

Glucagon-Like Peptide 1 and Type 1 Diabetes: NOD Ready for Prime Time?

Diabetes mellitus is considered the epidemic of the 21st century, affecting close to 170 million people in 2000, and expected to double in incidence by 2030 (1). The two major types of diabetes (types 1 and 2) are characterized by chronic hyperglycemia due to a loss of insulin secretion and/or defective insulin action; however, the etiopathogenesis of each type is distinct. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder, resulting from lymphocyte-mediated destruction of insulin-producing β -cells, whereas type 2 diabetes is a complex metabolic disorder, caused by a combination of insulin resistance and a concomitant impairment in insulin secretion. Currently, there is no cure for type 1 or 2 diabetes. The dominant treatment for T1DM continues to be insulin replacement therapy. Although multiple oral hypoglycemic and injectable agents are used for the treatment of type 2 diabetic patients, these agents do not appear to prevent the progressive loss of β -cell mass and function that accompanies the natural history of type 2 diabetes, so that eventually many patients require exogenous insulin administration.

Given the pathophysiology of T1DM, the most effective way to prevent the development of β -cell destruction and insulin deficiency is by interfering with the autoimmune attack. Unfortunately, due in part to difficulties inherent in identifying patients at high risk and lack of effective agents, most immune-based prevention trials have not yielded noteworthy results. Consequently, the majority of clinical trials are now focused on intervention therapies, targeting recent-onset diabetic patients to prevent further β -cell destruction (2). One of the most promising clinical trials involves short-term treatment with humanized anti-CD3 monoclonal antibodies. Treatment with anti-CD3 antibodies generated promising results in diabetic animal models (3, 4) and preserved β -cell function in two independent human trials (5, 6). The mechanism of action of the anti-CD3 therapy is not fully understood; however, the long-term beneficial effects are believed to be mediated via enhancement of specific populations of regulatory T cells, observed both in murine models (7) and in diabetic human subjects (8).

The work of Sherry *et al.* (9) in this issue of *Endocrinology* examines whether the addition of exendin-4 [a glucagon-like peptide 1 (GLP-1) receptor agonist] to anti-CD3 immunotherapy enhances diabetes remission. Given the reported decline in β -cell function after the first year of a single course of anti-CD3 monotherapy in human studies, the authors reasoned that a major determinant of successful response to

treatment is the extent of β -cell function at diagnosis. Hence, the authors hypothesized that the addition of exendin-4 (a molecule that stimulates β -cell proliferation and inhibits apoptosis in preclinical studies) after induction of immune tolerance may improve β -cell mass and function, and thus result in an improved response to the anti-CD3 immune therapy. Indeed, the rate of diabetes reversal was higher in NOD mice (a model of T1DM) receiving a 5-d course of anti-CD3 antibody in combination with a 10-d course of exendin-4 upon onset of hyperglycemia, compared with mice receiving the corresponding monotherapies and control treatments. However, the addition of exendin-4 to the anti-CD3 immunotherapy was only beneficial when there was residual functional β -cell mass upon initiation of treatment (reflected by ambient glucose levels < 350 mg/dl).

Contrary to expectations, the authors did not observe an enhancement of β -cell area or positive effects on the rate of β -cell proliferation and β -cell death when exendin-4 was added to the anti-CD3 immunotherapy. Furthermore, the authors showed that exendin-4 treatment had no detectable effects on the autoimmune response because modulation of immune function could have been an alternative explanation for the improved remission rate of diabetes. Rather, what Sherry *et al.* (9) discovered is that the mechanism accounting for the beneficial effects of combining exendin-4 with immunotherapy involves the recovery of residual β -cell function by replenishing and enhancing the insulin stores. The effects of exendin-4 were demonstrated by the authors both *in vivo*, by performing glucose tolerance tests, as well as by measuring the total pancreatic insulin content, and *in vitro*, by measuring glucose-stimulated insulin release and insulin content of cultured islets upon treatment with exendin-4. In both settings, exendin-4 resulted in a significantly improved insulin response to glucose stimulation as well as significantly enhanced total islet insulin content. Thus, the authors conclude that the beneficial effects of adding exendin-4 to the anti-CD3 antibody are accounted for via revival of β -cell function.

What are the implications of this preclinical study for clinical efforts directed at preservation of β -cell function in newly diagnosed subjects with T1DM? Although the results from the anti-CD3 clinical trials are encouraging, deterioration in the levels of C peptide in patients 18 months after the single-course treatment indicates that the efficacy of the treatment needs to be improved. Due to safety concerns, an increase in the dose of the drug may not be recommended because anti-CD3 may induce reactivation of latent Epstein-Barr virus infection (5). An alternative approach to overcome the limitations of anti-CD3 monotherapy is the addition of agents with complementary methods of action that promote β -cell regeneration. There is considerable attention focused on β -cell growth factors, including gastrin, epidermal

