DECEMBER 2015

diabetes®

In This Issue of *Diabetes*

By Max Bingham, PhD

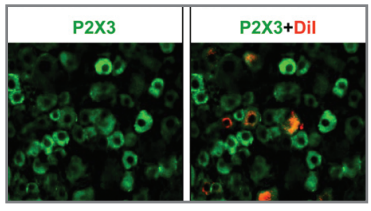
Diabetes and the Microbiome: ADA/JDRF Joint Symposium Report and Recommendations

The outcomes of a joint research symposium between the American Diabetes Association and JDRF entitled "Diabetes and the Microbiome" are reported in a Perspectives in Diabetes article by Semenkovich et al. (**p. 3967**) in this month's issue of *Diabetes*. The article highlights the sheer extent of research still needed to fully understand the microbiome, and particularly its role in diabetes. Meeting in October 2014 in Chicago, IL, ~100 experts from a diverse set of disciplines met to consider the relationship between the host and the microbiome. More specifically, they met to define critical research questions that are needed to potentially target the microbiome for the prevention and treatment of diabetes. The report touches on six areas of research including the potential relationships between the microbiome and both type 1 and type 2 diabetes. Notable discussions included whether early intervention to alter the microbiome could prevent diabetes, and whether there are microbiome characteristics that are predictive for autoimmunity or metabolic dysfunction. The researchers call for the strengthening of collaborations and the need for standardization in animal research and bioinformatics. But, perhaps most importantly, they call for longitudinal cohort studies to track the development of the microbiome and health status in diabetes. Commenting more widely on the outcomes of the symposium, author Tamara Darsow said: "The impact of microbes and their genes on human health offers a fascinating and potentially unifying hypothesis on a major driver of the increase in prevalence of diabetes and obesity. It is possible that modern human environments—including food supply, hygiene, and antibiotic use—have altered our commensal microbes to an extent that increases inflammation, affecting both the immune system, potentially leading to type 1 diabetes, and metabolism, potentially leading to obesity and/or type 2 diabetes. We have so much more to learn in this realm, and the American Diabetes Association is committed to supporting hypothesis-driven research to identify how much and what kind of microbes contribute to human health. Definitive information on causal relationships could lead to the development of probiotics or antibiotics that can develop or sustain a healthy microbial profile and prevent or treat diabetes."

Semenkovich et al. American Diabetes Association and JDRF Research Symposium: Diabetes and the Microbiome. Diabetes 2015;64:3967–3977

Molecular Mechanisms Underlying Pain in Diabetic Neuropathy Uncovered

Pain associated with diabetic neuropathy is a common complaint by individuals with both type 1 and type 2 diabetes, and yet the underlying molecular mechanisms have remained largely elusive. But, according to a report by Zhang et al. (**p. 4272**), DNA demethylation and enhanced interaction with nuclear factor-κB (NF-κB) in neurons might be central to explaining this type of pain. In a series of experiments in rats, the researchers demonstrated the likely role of a specific gene sequence in a purinergic receptor called P2X3R, which is located in dorsal root ganglia neurons, and its regulation by NF-κB. After inducing diabetes in the rats, the authors used standardized tests for pain threshold and hypersensitivity to test the effects of two purinergic receptor antagonists, finding that both significantly attenuated hypersensitivity in diabetic rats versus controls. Next, investigating at the molecular level, expression and function of P2X3Rs was found to be enhanced in neurons in the diabetic rats and specifically, a genetic island in the receptor's gene promoter region was demethylated. In a key follow-up finding, the authors then demonstrated that an active form of NF-κB called p65 could bind to this demethylated genetic region but only in rats with diabetes. Then, through inhibition of NF-κB via either a chemical or viral method, they showed that P2X3R activities could be suppressed, which led to attenuation of pain in diabetic neuropathy. Finally, insulin treatment could also reduce pain at the same time as suppressing p65 and P2X3R expression. In discussing the significance of the study, author Guang-Yin Xu said: "The present study may shed light on epigenetic mechanisms of diabetic neuropathic pain, and the added knowledge would provide new strategies for treatment for neuropathic pain in patients with diabetes. Further investigations are needed to explore the detailed epigenetic regulations of key nociceptive genes and the roles of inflammatory factors in the diabetic neuropathic pain state."



P2X3R-positive cells are shown in green (left panel) and the merge of P2X3R-positive staining and DiI labeling (right panel).

