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Thinking Outside the Cell: A Key Role for Hyaluronan in the Pathogenesis of Human Type 1 Diabetes

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> The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.
>
> —William Lawrence Bragg

For more than 50 years, chronic immunological processes have been considered central to type 1 diabetes pathogenesis. Studies in pancreata from patients with type 1 diabetes have revealed the presence of insulitis, identified histologically as immune cell infiltrates around and within the islets. Finding the insulitis lesion in a portion of patients with recent-onset type 1 diabetes indicates heterogeneity of the pathogenic process, while the uneven occurrence of the lesion within diabetic pancreata supports the view that the process of islet damage does not take place in all the islets concomitantly. The intermittent pattern of insulitis and the differential recruitment of islets into the pathological process despite the continuous presence of β-cell autoreactive immune cells in circulation suggest the islet pathological process may not be solely dependent on the presence of these cells. Changes in islet tissue-specific structural characteristics and in the local microenvironment may take place in the course of islet inflammation, which predisposes for islet invasion by the immune cells. Local tissue extracellular matrix (ECM) constituents are active participants in the regulation of in situ inflammatory processes during which the functional and structural properties of the local tissue components and of the immune cells themselves are continuously modulated. Recent studies in human diabetic pancreata have indicated the presence of greatly altered hyaluronan (HA), a major ECM component, in the diabetic islets, which is associated with the extent of invasive insulitis and β-cell loss. These novel observations led to the hypothesis that HA guides immune cell migration into the islets and regulates the immune cell phenotype and that alterations in islet HA contribute to the increased vulnerability of the β-cells to inflammatory insult. This Perspective reviews the evidence supporting a key role for this ECM component in type 1 diabetes pathogenesis.

COMPOSITION AND PROPERTIES OF THE ECM

> Most cells are surrounded by a … network of outer defenses and scaffoldings … . Such structures are not part of the cells but are built from precursor material that are secreted by the cells and that subsequently join together into a variety of combinations of almost every possible shape or consistency … they provide every sort of visible form that life creates on our planet. Without them there would be nothing but an amorphous covering of oozy slime made of a myriad of naked cells crawling over each other. (1)

With these words, Christian de Duve defines the extracellular substance, termed the ECM, and the essential function of the ECM structures as the physical support to the living matter. ECM functions extend beyond simply supporting and buttressing the tissue parenchymal cells and provide biochemical and biomechanical cues to the cells crucial for tissue development, function, and homeostasis. The extracellular matter exerts an active role in the regulation of a variety of cellular activities such as cell adhesion, migration, proliferation, and differentiation.

While there is considerable variety in the composition of different tissue ECM, fundamentally, the ECM components form two morphologically distinct types of matrices, the basement membrane (BM) and the interstitial matrix (IM), which occur as adjacent structures in vivo (Table 1) (2). The ECM forms a reciprocal relationship with the cells in contact with it, which significantly influences cellular behavior (3,4) and it is critical for tissue development and maintenance of mature tissue homeostasis as illustrated by the consequences of genetic abnormalities in the ECM proteins. Genetic deletion of HAS2,

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Table 1—The major components of the ECM

IM
Nonproteoglycan polysaccharides, HA
Proteoglycans HS Chondroitin sulfate Keratan sulfate Other (versican, aggrecan, neurocan, brevican, biglycan, decorin, lumican)
Collagen Fibrillar (type I, II, III, V, XI) Short chain (type VIII, X) FACIT (type IX, XII, XIV, XVI, XIX-XXII) Other (type VI, VII, XIII, XVII, XXIII-XXIX)
Elastin
Fibronectin
BM
Collagen type IV, XV, XVIII
Laminin
HS proteoglycans
Nidogen/entactin

FACIT, fibril-associated collagens with interrupted helices.

the major HA synthesizing enzyme, or the absence of different types of laminin or of both chains of the collagen IV isoform causes embryonic lethality due to defective cardiac and neural morphogenesis or defects in BM stability (5–7). Deletion of the genes encoding the enzymes involved in the synthesis or undersulfation of heparan sulfate (HS) cause cell growth arrest early in embryonic development (8,9).

ECM IN INFLAMMATION

An increased rate of ECM remodeling is observed under pathological conditions such as fibrotic diseases and cancer and is particularly high during inflammation (10). The tissue ECM could be envisioned as the "ground" for the passage of the immune cells from the blood into the injured tissue. Migrating immune and damaged parenchymal cells at the site of inflammation release inflammatory mediators that affect the expression of different ECM molecules and enzymes that break down ECM. As a result, ECM components are either generated in excess and deposited, fragmented, or lost. These modifications in the amount and composition of ECM result in a remodeled matrix endowed with the capacity to amplify immune cell recruitment in a feed-forward manner and to affect the behavior of these cells.

