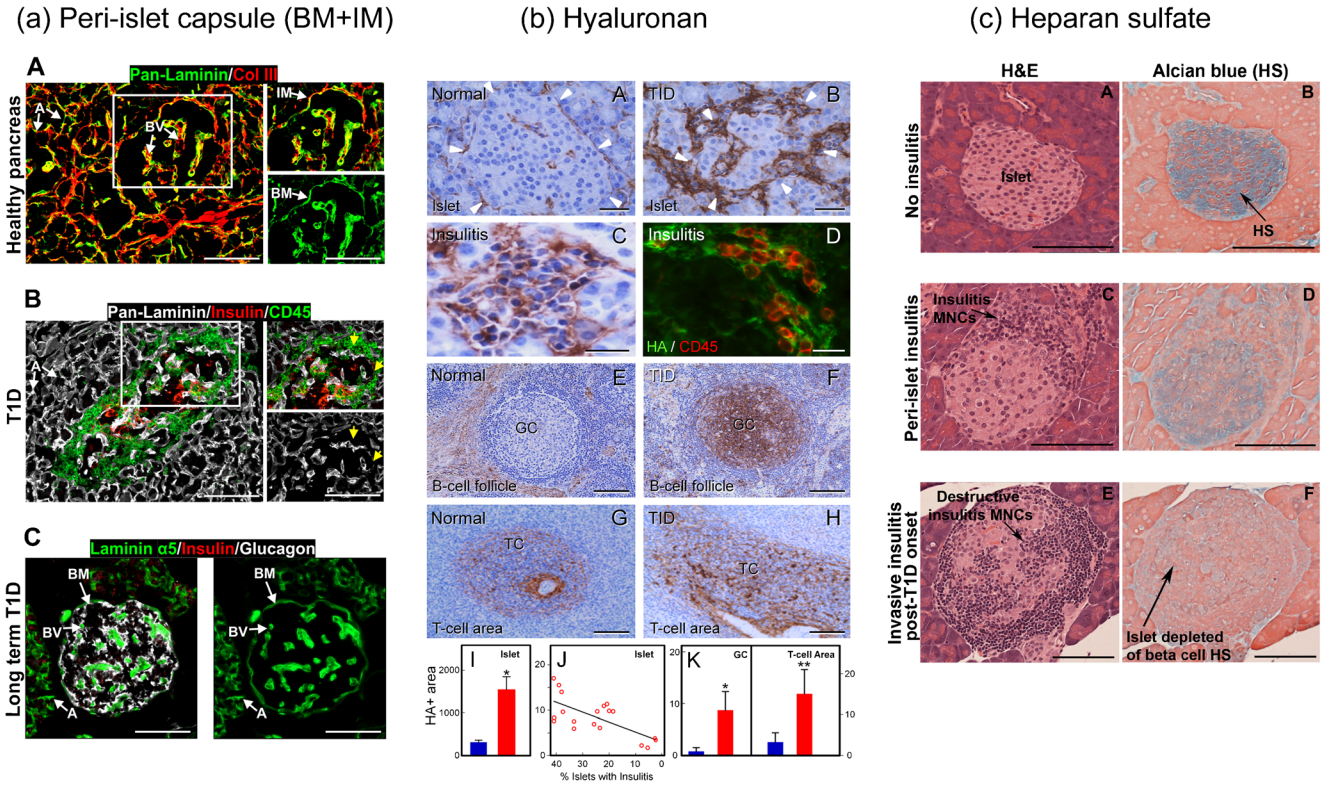

Fig. 1 Altered morphologic patterns of specific islet extracellular matrix components in T1D. **a** Disruption of the peri-islet basement membrane (BM) in T1D. (*A*) Immunofluorescence staining of the healthy human pancreatic islet for collagen type III to visualize the interstitial matrix (IM) of the peri-islet capsule and pan-laminin to mark the peri-islet basement membrane (BM). Panels A is from [15••]. Copyright © 2013, American Diabetes Association. Copyright and all rights reserved. Material from the publication has been used with the permission of American Diabetes Association. (*B*) In the inflamed islet of a T1D patient, the peri-islet BM staining (*arrows*) is lost only at the sites of leukocyte penetration into the islet. *Insets on the right hand side* show high magnifications of healthy and T1D pancreatic islets. (*C*) In long-term T1D islets, which are insulin-negative/glucagon-positive, the peri-islet BM is regenerated once inflammation has subsided, as shown by immunofluorescence for pan-laminin. *BV* blood vessels, *A* acinar BM. *Scale bars* 100 μm. **b** Hyaluronan (HA) accumulates in human pancreatic islets and insulitis areas, and lymphoid tissues in T1D. HA localizes outside the endocrine cells in normal human islets (*A*) and accumulates along the islet microvessels in T1D (*B*). HA also accumulates in regions of insulitis, (*C*) where it forms a meshwork around the CD45-positive inflammatory cells (*D*). HA is sparse in follicular germinal centers (GC) in normal pancreatic lymph nodes (*E*) while it is abundant in follicular GCs in T1D (*F*). HA appears to form a network in the T cell (TC) areas in normal spleen tissues (*G*) and its patterns are altered in these areas in T1D (*H*). *Scale bars* 25 μm (*A–D*) and 100 μm (*E–H*). Quantitative analysis of HA-stained areas in pancreatic islets (*I*) and specific regions of immune cell activation in lymphoid tissue (*K*) in T1D. (*J*) Correlation analysis between HA accumulation and prevalence of insulitis. *Red bars* and *circles* diabetic