Abbreviations: GLP-1, Glucagon-like peptide 1; GLP-1R, glucagon-like peptide 1 receptor; T1DM, type 1 diabetes mellitus.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

growth factor, IGF-I, and GLP-1, a gut hormone secreted in response to food ingestion. GLP-1 acts as an incretin, promoting glucose-stimulated insulin release but also increases both proinsulin gene transcription and mRNA stability, leading to an increase in insulin biosynthesis. Administration of GLP-1 or the more potent degradation-resistant lizard-derived peptide exendin-4 to normal and diabetic rodents results in expansion of β -cell mass via stimulation of β -cell proliferation and neogenesis, and a decrease in β -cell apoptosis (reviewed in Refs. 10 and 11). Exendin-4 has also been administered to NOD mice alone or in combination with two different immune modulators, lisofylline (12) or antilymphocyte serum (13); the highest frequency of diabetes remission was observed in animals that received the combination treatments, suggesting a beneficial synergistic effect between immunomodulators and regenerative agents (14). However, neither of these studies was clearly able to elucidate the mechanisms underlying the beneficial actions of exendin-4 in the setting of experimental T1DM.

Although regenerative and antiapoptotic actions of GLP-1 and exendin-4 have been demonstrated in both normoglycemic and diabetic animal models (15–19), the majority of these studies were conducted in animal models of type 2 diabetes. In contrast, much less is known about whether the β -cell actions of glucagon-like peptide 1 receptor (GLP-1R) agonists are sustained in the setting of an ongoing autoimmune attack, as is the case in the NOD mouse and in human subjects with T1DM. Recently, Zhang *et al.* (20) have shown that continuous delivery of GLP-1 using an osmotic mini pump in prediabetic NOD mice results in significant increases in β -cell mass and replication rate, and a significant reduction in the rate of β -cell apoptosis. Hence, it seems possible that GLP-1R activation may be able to enhance β -cell mass even in the presence of an autoimmune attack, if therapy is initiated before the onset of hyperglycemia.

The implications of the work by Sherry *et al.* (9) may inform future attempts to reverse the clinical onset of T1DM in human studies. Residual β -cell mass and function upon diagnosis are decisive attributes in determining the success of experimental therapies for T1DM. However, the degree of variability of β -cell mass and function between patients at diagnosis makes the success of a single therapeutic approach extremely difficult. To circumvent this problem, combination therapies may need to be customized to the degree of residual β -cell function present upon entry into clinical trials. Such therapies can include the use of more than one immunomodulating agent, in conjunction with β -cell regenerative or antiapoptotic agents, or in cases of extremely low β -cell mass, islet transplantations might be required in combination with immunotherapy (14).

Several lines of evidence suggest that GLP-1R agonists may be effective in subjects with T1DM (Fig. 1). GLP-1 and exendin-4 inhibit gastric emptying and glucagon secretion, and promote satiety in human subjects with T1DM (21, 22). Although administration of exendin-4 for 3 months to human subjects with islet transplants improved β -cell function while patients were receiving the drug (23), there is little evidence available as to whether chronic exendin-4 may produce a sustained improvement in β -cell function in subjects with T1DM. A study examining the effects of combining ex-

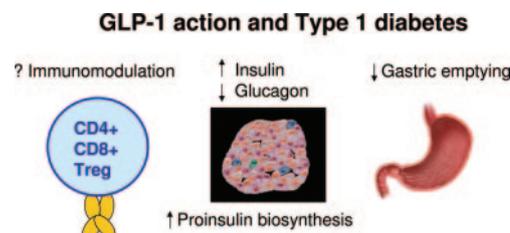


FIG. 1. GLP-1R agonists may improve glucose control in subjects with T1DM via enhancement of insulin secretion from residual β -cells, reduction of glucagon secretion, and inhibition of gastric emptying. Whether GLP-1 also exerts direct or indirect immunomodulatory actions remains unknown. Treg, Regulatory T cells.

endin-4 several times daily with immunosuppression in human subjects with newly diagnosed T1DM has been completed, and the results will likely further our understanding of whether GLP-1R activation alone is sufficient to augment β -cell function in patients with T1DM. Because even small improvements in endogenous insulin secretion may be associated with improved glucose control in subjects with T1DM, an ongoing study of agents such as the GLP-1R agonist exenatide in subjects with newly diagnosed T1DM seems warranted.

Irene Hadjiyanni and Daniel J. Drucker
Department of Medicine
Samuel Lunenfeld Research Institute
Mt. Sinai Hospital
University of Toronto
Toronto, Ontario, Canada M5G 1X5

Acknowledgments

Received August 13, 2007. Accepted August 15, 2007.

Address all correspondence and requests for reprints to: Dr. Daniel J. Drucker, Mt. Sinai Hospital, SLRI 975C, 600 University Avenue, Toronto, Ontario, Canada M5G 1X5. E-mail: d.drucker@utoronto.ca.

Work on glucagon-like peptide 1 and T1DM in the Drucker Laboratory is supported in part by an operating grant from the Juvenile Diabetes Research Foundation.