Accumulation of ECM results from altered enzymatic activity and is a major histopathological feature of inflammatory conditions such as autoimmune diseases, granulomatous diseases, and fibrosis. Deposition of collagen and fibronectin in tissues takes place in inflammatory bowel disease, asthma, scleroderma, lupus nephritis, and glomerulonephritis (11–14). Significant increases in HA and proteoglycans have been observed in rheumatoid arthritis, inflammatory bowel disease, chronic inflammatory vascular disease, lupus nephritis, Graves ophtalmopathy, and type 1 diabetes (15–20). The marked accumulation of the ECM in human tissues in different diseases and in corresponding experimental animal models (21–24) precedes or coincides with an influx of inflammatory cells, suggesting that the altered ECM influences the trafficking and recruitment of leukocytes into the site of inflammation.

Infiltrating leukocytes and the resident cells in the inflamed tissues release proteolytic and degrading enzymes that cause degradation of intact ECM molecules into fragments. The ECM fragments are bioactive and may serve as chemoattractants for leukocytes to the site of inflammation. Collagen, fibronectin, HA, HS, and elastin fragments are chemotactic for neutrophils, monocytes, and lymphocytes; augment phagocytic functions of macrophages; and modulate gene expression of mononuclear cells (22,25–27). Instillation of elastin fragments or intratracheally administered collagen I peptides recruit monocytes and neutrophils in the rat lung in vivo, while HA fragments generated by overexpression of hyaluronidase 1 activated migration of skin dendritic cells toward regional lymph nodes (28–30).

Other data indicate that besides promoting immune cell migration, the ECM has the capacity to regulate immune cell activation, gene expression, proliferation, survival, and differentiation (31–33).

Given the engagement of the ECM in chronic inflammatory responses, it is likely that the ECM is involved in islet inflammation, a chronic process that is associated with altered local tissue integrity and loss of insulin-producing β-cells, brought about by infiltrating immune cells (34).

IMMUNE CELL MIGRATION INTO THE INFLAMED ISLET IN TYPE 1 DIABETES

Entry of immune cells from blood through the blood-islet endocrine cell barrier of microvasculature and the ECM into the islets is a key step in the development of insulitis. Immune cell trafficking (35,36) is a three-step process in which the selectin-dependent initial adhesion of leukocytes to vascular endothelium (step 1) is a prerequisite for their subsequent chemokine- and integrin-regulated firm adhesion (step 2). Finally, utilizing protease-dependent or -independent mechanisms, leukocytes migrate through the endothelial wall and the BM into the underlying tissue (step 3; transmigration) where they receive additional signaling cues that guide them to specific tissue environments.

L-selectin, chemokines, and integrins have been implicated in the development of diabetes in NOD mice. Blockade of L-selectin by early administration of anti–L-selectin monoclonal antibody impaired the development of adoptively transferred diabetes (37), yet other studies (38) indicate that L-selectin may not be required for leukocyte migration in insulitis.

Chemokines involved in the firm adhesion step of leukocyte transmigration have been associated with progression to type 1 diabetes. Serum levels of chemokine CXCL10

were elevated in patients recently diagnosed with type 1 diabetes (39,40). CXCL10 was present in human diabetic islets, while its receptor CXCR3 was expressed in insulitic cells (41,42). Development of spontaneous insulitis in RIP-CXCL10 transgenic mice and prevention of diabetes in mice by antibody blockade of CXCL10 or genetic deletion of CXCR3 indicated that the CXCL10–CXCR3 axis could be important in type 1 diabetes (43,44). However, a recent study challenged this view (45). CXCR3 was also expressed by autoreactive preproinsulin-specific $CD8^+$ cell clones derived from patients with type 1 diabetes (46). When such islet-reactive $CXCR3^+$ T cell clones isolated from patients prior to or at clinical onset of type 1 diabetes were transferred into hyperglycemic NOD-SCID mice, the $CXCR3^+$ clonal cells were observed around blood vessels in the exocrine pancreas but not in the inflamed islets, indicating that CXCR3 interactions per se are not sufficient to guide the entry of T cells into the inflamed islets (47). CCL19 and CCL21, which interact with CCR7 to direct the migration of T cells into lymphoid tissues, were also highly expressed in insulitis areas, and conversely CCR7-deficient NOD mice did not develop diabetes (48,49).