tissues, *blue bars* control tissues. *$P<0.005$ vs. normal tissues. Panels are from [20]. Copyright © 2014, American Diabetes Association. Copyright and all rights reserved. Material from the publication has been used with the permission of American Diabetes Association. **c** Loss of heparan sulfate (HS) in islets of diabetic NOD mice. NOD mouse pancreas specimens show strong histochemical staining of intra-islet HS by Alcian blue in an islet without insulitis, as indicated by histological staining with hematoxylin and eosin (H&E) (*A*, *B*), intra-islet HS present in an islet with predominantly nondestructive insulitis mononuclear cells (MNCs) (*C*, *D*), and dramatic loss of HS in islet tissue with destructive insulitis post-T1D onset (*E*, *F*). *Scale bar* 100 μm

Barrier Function of Basement Membrane in T1D

Leukocyte Extravasation Occurs Only at Postcapillary Venules

Autoreactive T cells in T1D develop in the pancreatic lymph nodes and subsequently migrate to the pancreas where they first must extravasate from the postcapillary venules (PCVs) that surround the islets and subsequently penetrate the peri-islet BM before they gain access to the insulin-producing β cells (Figs. 1 and 2). In most inflammatory situations, with the possible exception of the lung [32], leukocyte extravasation occurs only at PCVs [5], where the blood flow is relatively slow, the shear forces are decreased and where the appropriate adhesion molecules are expressed by the endothelial cells [33].

Vascularization of pancreatic islets shows similarities to kidney glomeruli; the arterioles penetrate the islet, capillarize, and leave the islet as PCVs, which collect into venules [34]. Although the identification of the blood vessels from which the first autoreactive T cells extravasate is extremely difficult due to high degree of islet vascularization, the first inflammatory cells recruited to the islet in both mice and humans are always apparent outside of the peri-islet BM, and it is therefore considered that leukocyte extravasation in T1D takes place at the

