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The Immune Pathogenesis of Type 1 Diabetes: Not Only Thinking Outside the Cell but Also Outside the Islet and Out of the Box



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Around 1960, observations of lymphocytic insulinitis and the presence of islet cell autoantibodies in patients with type 1 diabetes led to the concept of type 1 diabetes being an organ-specific autoimmune disease. Much work has since been done to unravel the mechanisms behind the aberrant self-directed immune response and to predict disease progression, as it appeared that the autoimmune reaction preceded clinical diabetes, often for many years. Research focused on the identification of the primary autoantigen(s) to which the antibodies were directed. Important targets, such as insulin, GAD65, and IA-2, were discovered. However, research into the pathogenic mechanisms was hampered because the pancreas tissue of patients in the initial phases was rarely available. For that reason, animal models, such as the NOD mouse and the diabetes-prone BioBreeding (BBDP) rat, that developed autoimmune diabetes spontaneously were used to study prestages of the disease and to test therapeutic interventions. Studies in these animal models have mainly focused on autoreactive T-cell-mediated inflammatory destructive reactions to the islets of Langerhans, and balances between a multitude of regulatory and effector mechanisms became gradually apparent (1). However, translation of these animal data to the human disease was a controversial issue. Nevertheless, from all this work the view developed that the central pathogenic mechanism is an antigen-driven T lymphocyte-mediated inflammatory reaction of the islets, which specifically affects the β -cells disappearing in the reaction. This excessive reaction is allowed by defective tolerance mechanisms, and particular emphasis has been on the population of thymus-derived $CD4^+CD25^+FOXP3^+$ T regulatory cells (excellently reviewed in ref. 1). However, many questions remained (1), among which were, “What are the initiating factors?” and “How are innate immune responses involved?”

A consortium of research groups (Network for Pancreatic Organ Donors with Diabetes [nPOD]) embarked on collecting relevant tissues from organ donors to overcome the problem of availability of patient pancreas tissue in the early phases of disease. Studies on pancreatic tissues from preclinical cases have now led to new insights and views indicating that complex mechanisms are involved with more players in the field (excellently reviewed in ref. 2).

In this issue of *Diabetes*, Bogdani (3) reviews her work on the nPOD collection, the NOD mouse, and BBDP rat and asks for attention to another important player in the field, the extracellular matrix (ECM), and in particular a component thereof, hyaluronan (HA). Increased deposits of HA were found perivascularly in and at the edges of the islets in early cases of type 1 diabetes, the NOD mouse, and BBDP rat. Because HA itself is anti-inflammatory but its breakdown products are proinflammatory, a cross talk with and a regulation of immune cell activity in the islets can take place via this ECM component. Therapeutic interventions via this mechanism can also be envisaged. Inhibition of HA synthesis has resulted in the restoration of tolerance in an animal model (4).

On the basis of her ECM findings, Bogdani invites us to think outside the cell and in fact out of the box and proposes and depicts a model in which deviant ECM regulation plays a prominent role: inflamed intraislet endothelial cells are the aberrant producers of HA and the inflammatory breakdown of HA results in enhanced diapedesis and activation of leukocytes in the intraislet extracellular space, creating a perpetual loop for inflammation (see Fig. 2 in ref. 3).

Other nPOD data also suggest a more complex pathogenesis of type 1 diabetes, among which are

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1. The involvement of the exocrine pancreas with signs of atrophy and/or underdevelopment. Numerous CD4⁺ and CD8⁺ T cells and CD11c⁺ dendritic cells (DCs) accumulate in the exocrine pancreas of individuals with type 1 diabetes (2). It is noteworthy that HA is abundantly present in the exocrine pancreas (5,6).
2. The considerable numbers of residual β -cells, in particular in the first years after diagnosis (2).

Questions arise as to why β -cell regeneration is insufficient and why the exocrine pancreas is less developed. Disease pathways involving aberrancies in the growth and development of the endocrine and exocrine pancreas are thus most likely part of the early immune pathogenesis of type 1 diabetes (supported by a proteomics study [7]).