Lymphocyte integrins LFA-1, VLA-4, and LPAM-1 and the cell surface receptors they interact with to enable firm adhesion of leukocytes on the vascular endothelium may regulate immune cell migration in the initial phase of insulitis. Treatment of neonatal NOD mice with a combination of antibodies against α4, β2, and β7 integrin subunits and their ligands VCAM-1, MadCAM-1, and ICAM-1 led to retention of $CD4^+$ and $CD8^+$ T cells and macrophages at the islet periphery (50,51). This treatment was less effective when administered to young adult NOD mice and ineffective following the adoptive transfer of diabetogenic cells (52,53). It is possible that integrin-mediated immune cell adhesion is important during the early phase of insulitis and that the inflammatory islet microenvironment may give rise to new cell–cell and cell–matrix interactions that facilitate integrin-independent cell trafficking.

Additional chemokines, chemokine receptors, integrins, and cytokines, such as CCL2, CCL3, CCL5, CXCL12, CCR2, CXCR4, IFN-γ, and integrin αLβ2, have been considered as modulators of the interactions of diabetogenic T cells with islet vascular endothelium (43,54–56), but whether these molecules are essential to these interactions is not known.

The sequence of the events and the precise mechanisms of islet immune cell infiltration in human insulitis are still unknown. In the process of extravasation, leukocyte cross talk with the tissue ECM present on the surface of endothelial cells and in the extracellular environment suggests that interactions between leukocytes and the ECM are important in the regulation of immune cell trafficking.

CONTRIBUTION OF ISLET ECM TO INSULITIS

The Components of the Islet ECM

In normal human islets, IM (Table 2) locates along and in intimate association with the islet microvessels separating them from the endocrine cells. Differently from IM collagen and fibronectin that lie along the islet capillary pathway, IM HA occurs in a discontinuous pattern around the periphery of human islets and appears sparsely distributed within the islets (20). Quantitative analysis indicated that HA is similarly distributed within the "peri-islet" and "intra-islet" sites and that the distribution and relative amounts of HA in normal human islets did not change significantly with age (20). HA-binding molecules versican and interα-inhibitor (IαI), two molecules that serve to cross-link HA and help stabilize the HA complexes, are present in normal human islet and locate in the HA-rich areas (20). The islet BM displays a peculiar structure composed of two layers, the vascular endothelial BM surrounding the microvessels, and a second distinct peri-islet BM which penetrates into the islet along the microvessels and forms an endocrine BM lying outside the vascular endothelial BM (57). HS and the proteoglycans syndecan-1 and syndecan-4 have been detected in rodent β-cells but not in the other islet hormone-producing cells (58,59). The HA-binding molecule tumor necrosis factor-stimulated gene 6 (TSG-6), the heavy chains of IαI, and the proteoglycan bikunin also locate intracellularly in the human and mouse pancreatic endocrine cells (20,60).

Table 2—The ECM components identified in human islets

IM
HA
HA-binding proteins versican, IαI
HS
Collagen type I, III, V, VI
Fibronectin
BM
Collagen type IV
Laminin isoforms 411/421, 511/521
Perlecan
Islet endocrine cells
HA-binding protein TSG-6
HS

Islet ECM in Type 1 Diabetes

Studies in tissue specimens from patients with type 1 diabetes show that the β-cell mass is reduced at the time the disease becomes clinically overt and that the loss of residual β-cells continues over time, which is consistent with a chronic inflammatory process, probably driven by islet-associated macrophages and lymphocytes (34,61). Mediators of inflammation released locally by infiltrating macrophages, endothelial, and ductal cells have been shown to be detrimental to β-cell function and survival (62). It is not clear how these immune cells find their way from the blood through the islet vascular wall into the islet interior.

Earlier studies in NOD mice showed that dispersed immune cells, mainly dendritic cells (DC), histiocytic-like macrophages, and macrophages with scavenging potential,

were present at birth and persisted in the peri-islet, periductular, and perivascular areas during the first month of life in NOD mice (63,64). Concomitantly, fibronectin levels were increased in the pancreas from newborn NOD mice, and a strong fibronectin immunostaining was observed in the interlobular septa, at the islet periphery, and at the islet-ductal pole, concurrent with increased laminin labeling in the BMs of the vascular and ductal structures and of exocrine acini (65). The increase in the pancreatic fibronectin and laminin was associated with altered islet morphology, as indicated by larger relative islet areas and larger islets that were also of irregular shape. Further macrophage and DC accumulations were observed at the islet-ductal pole in young adult NOD mice, which preceded the later lymphocyte accumulation in these areas. The increased number of macrophages in the fibronectin-positive peri-islet regions was likely a result of their defective migratory capacity due to inadequate α4β1 fibronectin receptor expression (66,67), which could cause these cells to be entrapped in the islets. Since increased peri-islet fibronectin and accumulation of macrophages were concurrent in the neonatal NOD mice, it is unclear whether macrophages were halted by the already altered fibronectin or whether the arrested macrophages themselves were the source of accumulated fibronectin. Accumulation of abnormal DC and macrophages in fibronectin-containing peri-islet areas and in association with altered islet morphology early in life, during the period of rodent endocrine pancreas remodeling (65,68,69), suggested that functionally impaired macrophages and DC and altered islet ECM impact islet morphology and may be involved in the generation and/or progression of the autoimmune response in NOD mice (70).