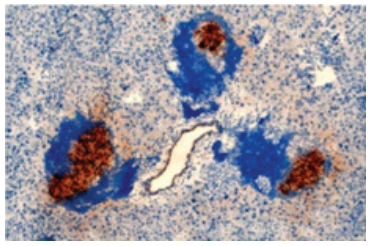
Zhang et al. Promoted interaction of nuclear factor-κB with demethylated purinergic P2X3 receptor gene contributes to neuropathic pain in rats with diabetes. Diabetes 2015;64:4272–4284

DECEMBER 2015

diabetes®

Combination Antibody Therapy for Persistent Remission of Type 1 Diabetes: Animal Studies

A candidate combined antibody therapy is the focus of a report by Lasch et al. (**p. 4198**) that suggests it might be possible to induce persistent remission of type 1 diabetes. The combination of anti-CD3 and anti-CXCL10 antibodies resulted in substantial remission in mice. Partial T-cell inactivation and subsequent blockading of T-cell migration in the pancreas appeared to be key in terms of mechanism, according to the authors. Focusing initially on the diabetic mouse model RIP-LCMV, they found that it was possible to achieve a remission rate of 60% with the combined therapy—a significantly higher rate than was achieved with either antibodies alone or control. Most of the mice receiving the combined therapy also achieved normal blood glucose levels. Subsequent molecular analyses revealed the likely mechanism of action relating to inactivation and blocking of T-cell migration and that islet destruction could be controlled or prevented in many cases by the combined approach. To confirm the findings the authors then generated CXCL10-deficient RIP-LCMV mice and found that it was possible to achieve complete remission of type 1 diabetes with anti-CD3 antibodies alone, supporting the notion that anti-CXCL10 antibodies appear to play a supporting role in the approach. Similar experiments in the second mouse model (NOD) appear to confirm the observations, although with some variation according to the authors. When commenting on the future of this work, author Urs Christen stated: "Our study has two major implications: first, it reestablishes critical chemokines, such as CXCL10, as targets for immune intervention in autoimmune disease. Second, it demonstrates that targeting several aspects of the disease pathogenesis with differential treatments might be more successful than monotherapies. Since such combination therapies also require lower doses of the individual drugs, possible side effects can be minimized. Thus, in the future, combination therapies should be more often considered for clinical trials."

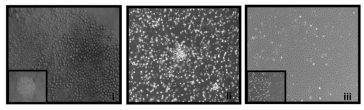


Pancreas tissue section obtained from mice treated with isotype-matched control antibody aCD3.

Lasch et al. Anti-CD3/anti-CXCL10 antibody combination therapy induces a persistent remission of type 1 diabetes in two mouse models. Diabetes 2015;64:4198–4211

Autologous Cell Therapy: Is It Possible After Restoring Specific MicroRNA in CD34$^+$ Cells?

A small noncoding microRNA (miRNA) molecule termed miR-92a may have the ability to correct dysfunctioning CD34$^+$ cells that are typically associated with diabetic patients that have microvascular complications. The significance of this, according to the authors of the report by Bhatwadekar et al. (**p. 4226**), is that it would enhance the usefulness of these cells in autologous cell therapy, an approach that is used for vascular repair. The authors report that they initially examined how CD34$^+$ cells differentiate and specifically the influence of so-called clock genes. According to the report, differentiation of the cells resulted in "robust oscillations" in gene expression. Then, using small interfering RNA molecules to probe the system, it became clear that the gene *Per2* was the only one necessary to maintain the cells in an undifferentiated state. The significance of this is that when cells of diabetic origin were exposed to the same conditions they did not differentiate in the same way, leading to the suggestion that they had somehow lost their "stemness" (or ability to differentiate into different cell types). To understand this, the authors then probed the cells with miRNA molecules and found a number that were only associated with diabetic origins. Moving on to individuals with diabetes with or without diabetic retinopathy (DR) or healthy controls, they subsequently found that the expression of *Per2*-regulatory miRNA, miR-92a, was significantly reduced but only in cells from individuals with diabetes and DR. Restoring the level of miR-92a in the CD34$^+$ cells then reduced their inflammatory phenotype and restored their propensity to differentiate in a normal manner. Commenting more widely on the study, author Maria B. Grant said: "Overall, our studies suggest that clock regulatory miRNAs play an important role in directing the fate of stem cells toward differentiation. miRNA-mediated repair of stem cells may represent a novel therapeutic strategy that may be utilized for the correction of defects in diabetic stem cells, and miR-92a in particular may serve this important role."



Differentiation pattern and clock gene expression in freshly isolated CD34$^+$ cells (i), cultured early endothelial progenitor cells (ii), and endothelial colony–forming cells (iii).

Bhatwadekar et al. miR-92a corrects CD34$^+$ cell dysfunction in diabetes by modulating core circadian genes involved in progenitor differentiation. Diabetes 2015;64:4226–4237

DOI: 10.2337/db15-ti12