In view of this, it is interesting that Bogdani refers to our work in the NOD mouse published in the 1990s showing extensive ECM abnormalities other than HA abnormalities. We described ECM abnormalities particularly in the extended vascular-ductal pole adjacent to the actual islet (reviewed in ref. 8). The vascular-ductal pole is involved in the generation of islets during fetal life and in postnatal periods, such as postweaning and pregnancy (8). We focused in our studies on two other ECM components, i.e., laminin and fibronectin, and found fibronectin overexpressed, particularly prominently at the ductal-vascular poles but also in the exocrine pancreas. HA was also found at these locations by Bogdani. Also similar to the data of Bogdani (5,6), myeloid cells (DCs and macrophages [M Φ]) started to accumulate particularly at this pole as the first sign of insulinitis and to encircle the islet later at the islet edges (8,9). Indeed, we considered myeloid cells (and also fibroblasts) as important extra players in this process, as they are producers and degraders of the ECM par excellence (8).

Aberrant activities of pancreas DCs and M Φ can be noticed in the NOD mouse model not only at the onset of insulinitis but also at the early neonatal stage (i.e., weeks before the initial para-islet accumulation of myeloid cells) (10,11). There are waves of M Φ accumulations in the pancreas linked to aberrant signs of islet cell apoptosis and islet regeneration at weaning.

In the classic view of the immunologist, DCs and M Φ are bone marrow-derived cells, of which the prime task of the precursors is to infiltrate from the bloodstream into zones of danger to differentiate into well-developed DCs and M Φ to eliminate the dangerous stimulus and/or to recruit the adaptive immune system to aid in the elimination job. However, we invite you to think a little further outside the box: there is now extensive literature that DCs and M Φ are important homeostatic support cells under steady state, nondangerous normal situations in essentially all organs (excellently reviewed in refs. 12,13), including our early descriptions in neuroendocrine organs (14,15). The steady-state M Φ and DCs provide growth factors and are regulators of hormone production and play important roles in embryonic and adult developmental processes. Indeed, M Φ and DCs are also essential

for normal islet development and for sufficient numbers of insulin-producing cells (16,17). Interestingly inflammatory processes are not necessary to get these M Φ and DCs in the pancreas, local precursors for M Φ and DCs do exist in the pancreas (18), as is also well known for other organs, such as the brain and lung (12).

Together, these data make a very early, noninflammatory starting point for the type 1 diabetes pathogenic process possible and lead to a hypothetical model for debate depicted in Fig. 1 (adjusted and extended from the Fig. 2 in Bogdani [3]). This model illustrates that not only inflammation driven by autoantigen-specific T cells and ECM breakdown products but also early aberrancies in the development of the myeloid lineage and most likely other cell lineages contribute to type 1 diabetes pathogenesis.

The model depicted in Fig. 1 also involves the preinsulinitis phase as described in the NOD mouse model. Before the development of the islet autoimmune reaction and even from fetal life onward, an abnormal intrapancreatic development of DCs and M Φ from precursors can be detected in the NOD pancreas. These aberrancies impact the growth and development of adjacent parenchymal endocrine and exocrine structures, including ECM components, and the migration and tolerogenic capacity of the cells, favoring autoimmunization. The reported preinsulinitis abnormalities include the following

1. An abnormally high proliferation of intrapancreatic local myeloid precursors (18). It must be noted that in NOD mice myeloid bone marrow precursors (19), other myeloid lineages (microglia [20]), and T cells also show extensive intrinsic abnormalities in growth and development (1,21). Do parenchymal cells in NOD mice and patients with type 1 diabetes share the same molecular aberrancies, leading to abnormalities in growth and development? In a related disease, thyroid autoimmune disease, which shares risk genes with type 1 diabetes, prestages are characterized by various aberrancies in serum growth factors such as IGFBP2, PDGF-BB, and SCF (22).
2. The development of irregularly shaped islets (small and mega-islets), with mega-islets in particular being the target of initial DC and M Φ para-islet accumulation. This is also the case in the NOD-*scid* mouse, ruling out the involvement of T cells (24).
3. A low-level expression of growth factors, including islet-regenerating factors (reg 2, reg 3) by the main population of local pancreas DCs, the CD8 α^- DCs (23).
4. A reduced expression at the gene and protein level of tolerance-inducing molecules (such as CD200R and CCR5) by these local CD8 α^- DCs (23).
5. A hypersensitivity of the defective local CD8 α^- DCs to environmental danger signals, such as lipopolysaccharide, with an enhanced production of proinflammatory cytokines (23).
6. A significant reduction of the small subset of local tolerogenic CD8 α^+ CD103⁺Langerin⁺ DCs, with a reduced