Recent systematic studies in human diabetic pancreata have implicated other specific ECM components in the regulation of leukocyte trafficking (20,71,72). These studies suggest that ECM components impact β-cell function and survival, and thus contribute to β-cell damage in diabetes (58,72). Immunohistochemistry for laminin, perlecan, and collagen showed that these components of the peri-islet BM were lost at sites of leukocyte infiltration in islets in NOD mice and in pancreata from type 1 diabetes donors (71,73). Time-course analysis of pancreatic islets during development of insulitis in NOD mice revealed an increase with age in the proportion of islets showing disruption of the BM along with an increasing number of islets infiltrated by immune cells (71). Association of invasive insulitis with degradation of the peri-islet BM indicates that removal of the BM physical barrier takes place during leukocyte entry into the islets. In addition, expression of the proteolytic enzymes cathepsin C, H, S and W was upregulated in inflamed islets versus healthy islets of NOD mice, indicating a possible direct involvement of cathepsins in peri-islet ECM degradation.

These studies indicate that the peri-islet BM serves as a physical barrier to insulitic leukocytes accumulated at islet periphery. Yet the mechanisms that control immune cell adhesion and accumulation at the islet border, what modifications take place in the islet microenvironment that would confer migratory properties to leukocytes, and whether and how islet ECM contributes to the directed migration and phenotype of the immune cells within the islet are unknown.

THE ROLE OF HA IN TYPE 1 DIABETES PATHOGENESIS

HA, a Regulator of Immune Responses

HA is a linear, high–molecular-weight glycosaminoglycan consisting of repeating disaccharides of 4-D-glucuronic acid and 3-*N*-acetyl-D-glucosamine ubiquitously present in the ECM of vertebrate tissues. HA participates in the regulation of cellular responses elicited by the microenvironment (74,75). Occurrence of HA in variable molecular sizes and configurations leads to a diversity of interactions of HA with various ECM molecules. These modify the structure and the properties of HA and promote the formation of multimolecular assemblies with distinct structural organization endowed with different physiological and biological functions (76).

HA synthesis, sizing, and removal are highly regulated to maintain its physiological concentration in tissues, which is essential to ECM stability and tissue homeostasis (74). Importantly, HA has been increasingly implicated in the regulation of immune responses (26,74,77,78). Intact HA in its high–molecular-weight form (HMW-HA) is intrinsically anti-inflammatory (26,79). The large HA polymers function as tissue integrity signals and serve to suppress the inflammatory response. HMW-HA present in the pericellular matrix protects tissue-resident cells from lymphocyte-mediated cell killing, prevents immune cell recognition, promotes the maintenance and enhances the activity of regulatory T cells, and inhibits angiogenesis (31,79). In contrast, altered HA generated during inflammation is proinflammatory. HA-rich ECM formed in response to inflammatory stimuli controls vascular permeability, edema, angiogenesis, leukocyte extravasation, and leukocyte phenotype (26,78,80,81).

Increased accumulation of HA in tissues occurs during cellular stress responses or viral infection and in a variety of inflammatory diseases (23,82,83). Following tissue injury, intact HMW-HA (>1,000 kDa) breaks down into fragments of low–molecular-weight HA (LMW-HA) through enzymatic degradation by endogenous or microbial hyaluronidases and nonenzymatic processes such as mechanical forces and oxidative stress (26,77,84). The LMW-HA fragments have proinflammatory effects, and their persistence leads to unremitting inflammation. Exogenously added LMW-HA and HA oligomers (<30 kDa) have been shown to activate macrophages and to increase chemokines, cytokines, growth factors, proteases, and nitric oxide (85–88). HA oligomers induced the phenotypic maturation of human monocyte-derived dendritic cells and promoted endothelial cell proliferation (89). LMW-HA facilitates the differentiation of several types of mesenchymal cells that

are activated following injury and influence macrophage polarity toward an M1 proinflammatory phenotype.