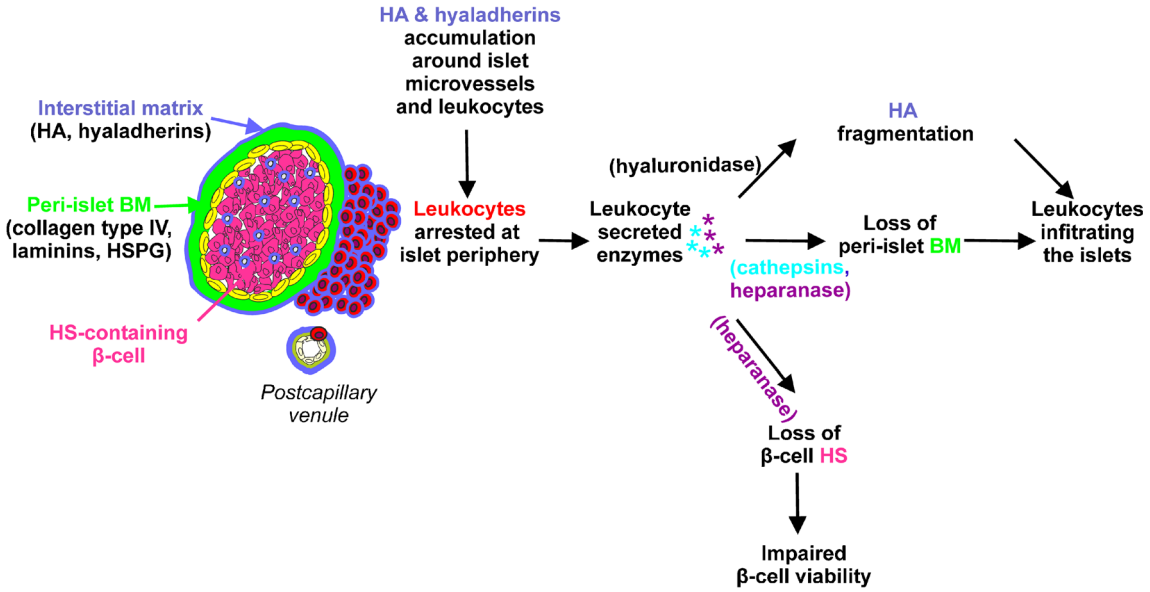

Fig. 2 Schematic representation of the possible contributions of the extracellular matrix to inflammatory cell infiltration of the pancreatic islet in T1D. Following islet injury, induced changes in the local environment promote an inflammatory process resulting in the migration of leukocytes from the circulation into the pancreatic islet. During this process, HA accumulates along the islet microvasculature and serves as "glue" for the leukocytes extravasating from the PCVs, causing leukocyte arrest at the islet periphery. Degrading and proteolytic enzymes released by the arrested leukocytes breakdown islet ECM constituents. Consequently, molecular interactions among the ECM components are destabilized, leading to disruption and finally loss of the peri-islet basement membrane allowing leukocyte invasion of the islet. Leukocyte cell surface-associated or vicinal HA and fragmented HA provide cues for leukocyte activation and phenotypic changes, further promoting islet inflammation. In addition, loss of HS from the pancreatic β cells contributes to a decrease in β cell survival

PCVs that are localized at the periphery of the islets. In other tissues, the laminin α4/α5 content of the PCVs has been shown to define sites of extravasation, with laminin α5 low sites defining sites of preferred extravasation [35–38]; whether this is also the case in the pancreas is difficult to define because of the high density and tortuosity of the peri-islet vessels.

Penetration of Peri-Islet BM Barrier

Upon extravasation from blood vessels, the leukocytes migrate through the thin IM and must then penetrate the barrier presented by the peri-islet BM. Immunofluorescence studies have revealed a global loss of peri-islet IM and BM components only at sites of leukocyte infiltration into the islet (Figs. 1 and 2) in both mice [12, 13, 15••] and humans [15••]. Stereological analyses revealed a correlation between incidence of insulitis and the number of islets showing loss of peri-islet BM vs. islets with intact BMs, suggesting that leukocyte penetration of the peri-islet BM is a critical step in disease development. This general loss of the peri-islet ECM suggests either involvement of several proteases with different substrate specificity or proteases with broad proteolytic activity. Using protease- and protease-inhibitor-specific microarray analyses (CLIP-CHIP) [39] of laser dissected islets showing leukocyte infiltration or no infiltration, we have identified members of the cathepsin family, cysteine proteases, only in cases where peri-islet BMs were penetrated by leukocytes [15••]. Cathepsins are best known as lysosomal proteases, active at low pH in the lysosomes; however, in certain situations, some members of this family can be secreted extracellularly and can be active at neutral pH. Cathepsins C, S, H, and W are all upregulated at the messenger RNA (mRNA) level in inflamed islets, and immunofluorescence microscopy has revealed their expression by a subset of macrophages and dendritic cells (DC) localized specifically at the infiltrating front of leukocytes moving into inflamed islets. This suggests that cathepsins secreted by macrophages and DCs may be involved in leukocyte penetration of the peri-islet BM. Whether cathepsins are involved directly in the cleavage of BM components or whether they exert an indirect effect by activating other proteases or degrading some protease inhibitors is not yet clear, although several ECM molecules, including laminins and collagens, have been reported to be cleaved by cathepsin S [40–43]. Several matrix metalloproteinases (MMPs), specifically the gelatinase MMP-2, MMP-7, and MMP-14, are upregulated at the mRNA level in CLIP-CHIP analyses but could not be confirmed at the protein level. Rather, MMP-2 (and potentially also MMP-9) activity was associated with the healthy part of the islet. Nevertheless, MMPs may still promote inflammation by cleaving non-ECM substrates, such as chemokines and cytokines [44, 45••].