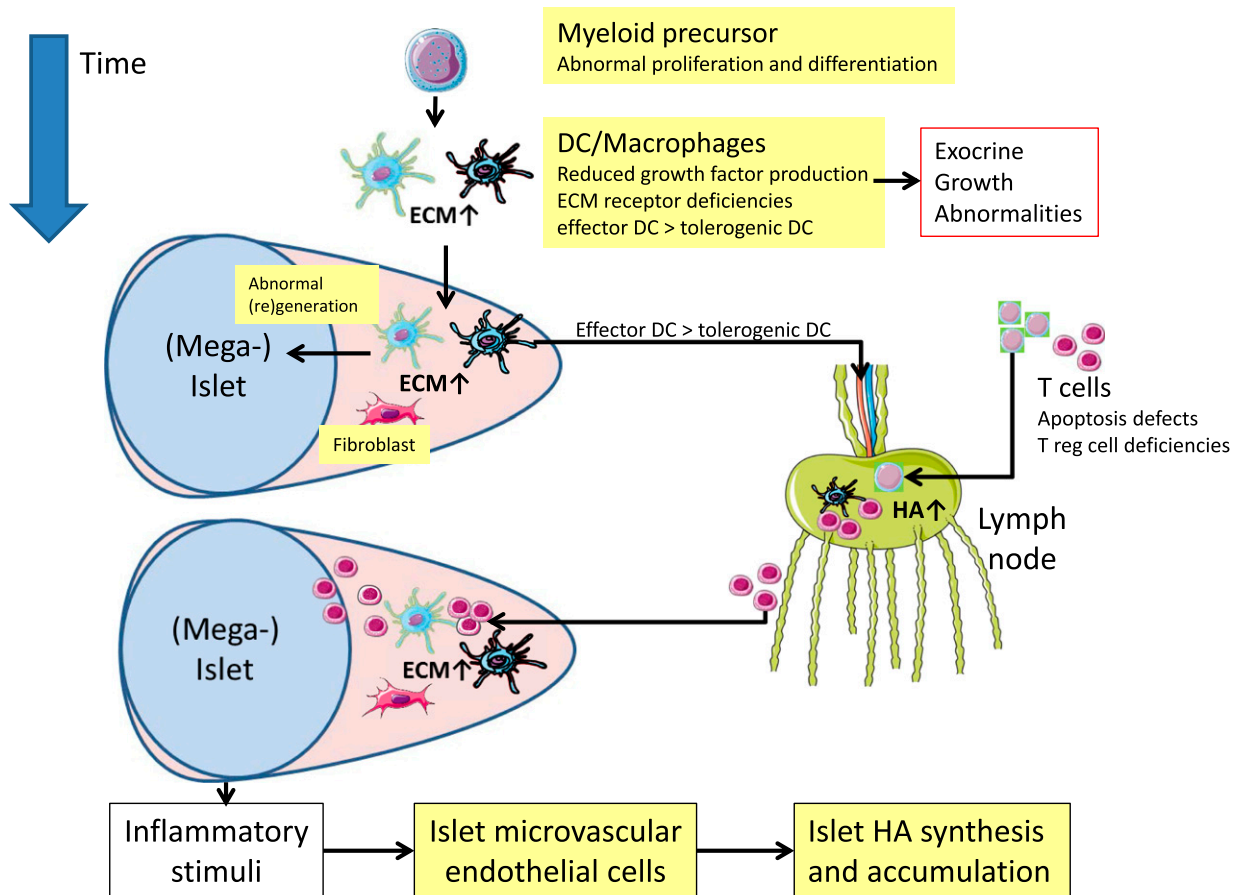


Figure 1—Hypothetical model for the pathogenesis of type 1 diabetes. T reg cell, regulatory T cell.

expression of CCR5, CLEC9A, and interleukin-10 in the prediabetic pancreas of NOD mice (25).

- Fibronectin receptor deficiencies on local MΦ, suggesting an abnormal adhesion (26), poor migratory and infiltration capacity (26), and poor differentiation of the cells on ECM (27).
- In human prediabetic stages, we reported abnormalities in monocyte gene expression (28).