An active role of HA in inflammation can be demonstrated by studies in animal models of inflammatory diseases such as chemically induced colitis, experimental autoimmune encephalomyelitis, and type 1 diabetes, in which large HA deposits are present in the intestine, brain, or pancreatic islets, respectively. Reducing HA accumulation attenuated the inflammatory infiltrates in these tissues and delayed the development or the onset of the disease. Disruption of the HA synthase 3 gene led to minor leukocyte infiltrate in the dextran sulfate sodium–induced experimental colitis model (90). Injection of hyaluronidase transiently ameliorated the symptoms and delayed the onset of experimental autoimmune encephalomyelitis due to degradation of HA and impaired $CD4^+$ T-cell extravasation, while administration of an inhibitor of HA synthesis to DORmO mice prevented development of invasive insulitis and hyperglycemia (21,24).

In inflamed tissues, HA interactions with leukocytes are governed by a diverse group of HA-binding proteins called hyaladherins, such as IαI, versican, and TSG-6 (76). TSG-6 is a secreted glycoprotein that is expressed at sites of inflammation and injury (91). IαI is a component of the pericellular HA matrix of different cells that accumulates in inflamed tissues along with HA (16,92). During the inflammatory process, TSG-6 catalyzes the covalent transfers of heavy chains (HCs) from IαI to HA leading to the formation of a specific HC–HA complex that is highly adhesive for leukocytes (80,93). Versican, another proteoglycan that binds HA with high affinity, also contributes to the formation of a cross-linked HA/versican-rich complex with proinflammatory properties (94,95). Thus, the hyaladherins cross-link HA into complexes that interact with a variety of cell surface and secreted proteins to regulate leukocyte recruitment into the site of injury and inflammatory gene expression (23,80,93). HA macromolecules on the cell surface can ligate tissue- or cell-specific HA protein receptors such as CD44, RHAMM, HARE, LYVE-1, laylin, and different members of the toll-like receptor family. Through these interactions, HA can trigger a network of signal transduction from the ECM to the nucleus that affects the transcriptional activation of genes involved in a variety of cellular processes during inflammation including cell activation, proliferation, differentiation, migration, and extravasation (77).

Briefly, the ability of HA to exert proinflammatory properties is dependent upon its molecular size, availability of specific HA-binding molecules and the structure of the complexes they form with HA, and the organization and composition of tissue-specific microenvironment.

HA and Hyaladherins in Human Islets and Lymphoid Tissue in Type 1 Diabetes

We recently demonstrated that HA and hyaladherins accumulated in areas of insulitis in human type 1 diabetes pancreatic tissue (20) (Fig. 1*A*–G). HA deposits occurred along the edge capillaries of diabetic islets, where leukocytic infiltrates in insulitis are frequently observed, and along the intraislet microvessels. The increase in islet HA mass was more pronounced in tissues of younger donors with type 1 diabetes and those collected within the first year from diagnosis. HA morphological patterns in insulitis-free tissues from donors with long-standing diabetes were comparable to those observed in normal islets. HA also amassed within the clusters of leukocytes situated at the islet periphery, adjacent to the endocrine cells. The leukocytes were surrounded by HA, seemingly entrapped in the HA-rich meshwork. The proportion of islets with leukocytic infiltrates correlated with the islet HA mass. Tissues were characterized by changes in the distribution and quantity of hyaladherins, IαI, and versican, which amassed in HA-rich regions in diabetic islets, while TSG-6 was decreased. These observations strongly indicate an association between HA deposits, pancreatic β-cell loss, and insulitis. Concomitant occurrence of HA, versican, and IαI with insulitic leukocytes suggests that HA and proteins that associate with HA form a matrix that interacts with myeloid and lymphoid cells.

We also observed HA changes in human secondary lymphoid organs (20) (Fig. 1*H–N*) where substantial accumulations of HA and IαI were found within the follicular germinal centers and T-cell areas, suggesting that HA accumulation in these specific immune cell regions induces T-cell phenotype changes by altering immune cell interactions or their migratory and adhesive properties. In addition, HA accumulation was not evident in other regions of the pancreatic lymph nodes (PLN) and spleen, such as PLN medulla or splenic red pulp, or in thymus. Also, HA did not appear to accumulate in intestine tissue or in the exocrine pancreas surrounding the islets in human type 1 diabetes. Further, circulating HA levels did not increase in patients with type 1 diabetes with recent disease onset. Altogether, these observations point to HA accumulation specifically in the tissues directly involved in type 1 diabetes pathogenesis. Such observations raise important new questions regarding the functional significance of these specific ECM components in the pathogenesis of human type 1 diabetes.