Disclosure Statement: D.J.D. has served as an advisor or consultant within the past 12 months to Amgen Inc., Amylin Pharmaceuticals, Arisaph Pharmaceuticals Inc., Chugai Inc., Conjuchem Inc., Eli Lilly Inc., Emisphere Technologies Inc., GlaxoSmithKline, Glenmark Pharmaceuticals, Isis Pharmaceuticals Inc., Johnson & Johnson, Merck Research Laboratories, Merck Fr., Novartis Pharmaceuticals, Novo Nordisk Inc., NPS Pharmaceuticals Inc., Phenomix Inc, Takeda, and Transition Pharmaceuticals Inc. I.H. has nothing to disclose.

References

1. Wild S, Roglic G, Green A, Sicree R, King H 2004 Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27:1047–1053
2. Staeva-Vieira T, Peakman M, von Herrath M 2007 Translational mini-review series on type 1 diabetes: immune-based therapeutic approaches for type 1 diabetes. *Clin Exp Immunol* 148:17–31
3. Chatenoud L, Thervet E, Primo J, Bach JF 1994 Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci USA* 91:123–127
4. von Herrath MG, Coon B, Wolfe T, Chatenoud L 2002 Nonmitogenic CD3 antibody reverses virally induced (rat insulin promoter-lymphocytic choriomeningitis virus) autoimmune diabetes without impeding viral clearance. *J Immunol* 168:933–941
5. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L 2005

- Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med* 352:2598–2608
6. **Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, Rother K, Diamond B, Harlan DM, Bluestone JA** 2005 A single course of anti-CD3 monoclonal antibody hOKT3 γ 1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes* 54:1763–1769
 7. **Belghith M, Bluestone JA, Barriot S, Megret J, Bach JF, Chatenoud L** 2003 TGF- β -dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat Med* 9:1202–1208
 8. **Bisikirska B, Colgan J, Luban J, Bluestone JA, Herold KC** 2005 TCR stimulation with modified anti-CD3 mAb expands CD8 $^{+}$ T cell population and induces CD8 $^{+}$ CD25 $^{+}$ Tregs. *J Clin Invest* 115:2904–2913
 9. **Sherry NA, Chen W, Kushner JA, Glandt M, Tang Q, Tsai S, Santamaria P, Bluestone JA, Brillantes AM, Herold KC** 2007 Exendin-4 improves reversal of diabetes in NOD mice treated with anti-CD3 monoclonal antibody by enhancing recovery of β -cells. *Endocrinology* 148:5136–5144
 10. **Drucker DJ** 2003 Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. *Mol Endocrinol* 17:161–171
 11. **Drucker DJ** 2006 The biology of incretin hormones. *Cell Metab* 3:153–165
 12. **Yang Z, Chen M, Carter JD, Nunemaker CS, Garmey JC, Kimble SD, Nadler JL** 2006 Combined treatment with lisofylline and exendin-4 reverses autoimmune diabetes. *Biochem Biophys Res Commun* 344:1017–1022
 13. **Ogawa N, List JF, Habener JF, Maki T** 2004 Cure of overt diabetes in NOD mice by transient treatment with anti-lymphocyte serum and exendin-4. *Diabetes* 53:1700–1705
 14. **Bresson D, von Herrath M** 2007 Moving towards efficient therapies in type 1 diabetes: to combine or not to combine? *Autoimmun Rev* 6:315–322
 15. **Xu G, Stoffers DA, Habener JF, Bonner-Weir S** 1999 Exendin-4 stimulates both β -cell replication and neogenesis, resulting in increased β -cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270–2276
 16. **Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ** 2003 Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 278:471–478
 17. **Wang Q, Brubaker PL** 2002 Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 45:1263–1273
 18. **Tourrel C, Bailbe D, Meile M-J, Kergoat M, Portha B** 2001 Glucagon-like peptide-1 and exendin-4 stimulate β -cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 50:1562–1570
 19. **Stoffers DA, Desai BM, DeLeon DD, Simmons RA** 2003 Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* 52:734–740
 20. **Zhang J, Tokui Y, Yamagata K, Kozawa J, Sayama K, Iwahashi H, Okita K, Miuchi M, Konya H, Hamaguchi T, Namba M, Shimomura I, Miyagawa JI** 2007 Continuous stimulation of human glucagon-like peptide-1 (7–36) amide in a mouse model (NOD) delays onset of autoimmune type 1 diabetes. *Diabetologia* 50:1900–1909
 21. **Behme MT, Dupre J, McDonald TJ** 2003 Glucagon-like peptide 1 improved glycemic control in type 1 diabetes. *BMC Endocr Disord* 3:3
 22. **Dupre J, Behme MT, McDonald TJ** 2004 Exendin-4 normalized postcibal glycemic excursions in type 1 diabetes. *J Clin Endocrinol Metab* 89:3469–73
 23. **Ghofaili KA, Fung M, Ao Z, Meloche M, Shapiro RJ, Warnock GL, Elahi D, Meneilly GS, Thompson DM** 2007 Effect of exenatide on beta cell function after islet transplantation in type 1 diabetes. *Transplantation* 83:24–28

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.