Selective cleavage of ECM molecules, resulting in bioactive fragments with functions distinct from the parent molecule, has been proposed in several inflammatory models [46, 47]. Given the extensive loss of ECM at sites of leukocyte

infiltration of the pancreatic islet, there is the potential that similar processes may occur in diabetes; however, further investigation using mass spectrometry for identification of such bioactive matrix fragments is required.

In addition to proteases, several protease inhibitors are upregulated in inflamed islets as defined by CLIP-CHIP analyses, including TIMP-2, a specific inhibitor of MMP-2, and the serine protease inhibitors (serpins), which are most likely to counteract the proteolytic activity of proteases [15••]. This substantiates the hypothesis that several different proteases are likely to be active concurrently at sites of leukocyte penetration of the peri-islet BM.

Peri-Islet Capsule Regeneration

Pancreases of long-term non-obese diabetic (NOD) mice and T1D patients contain pancreatic islets devoid of insulin staining but harboring an intact peri-islet capsule (Fig. 2). This suggests that the peri-islet BM-producing cells are not lost during disease development and are able to reconstitute the peri-islet BM once the inflammation has subsided [15••]. The close association of glucagon producing α cell with the peri-islet BM [15••] may suggest that these cells secrete the BM components; however, other cellular sources such as Schwann cells and myofibroblasts, which are abundant around the islet, cannot be excluded. Identification of the cells responsible for secretion of the peri-islet BM is likely to open new avenues in the development of islet transplantation strategies.

Concluding Remarks and Future Perspectives

In contrast to leukocyte extravasation from PCVs where there is no evidence for extensive proteolysis [37], leukocyte penetration of the peri-islet BM is associated with broad loss of several ECM molecules which can only be achieved by the activity of different proteases or broad specificity proteases, one of which is the cathepsin family [15••, 37]. As BMs of the PCVs and those surrounding the pancreatic islets are biochemically distinct, this suggests that leukocytes can recognize such differences and adjust their mode of penetration accordingly. Understanding the mechanisms of loss of the peri-islet BM is important for the development of strategies to prevent autoreactive T cell infiltration into the islet and, as such, the destruction of β cells.

Heparan Sulfate

Heparan Sulfate Is a Major Glycosaminoglycan in Basement Membranes and Extracellular Matrix

HS is an unbranched glycosaminoglycan or linear polysaccharide that is attached to core proteins and present in the ECM, including BM, IM, and on the surface of cells. HS chains contain repeating disaccharides of uronic (glucuronic or iduronic) acid and glucosamine, with each chain being covalently attached to the core protein of HSPGs [28, 48–50]. The core proteins of HSPGs commonly consist of syndecan (1–4) and glypican (1–6) on the cell surface and collagen type XVIII, agrin, and perlecan in BMs [51]. During HS assembly, a process that takes place in the Golgi compartment of cells, the chains undergo chemical modification, including epimerization of glucuronic acid to iduronic acid, *N*-deacetylation/*N*-sulfation, 3-*O*- and/or 6-*O*-sulfation of glucosamine, and 2-*O*-sulfation of iduronic acid. HS chains are heterogeneous in their chemical modification and length (14–45 kDa). However, invariably, they contain regions that are highly sulfated and other regions that are non-sulfated or under-sulfated [49, 50, 52]. The highly sulfated regions, together with the carboxyl groups of glucuronic acid, make HS chains highly negatively charged, a property that permits binding to a wide range of signaling proteins including growth factors, chemokines, and cytokines, as well as certain enzymes [28, 48, 50, 53]. The function of HS as a local reservoir for proteins is characteristic for cell surface HSPGs and HSPGs in the ECM, with the bound proteins being liberated and rendered biologically active following cleavage of the HS chains by the HS-degrading enzyme, heparanase [28, 49, 50]. In BMs of the pancreas, HS exists predominantly in the form of the HSPG perlecan and interacts with other major matrix proteins, particularly laminin and collagen type IV, to provide mechanical support and to act as a barrier to cell migration [28, 50, 54]. In fact, to enable cells of the immune system (leukocytes) to migrate from the vasculature to sites in underlying tissue, both proteinases and heparanase need to be produced locally to degrade matrix proteins and HS, respectively, in the subendothelial BM [28].