The model as depicted in Fig. 1 also shows that after the preinsulinitis phase, DCs and MΦ start to accumulate at the vascular ductal pole to initiate early insulinitis. The vascular ductal pole is known to be involved in islet regeneration. At the time of early insulinitis, it is characterized by fibronectin and HA accumulations. In the thyroid of the NOD mouse, cell necrosis, induced by abnormal metabolic iodine stress, induces the local DC and MΦ accumulations (29). It remains to be established if viruses and/or metabolic growth abnormalities of the islets are also capable of inducing necrosis-linked nonspecific attracting stimuli (2). Fibroblasts are also well-known producers of ECM and regulators of the growth and development of parenchymal cells, providing numerous growth factors. Interestingly, in Graves thyroid autoimmune ophthalmopathy, the excessive production of HA by orbital

fibroblasts is the hallmark of the disease and the orbital fibroblasts are the targets of an autoimmune response (30). Intra-orbital MΦ, DCs, and mast cells are the drivers of the excessive HA production of the fibroblasts, showing an intricate relationships of these cells and the ECM (30).

In the later sensitization phase, DCs and MΦ reach the lymph node. Most likely due to the imbalance between regulatory and suppressor forces (i.e., effector DC > tolerogenic DC, relative deficiencies of regulatory T cells, and abnormal ECM accumulations) an effector T-cell response is initiated in the draining lymph node in the NOD mouse.

In the final effector phase of the response, autoimmune T cells start to accumulate and together with the DCs and MΦ break the basal membrane barrier and infiltrate the islets from the islet edges or infiltrate from the in-traislet capillaries, according to the hypothetical mechanisms as proposed by Bogdani (3).