HA also impacts different events associated with immune regulation in type 1 diabetes. In vitro studies showed that a HA-rich matrix controls human T-cell motility (81). Further, intact HMW-HA enhances the suppressor activity and viability of human regulatory T cells and induces phenotypic maturation of the dendritic cells and their cytokine production. The occurrence of HA in the immune synapse suggests a crucial role for the molecule in antigen presentation (31,32). We found increased islet HA and HA deposition in insulitis areas in different autoimmune models of type 1 diabetes, the NOD mouse (31), the BB rat (M.B., unpublished data), and the DORmO mouse (24). We have also found that inhibiting the synthesis of HA using a chemical inhibitor blocks the development of type 1 diabetes in DORmO mice (24). Antibody blocking of the HA receptor

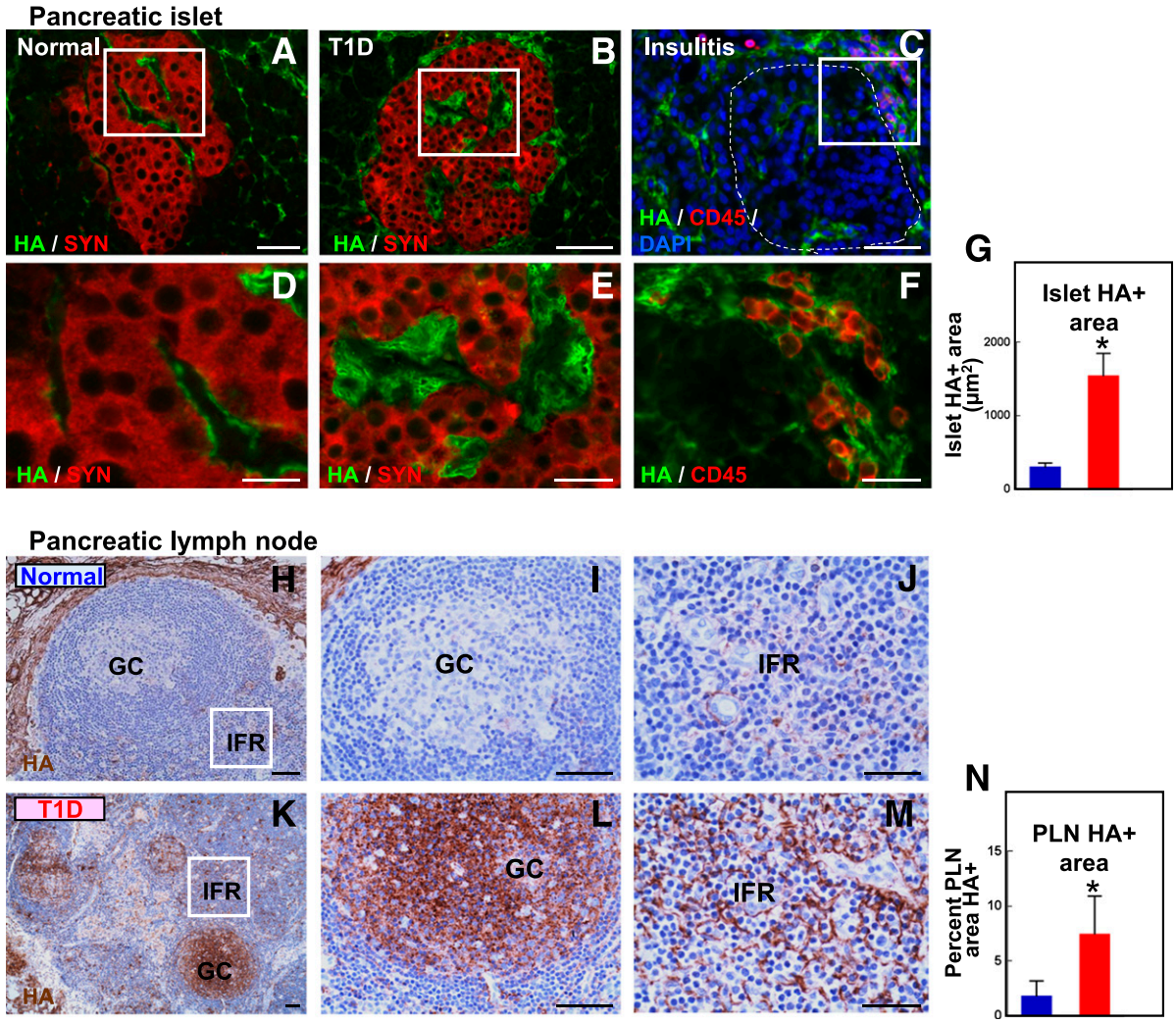

Figure 1—HA accumulates in human pancreatic islets and insulitis areas and in PLN in type 1 diabetes. *A–G*: Pancreas tissue. Staining for HA (green) and synaptophysin (SYN, red) of normal (*A* and *D*) and diabetic (*B* and *E*) islets shows accumulation of HA around and within the diabetic islet. Colabeling of HA (green) with the leukocyte common antigen CD45 (red) confirms the presence of HA in the site of inflammatory infiltrate (*C* and *F*). The islet is delineated with a white dashed line. Morphometric quantification of HA in pancreatic islets is shown in *G*. Panels *D*, *E*, and *F* show higher magnification of the boxed areas in *A*, *B*, and *C*, respectively. *H–N*: PLN tissue. Histochemistry for HA (brown) in normal (*H–J*) and diabetic (*K–M*) islets is shown. Higher magnification of B-cell–rich germinal centers (GC) and T cell–rich interfollicular regions (IFR) present in *H* and *K* are shown in *I* and *J* and in *L* and *M*, respectively. Morphometric quantification of HA in PLN is shown in *N*. Scale bars: 100 μm (*H*, *I*, *K*, and *L*), 50 μm (*A–C*, *J*, and *M*), 25 μm (*E* and *F*), and 10 μm (*D*). Blue bars, normal tissues; red bars, diabetic tissues. T1D, type 1 diabetes. Panels *A*, *C*, *D*, *F*, *G*, *K*, *L*, and *N* are reproduced from Bogdani et al. (20). *$P < 0.001$ vs. normal tissues.

CD44 conferred resistance to diabetes development, and administration of hyaluronidase partially prevented adoptive transfer of diabetes (96). Human mesenchymal stem cells secreting the HA-binding molecule TSG-6 delayed onset of type 1 diabetes in NOD mice, in part by the suppressive effects of TSG-6 on antigen presentation and cytotoxic T-cell activation (97). Altogether, these studies indicate multiple mechanisms by which HA and associated proteins can regulate events associated with development of type 1 diabetes.