Heparan Sulfate and Normal Islet Biology

Studies of the pathology of islets during the development of autoimmune T1D in NOD mice have consistently demonstrated the peri-islet accumulation of mononuclear cells (MNCs) [55, 56]. This initial blockade to islet entry implicated a physical barrier around the perimeter of the islet tissue, most probably a peri-islet BM. Our studies demonstrated that mouse islets in situ are surrounded by a continuous peri-islet BM containing the conventional BM protein components, collagen type IV, laminin, and nidogen, as well as the HSPG perlecan [13]. In addition, extensive immunohistochemical and histochemical studies from our group of the distribution of HS in mouse islets in situ revealed the novel finding that HS is expressed at extraordinarily high levels throughout normal islet tissue (Fig. 1). Flow cytometry analyses of isolated mouse β cells demonstrated that HS was localized intracellularly in islet β cells [14••]. This high intracellular level of HS

in β cells in situ is a unique property of islet tissue, because normally HS is localized on the cell surface or extracellularly. We found that during islet isolation in vitro, islets lose approximately 50 % of their intra-islet HS, a property that correlated with the rapid death of β cells in culture. β cells were rescued from dying in vitro and from acute oxidative damage (hydrogen peroxide-mediated death) by replacing the lost HS with an exogenous source of HS, including HS mimetics such as heparin [14••]. Together, these studies indicated that intracellular HS is essential for β cell survival, probably by capturing reactive oxygen species (free radicals) and neutralizing their activity [57]. Overall, HS distributed in the peri-islet BM and at unusually high levels inside islet β cells protects islets from cell invasion and preserves β cell viability, respectively [13, 14••], properties which substantially extend the previously reported function of HS in β cell differentiation [58].

Heparan Sulfate and T1D

We discovered that HS at both peri-islet and intra-islet sites represents a critical target for destruction during the development of autoimmune T1D in NOD mice, a recognized preclinical model for T1D in humans. Firstly, we found that intra-islet invasion by insulitis leukocytes correlated with solubilization of the peri-islet BM, including the HSPG perlecan [13], and the maximal production of catalytically active heparanase (an HS-degrading enzyme [28, 59]) at T1D onset [14••]. Beyond the BM breach, there was a progressive decline in islet β cell HS (Figs. 1 and 2). In parallel, we observed that HS depletion in β cells in vitro resulted in cell death, potentially via an increased susceptibility to oxidative damage. Directly supporting a central role for HS loss in T1D development, prediabetic female NOD mice treated with the heparanase inhibitor/HS mimetic PI-88 prevented T1D in 50 % of T1D-susceptible mice, significantly increased the population of intact islets, reduced the percentage of islets with destructive insulitis, and preserved intra-islet HS [14••]. Taken together, we established a novel role for heparanase-mediated degradation of HS in the onset of destructive insulitis and β cell death, ultimately leading to insulin insufficiency and T1D (Fig. 2). The strategic localization and biological roles of HS in islets therefore render β cells exquisitely vulnerable to heparanase-induced damage. This new paradigm for T1D pathogenesis, however, does not exclude a role for proteolytic enzymes (produced by leukocytes) from also contributing to the destruction of the peri-islet BM [15••].