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References

1. Herold KC, Vignali DA, Cooke A, Bluestone JA. Type 1 diabetes: translating mechanistic observations into effective clinical outcomes. *Nat Rev Immunol* 2013;13:243–256
2. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 2014;383:69–82
3. Bogdani M. Thinking outside the cell: a key role for hyaluronan in the pathogenesis of human type 1 diabetes. *Diabetes* 2016;65:2103–2114
4. Nagy N, Kaber G, Johnson PY, et al. Inhibition of hyaluronan synthesis restores immune tolerance during autoimmune insulinitis. *J Clin Invest* 2015;125:3928–3940
5. Bogdani M, Johnson PY, Potter-Perigo S, et al. Hyaluronan and hyaluronan-binding proteins accumulate in both human type 1 diabetic islets and lymphoid tissues and associate with inflammatory cells in insulinitis. *Diabetes* 2014;63:2727–2743
6. Bollyky PL, Bogdani M, Bollyky JB, Hull RL, Wight TN. The role of hyaluronan and the extracellular matrix in islet inflammation and immune regulation. *Curr Diab Rep* 2012;12:471–480
7. Liu CW, Atkinson MA, Zhang Q. Type 1 diabetes cadaveric human pancreata exhibit a unique exocrine tissue proteomic profile. *Proteomics* 2016;16:1432–1446
8. Homo-Delarche F, Drexhage HA. Immune cells, pancreas development, regeneration and type 1 diabetes. *Trends Immunol* 2004;25:222–229
9. Geutskens SB, Homo-Delarche F, Pleau J-M, Durant S, Drexhage HA, Savino W. Extracellular matrix distribution and islet morphology in the early postnatal pancreas: anomalies in the non-obese diabetic mouse. *Cell Tissue Res* 2004;318:579–589
10. Durant S, Geutskens S, Van Blokland SC, et al. Proapoptosis and antiapoptosis-related molecules during postnatal pancreas development in control and nonobese diabetic mice: relationship with innervation. *Lab Invest* 2003;83:227–239
11. Charré S, Rosmalen JG, Pelegri C, et al. Abnormalities in dendritic cell and macrophage accumulation in the pancreas of nonobese diabetic (NOD) mice during the early neonatal period. *Histol Histopathol* 2002;17:393–401
12. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol* 2013;14:986–995
13. Lavin Y, Mortha A, Rahman A, Merad M. Regulation of macrophage development and function in peripheral tissues. *Nat Rev Immunol* 2015;15:731–744
14. Hoek A, Allaerts W, Leenen PJ, Schoemaker J, Drexhage HA. Dendritic cells and macrophages in the pituitary and the gonads. Evidence for their role in the fine regulation of the reproductive endocrine response. *Eur J Endocrinol* 1997;136:8–24
15. Simons PJ, Delemarre FG, Drexhage HA. Antigen-presenting dendritic cells as regulators of the growth of thyrocytes: a role of interleukin-1beta and interleukin-6. *Endocrinology* 1998;139:3148–3156
16. Geutskens SB, Otonkoski T, Pulkkinen MA, Drexhage HA, Leenen PJ. Macrophages in the murine pancreas and their involvement in fetal endocrine development in vitro. *J Leukoc Biol* 2005;78:845–852
17. Calderon B, Carrero JA, Unanue ER. The central role of antigen presentation in islets of Langerhans in autoimmune diabetes. *Curr Opin Immunol* 2014;26:32–40
18. Welzen-Coppens JM, van Helden-Meeuwse CG, Drexhage HA, Versnel MA. Abnormalities of dendritic cell precursors in the pancreas of the NOD mouse model of diabetes. *Eur J Immunol* 2012;42:186–194
19. Nikolic T, Bunk M, Drexhage HA, Leenen PJ. Bone marrow precursors of nonobese diabetic mice develop into defective macrophage-like dendritic cells in vitro. *J Immunol* 2004;173:4342–4351
20. McGuinness B, Gibney SM, Beumer W, et al. An increased proliferation of microglia and an increased brain inflammatory response and sickness behaviour to LPS in autoimmune-prone NOD mice. *Neuroimmunomodulation* (in press)
21. Radosevic K, Casteels KM, Mathieu C, Van Ewijk W, Drexhage HA, Leenen PJM. Splenic dendritic cells from the non-obese diabetic mouse induce a prolonged proliferation of syngeneic T cells. A role for an impaired apoptosis of NOD T cells? *J Autoimmun* 1999;13:373–382
22. Massolt ET, Effraïmidis G, Korevaar TIM, et al. Aberrant levels of hematopoietic/neuronal growth and differentiation factors in euthyroid women at risk for autoimmune thyroid disease. *PLoS One* 2016;11:e0153892
23. Beumer W, Welzen-Coppens JM, van Helden-Meeuwse CG, Gibney SM, Drexhage HA, Versnel MA. The gene expression profile of CD11c+ CD8α-dendritic cells in the pre-diabetic pancreas of the NOD mouse. *PLoS One* 2014;9:e103404
24. Rosmalen JGM, Homo-Delarche F, Durant S, Kap M, Leenen PJM, Drexhage HA. Islet abnormalities associated with an early influx of dendritic cells and macrophages in NOD and NODscid mice. *Lab Invest* 2000;80:769–777
25. Welzen-Coppens JM, van Helden-Meeuwse CG, Leenen PJ, Drexhage HA, Versnel MA. Reduced numbers of dendritic cells with a tolerogenic phenotype in the prediabetic pancreas of NOD mice. *J Leukoc Biol* 2012;92:1207–1213
26. Bouma G, Nikolic T, Coppens JM, et al. NOD mice have a severely impaired ability to recruit leukocytes into sites of inflammation. *Eur J Immunol* 2005;35:225–235
27. Geutskens SB, Nikolic T, Dardenne M, Leenen PJ, Savino W. Defective up-regulation of CD49d in final maturation of NOD mouse macrophages. *Eur J Immunol* 2004;34:3465–3476
28. Beyan H, Drexhage RC, van der Heul Nieuwenhuijsen L, et al. Monocyte gene-expression profiles associated with childhood-onset type 1 diabetes and disease risk: a study of identical twins. *Diabetes* 2010;59:1751–1755
29. Many MC, Maniratunga S, Varis I, Dardenne M, Drexhage HA, Denef JF. Two-step development of Hashimoto-like thyroiditis in genetically autoimmune prone non-obese diabetic mice: effects of iodine-induced cell necrosis. *J Endocrinol* 1995;147:311–320
30. van Steensel L, Paridaens D, van Meurs M, et al. Orbit-infiltrating mast cells, monocytes, and macrophages produce PDGF isoforms that orchestrate orbital fibroblast activation in Graves' ophthalmopathy. *J Clin Endocrinol Metab* 2012;97:E400–E408