HA can generate a number of HA complexes that interact with cells through specific HA receptors. We showed that both IαI and versican closely associate with HA in normal human islets but only IαI occurs in the HA-rich regions in normal lymphoid tissue (20). In these tissues, it is possible that the HA-IαI-versican–rich and the HA-IαI–rich complexes may constitute ECM substrates with distinct properties, with the former repulsing immune cells from the islet endothelium surface and the latter facilitating homing and migration of immune cells in lymphoid tissue. By using a limited number of HA-binding molecules and HA receptors, HA may thus generate assemblies with tissue-specific structural and functional properties. In this way, although ubiquitously found in all tissues, HA may behave as a tissue-specific molecule.

HYPOTHESIS: REGULATION OF INSULITIS BY HA-RICH MATRIX

On the basis of our studies in human diabetes and in vitro and in vivo studies by other investigators, we propose a model for the role of HA in the regulation of insulitis (Fig. 2). Our model implies that enhanced production of islet HA and unceasing generation of bioactive HA fragments create a constantly HA-rich islet microenvironment that contributes to islet inflammation and continuous injury to β-cells.

The model, shown schematically in Fig. 2, represents the vicious cycle of HA changes contributing to initiation, promotion, and maintenance of islet inflammation. Inflammatory stimuli, such as inflammatory cytokines, viral infections, or ER stress, enhance HA synthesis by islet endothelial cells, leading to accumulation of HA in islet microvessels. Available plasma-derived or islet cell–synthesized hyaladherins cross-link HA to form an HA-hyaladherin–rich matrix around islet endothelium that is adhesive for leukocytes. Leukocytes arrested at the islet border release hyaluronidase and a variety of degrading and proteolytic enzymes that break down HA and other islet ECM constituents and finally destroy the islet vascular barrier, enabling leukocyte entry into the islet. The breakdown of HA results in formation of bioactive HA fragments that convey pro-migratory signals to leukocytes and enhance leukocyte activation and gene expression. Inflammatory stimuli generated inside the islets further induce synthesis of HA by endothelial cells and also by the recruited leukocytes themselves. The newly formed HA will enter the cycle of degradation and generation of new HA breakdown products, the persistence of which leads to continual leukocyte recruitment into the islet and their activation of gene expression, which contribute to ongoing islet inflammation. In addition, the HA-rich matrix deposited between the endocrine cells and islet capillaries constitutes a quantitatively and qualitatively altered islet ECM that in itself may impact islet endocrine cell function and viability possibly via altering biomechanical properties of the islet microenvironment and/or intracellular signaling pathways regulating β-cell function and survival.