Future Directions in T1D

Our preclinical studies of autoimmune T1D are supported in part by studies of human T1D pancreas specimens. Human T1D, as reviewed here, is characterized by a modified matrix in the islet microenvironment, including the striking extra-islet deposition of the glycosaminoglycan HA [20••] and breakdown of the peri-islet BM containing the HSPG perlecan [15••]. Our T1D investigations in NOD mice exemplify how both unintuitive and predictable discoveries in normal β cell biology, in particular the role of high intracellular levels of HS in maintaining islet β cell viability and HS as an integral constituent of the islet BM barrier, respectively, can unveil novel and potentially crucial targets for destruction in T1D development. Indeed, our experimental findings in NOD mice urge us to reconfigure our conventional understanding of "cytotoxic" T cell-induced β cell death in T1D and to embrace immune-mediated perturbations in islet and β cell "matrix" as critical components of human T1D pathogenesis (Fig. 2). Studies of the localization of HS in nPOD specimens of normal and T1D human pancreas are in progress and will elucidate whether β cell HS is also targeted in human T1D. Recent clinical trials of immune modulators have failed to sustain improved outcomes and long-term insulin-independence [60, 61]. We propose that dual activity drugs acting as both heparanase inhibitors and HS replacers may represent a novel therapeutic for preventing the progression of T1D and its associated vascular complications in newly diagnosed T1D patients, and for protecting high-risk individuals from T1D onset.

Hyaluronan and Hyaladherins

Hyaluronan Is an Active Participant in Inflammatory Responses

HA is a high-molecular-weight linear polysaccharide composed of disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid [62, 63]. The polysaccharide is a component of the cell surface and the extracellular environment and is ubiquitously distributed in all tissues. Initial studies described this polymer as an inert material filling the extracellular space and serving to stabilize the physical structure of the tissues [64]. Discovery of cellular receptors that specifically recognize HA led to unraveling of important regulatory functions of the molecule in different cellular responses elicited by changes in the microenvironment [63, 65]. The linear structural simplicity makes HA unique among the ECM glycosaminoglycan polysaccharides. The molecule does not contain any sulfate, is not found covalently attached to proteins, and can form polymers of variable molecular sizes and conformations. Occurrence of HA in different configurations leads to a diversity of specific interactions of HA with other ECM molecules. These interactions modify the structure of HA and the properties of the molecule, and promote formation of a variety of HA complexes with different physiological and biological

functions. A highly regulated equilibrium between the synthesis, sizing, and removal of HA is crucial to its functions in development, tissue homeostasis, and disease [66, 67, 68••, 69]. Importantly, HA has been increasingly implicated in the regulation of immune responses [28, 63, 67, 70]. Intact HA in its high-molecular-weight form is intrinsically anti-inflammatory [67, 68••]. High-molecular-weight HA contained in the pericellular matrix of tissue-resident cells protects them from lymphocyte-mediated cell killing and inhibits angiogenesis [68••]. Whereas enhanced HA production and fragmentation during inflammation has implied a role for HA in promotion and maintenance of inflammatory processes (Fig. 2) [67, 71, 72].

We and others have proposed that HA plays a decisive role in immune regulation [67, 73••]. HA regulates the major components of an inflammatory response such as vascular permeability, edema, and leukocyte extravasation [67, 74]. ECM enriched in HA is generated by tissue-resident cells in response to inflammatory mediators and influences the accumulation of myeloid and lymphoid cells during inflammation [72, 73••, 74–76, 77••, 78••, 80]. In inflamed tissues, HA interactions with leukocytes are governed by a diverse group of HA-binding proteins, called hyaladherins, such as inter-α-inhibitor (IαI), versican, and tumor necrosis factor-stimulated gene-6 (TSG-6) [66]. The hyaladherins cross-link HA into structures with different architectures and functions [72, 79, 80]. This cross-linked HA interacts with a variety of cell surface proteins, proteases, chemokines, and growth factors to regulate leukocyte migration, adhesion, and activation. For instance, during the inflammatory process, covalent transfer of heavy chains (HCs) from IαI to HA catalyzed by TSG-6 forms the HC-HA complex that promotes the adhesion of leukocytes to HA-rich matrices [72, 74, 79]. Versican, another proteoglycan that binds HA, also contributes to the formation of a cross-linked HA/versican-rich complex that is capable of regulating leukocyte adhesion, proliferation, and migration.

Both the relative amount of HA as well as the size of HA molecules are of physiological importance. Uninjured tissues contain high-molecular-weight (>1000 kDa) HA. Following tissue injury, intact HA breaks down into fragments of low-molecular-weight (<250 kDa) HA and short (<30 kDa) HA oligomers generated through enzymatic degradation of intact high-molecular-weight HA by endogenous hyaluronidases (Fig. 2) as well as catabolism by a diverse group of microbial hyaluronidases, mechanical forces, and oxidative stress [67, 70, 81]. The HA fragments predominate during injury and inflammation and their persistence leads to unremitting inflammation. The association of HA with chronic inflammation is found in many different tissues as well as in animal models, where HA has been implicated in key events in adaptive and innate immune responses [71, 82, 83].