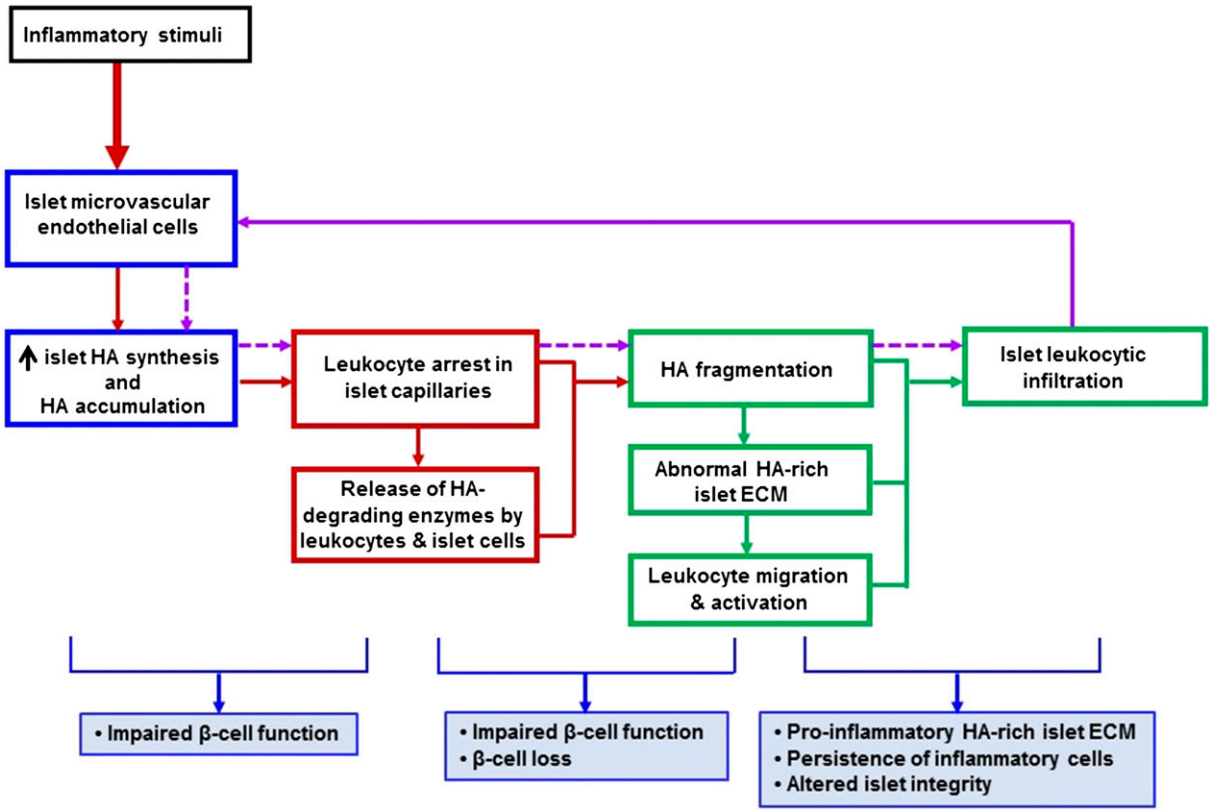

Figure 2—Proposed model for the role of islet HA in the regulation of insulitis and β-cell damage in type 1 diabetes. The model represents the vicious cycle of HA changes contributing to initiation, promotion, and maintenance of islet inflammation. Initiation (blue and red boxes and arrows): Inflammatory stimuli enhance HA synthesis by islet endothelial cells and generation of an HA-rich ECM that is adhesive for leukocytes, causing the leukocyte arrest at the islet border. Promotion (green boxes and arrows): HA-degrading enzymes released by the arrested leukocytes break down HA into bioactive HA fragments, which, by themselves and as structural components of islet HA complexes, conduct leukocyte migration into the islets and enhance leukocyte activation and gene expression. Maintenance (purple arrows): Leukocyte cell surface–associated or vicinal HA and fragmented HA provide structural and cell-signaling cues that maintain a vicious circle of islet inflammation. In addition, alterations in structural complexes of HA and other islet ECM components lead to altered islet integrity and impairment of β-cell function and viability.

CONCLUSIONS

Our observations and those of others highlight novel potential roles for different components of the ECM in the regulation of insulitis in human type 1 diabetes. Collective changes in the structural complexes of ECM components are proposed to create a proinflammatory microenvironment that regulates crucial steps in the pathogenic process of type 1 diabetes such as immune cell adhesion and migration, immune cell activation, and β-cell death. The JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD) ECM working group composed of three research teams (72) has initiated studies that will lead to our better understanding of the collective changes in the ECM that take place in human islets and lymphoid tissues during development of type 1 diabetes. Understanding the contribution of the ECM in type 1 diabetes could complete the "unfinished harmony" of the pathogenic process of the disease.

> If you have built castles in the air, your work need not be lost, that is where they should be. Now put the foundations under them.
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> —Henry David Thoreau

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