Involvement of Hyaluronan and Hyaladherins in T1D Pathogenesis

T1D results from an attack by immune cells on the insulin-producing β cells in the pancreas [84, 85]. Although the triggering mechanisms remain unknown, much of this immune response directed against islet autoantigens is initiated in the lymphoid tissue of pancreatic lymph node and spleen. The immune cells encounter the antigen and undergo phenotypic changes in specialized regions of B cell follicles and inter-follicular T cell areas, and then egress into the circulation to reach the target tissue. In this respect, the structure of the ECM in both lymphoid tissue and the pancreatic islet is important since it is the milieu within which the immune cells become activated and reach the pancreatic β cell. The precise role that HA plays in T1D pathogenesis has yet to be elucidated. However, evidence collected from in vitro and in vivo studies point to a role for HA in events associated with immune regulation in T1D. For example, we have found that a HA-rich matrix plays a role in controlling T cell movement [77••]. In addition, the suppressor activity and viability of regulatory T cells is augmented by intact HA which also induces phenotypic maturation of the dendritic cells and production of cytokines [86–90]. The localization of HA to the immune synapse suggests a key role in antigen presentation [88, 90]. In vivo studies targeting the HA receptor, CD44, with monoclonal antibodies led to resistance to diabetes development in the NOD mouse [91]. Weiss et al. also found that injections of hyaluronidase 1 h before cell transfer of diabetogenic splenocytes prevented the development of diabetes [92••]. Recently, Kota et al. reported that TSG-6, a HA-binding protein, secreted by human mesenchymal stem cells in vivo was one of the factors that led to delayed onset of T1D in NOD mice, in part by suppressing antigen presentation and activation of cytotoxic T cells [92••]. Collectively, these studies indicate multiple mechanisms by which HA and associated proteins can impact events associated with development of T1D.

Hyaluronan and Hyaladherins in Regulation of Insulitis

The insulitis lesion in human T1D tissues collected at disease onset represents infiltrates of cells of both myeloid and lymphoid lineage, with the CD8-positive cytotoxic T cells being the most abundant population [93, 94]. The constrained movement of cytotoxic T cells within the islets suggests that changes in the islet microenvironment surrounding the β cells have taken place prior to encounter of these cells with the β cell. This hypothesis is supported by our recent observations of altered morphologic patterns of HA and hyaladherins in human T1D pancreatic islets (Fig. 1) [20••]. We demonstrated that HA increased in islets of younger T1D donors within 10 years of disease onset. Moreover, HA preferentially

accumulated in insulin-containing diabetic tissues, in particular those collected within the first year from diagnosis and which had lost a portion of their β cells. The presence of smaller HA deposits in pancreatic tissues that did not contain insulin-positive islets indicates that changes in HA precede β cell loss. Different from the young donors, pancreas tissues of T1D donors with longer disease duration (28–66 years) showed HA morphologic patterns similar to those identified in control tissues. It is possible that the normal islet HA patterns in long-standing T1D result from an earlier process of HA remodeling leading to clearance of HA accumulated initially during the course of the disease. HA accumulation in tissues of younger T1D patients was accompanied by changes in the distribution and quantity of hyaladherins, IαI, and versican, which increased in HA-rich regions in human T1D islets, while TSG-6 was decreased. HA and hyaladherins also accumulated in insulitis areas, independent of disease duration. Dispersed or aggregated inflammatory cells were surrounded by HA, IαI, and versican (Fig. 1). We have observed similar accumulations in the islets of NOD mice [73••] and two other autoimmune models, the BB rat (Bogdani et al., unpublished data) and the DORmO mouse (Nagy et al., unpublished data). The functional significance of these events remains to be determined. However, physical association of HA, versican, and IαI with islet inflammatory infiltrates implies a possible relationship between the deposition of these ECM molecules and accumulation of leukocytes [63, 72, 74–76, 77••, 79, 80]. Such relationships have been observed during inflammatory events in other tissues. Clearly, HA and proteins that associate with HA form a matrix that interacts with myeloid and lymphoid cells.

We have also observed that HA and IαI occur in specific regions of pancreatic lymph nodes and spleens in T1D (Fig. 1) [20••]. HA and IαI accumulated in the follicular germinal center (GC) and T cell areas, specialized regions of lymphoid tissues in which B cells and T cells come into contact with their cognate antigens and subsequently undergo activation and differentiation. It is possible that HA present in these immune cell areas promotes T cell phenotype changes via modulation of immune cell interactions or their migratory and adhesive characteristics [73••, 95].

The observation that there are specific changes in HA and associated proteins, both in pancreatic islets as well as in pancreatic lymph nodes and spleen, suggests that in diabetic subjects, the HA within these tissues may potentiate immune responses and contribute to failures in immune tolerance. Our findings of increased amounts of HA and specific hyaladherins in islets and lymphoid tissues of T1D subjects suggest new areas of investigation regarding the functional significance of these ECM components in the development of T1D. Further, our observations suggest that events contributing to T1D are also taking place in tissues other than the pancreatic islets, which could provide a basis for a paradigm shift in the way we think about T1D pathogenesis.

Future Questions

What we do not know is the precise location of HA within the islets, lymph nodes, and spleen; the identity of the cells that produce it; how the organization of HA is altered in T1D; and whether size and amount of HA are important in regulating key immune events that lead to the destruction of pancreatic β cells in T1D. Answers to these questions will determine if this ECM molecule may represent a therapeutic target for the treatment of this disease in the future.

Concluding Remarks

This review highlights recent data pertaining to the involvement of different components of the islet ECM in immune cell infiltration of the pancreatic islet in T1D. It is clear that remodeling of the ECM occurs in pancreatic islet and lymphoid tissues in T1D. Alterations in the structural complexes of individual ECM components are postulated to create a permissive environment for immune cell infiltration into the pancreatic islets and for impairment of β cell survival (Fig. 2). Although the triggering mechanisms of immune cell attack of the pancreatic β cells are still unknown, the ECM may, in part, regulate key events in pathogenesis of T1D such as β cell death, immune cell migration, proliferation and invasion, cytokine expression and release, and antigen presentation.

In fact, the ECM may be a critical component in determining immune tolerance. The nature of ECM in insulitis and its role in T1D pathogenesis deserve to be explored further. Future studies should address whether and how targeting of specific ECM components can affect T1D progression.

Acknowledgments The basement membrane work was supported by the European Foundation for the Study of Diabetes/Juvenile Diabetes Research Foundation (JDRF)/Novo Nordisk A/S (BD21070), JDRF (1-2005-903), and German Research Foundation Collaborative Research Center (CRC) (1009 and SO285/9-1) (L.S.). The heparan sulfate work was supported by a National Health and Medical Research Council of Australia (NH & MRC)/JDRF Special Program Grant in Type 1 Diabetes (#418138; to C.R.P.), a NHMRC Project Grant (#1043284; to C.J.S.), and a research grant from the Roche Organ Transplantation Research Foundation (ROTRF)/JDRF (#477554991; to C.J.S.). The hyaluronan work was supported by JDRF nPOD grant 25-2010-648, and NIH/NIAID grants U01 AI101990 and U01 AI101984 (T.N.W.). This research was performed with the support of the Network for Pancreatic Organ Donors with Diabetes (nPOD), a collaborative type 1 diabetes research project sponsored by JDRF. Organ Procurement Organizations (OPO) partnering with nPOD to provide research resources are listed at http://www.jdrfnpod.org/for-partners/npod-partners/.

Compliance with Ethics Guidelines

Conflict of Interest Marika Bogdani declares that she has no conflict of interest.

Eva Korpos declares that she has no conflict of interest.

Charmaine J. Simeonovic has received research support through a grant from The Australian National University, is currently a shareholder of Beta Therapeutics, and currently has two patents pending.

Christopher R. Parish has received research support through a grant from The Australian National University, is currently a shareholder of Beta Therapeutics, and currently has two patents pending.

Lydia Sorokin declares that she has no conflict of interest.

Thomas N. Wight declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human subjects performed by any of the authors. Studies with animals were approved by the relevant institution's Animal Care and Use Committee and have been previously published.

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- • Of importance
- •• Of major importance

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