Successful transplantation of human pancreatic islets provides the prospect of a cure for type 1 diabetes (1); however, the lack of sufficient donor pancreata greatly limits the widespread use of this approach (2). Recent discoveries showing the regenerative capabilities of the endocrine pancreas suggest that β-cell regeneration may be a nonmutually exclusive alternative to allogeneic islet transplantation (3,4).

Pancreatic β-cell mass can increase in adult life in response to physiological stimuli such as pregnancy (5) and obesity (6). In addition, β-cells can regenerate under conditions of tissue injury and repair, such as partial pancreatectomy, pancreatic duct ligation, cellophane wrapping of the gland, administration of alloxan or streptozotocin, and transgenic overexpression of γ-interferon in β-cells (7–9). Although β-cell proliferation is increased before diabetes onset in NOD mice, this is not sufficient to keep up with the ongoing autoimmune response that decreases the β-cell mass (10). Normoglycemia can be restored in diabetic NOD mice, however, when the β-cell–directed autoimmune response is abrogated by treatments with anti–T-cell antibodies (11,12) or by replacing NOD immunocompetent cells with bone marrow or splenic cells from diabetes-resistant donors (13,14). These findings suggest that β-cell regenerative processes can occur after diabetes onset; therefore, therapies directed at stimulating β-cell regeneration may restore the β-cell mass in type 1 diabetes.

Many putative β-cell growth factors have been identified, including epidermal growth factor (EGF) and other EGF family members, such as transforming growth factor-α and betacellulin (15–19). In addition, gastrointestinal peptides such as glucagon-like peptide-1 (20,21) and gastrin (22,23) can stimulate β-cell neogenesis. In the rat pancreatic duct-ligated model of pancreas regeneration, gastrin enhances β-cell neogenesis from pancreatic duct cells (22); further, endogenous gastrin may be necessary for β-cell neogenesis in this model (23). Combined EGF and gastrin treatment has been found to increase β-cell mass and reduce hyperglycemia in streptozotocin-induced diabetic rats (24) as well as induce islet regeneration from pancreatic duct cells and restore normoglycemia in alloxan-induced diabetic mice (25). The choice of EGF and gastrin combination therapy in the current studies (24,25)
was based on a previous study in which an increase in islet mass was observed in double transgenic mice that expressed transforming growth factor-α, an EGF receptor ligand, and gastrin locally in the pancreas (26).

In the present study, we investigated whether combination therapy with EGF and gastrin could restore the pancreatic β-cell mass and reverse hyperglycemia in NOD mice with autoimmune diabetes. We report that a short course of EGF and gastrin combination therapy increases β-cell mass and reverses hyperglycemia in acutely diabetic NOD mice concurrently with the induction of immunoregulatory cells.

RESEARCH DESIGN AND METHODS

NOD female mice, age 6–8 weeks, were purchased from Taconic (Germantown, NY). The mice were housed and fed under specific pathogen-free conditions and were cared for according to the guidelines of the Canadian Council on Animal Care. The mice were monitored daily for diabetes onset by urine testing using Keto-Diastix (Bayer, Etobicoke, Canada). Diabetes onset was based on a previous study in which an increase in islet mass was observed in double transgenic mice that expressed transforming growth factor-α, an EGF receptor ligand, and gastrin locally in the pancreas (26).

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glucosuria, followed by blood glucose measurement; a blood glucose ≥12 mmol/l marked the onset of diabetes after splenic cell transfer.

**Statistical analyses.** Data are expressed as means ± SE. Differences between groups were analyzed by one-way ANOVA followed by the Bonferroni multiple comparisons test (for pancreatic insulin content and β-cell mass) and the unpaired Student’s t test (for insulitis scores). Correlations were analyzed by linear regression analysis using Spearman’s rank correlation test. P < 0.05 was considered significant.

**RESULTS**

**Effects of EGF and gastrin on blood glucose.** Female NOD mice had FBG concentrations of 10.0–13.2 mmol/l (normal FBG <6.5 mmol/l) 3–6 days after diabetes onset. The mice treated with vehicle for 2 weeks (controls) became progressively more hyperglycemic and had to be killed at 7 weeks because of severe hyperglycemia (FBG >25 mmol/l) and weight loss (Fig. 1). Treatment with EGF alone reduced FBG to normal levels in three of five mice, but hyperglycemia recurred in these mice when EGF treatment was stopped. Treatment with gastrin alone reduced FBG to near normoglycemic levels in three of five mice, but this effect did not last beyond 5 weeks after stopping gastrin treatment. In contrast, treatment with EGF plus gastrin reduced FBG in five of five mice (from 10.2 ± 0.2 to 6.9 ± 0.2 mmol/l; P < 0.01) after 2 weeks, and FBG remained normal (<6.5 mmol/l) or mildly elevated (<11 mmol/l) in five of six mice (83%) for 10 weeks after EGF and gastrin combination therapy was stopped (Fig. 1).

**Pancreatic histology.** Histological examination of NOD mice pancreata 3–6 days after diabetes onset (just before treatments were started) revealed some islets that still contained abundant insulin-stained β-cells with a halo of leukocytes and other islets that were almost devoid of β-cells and heavily infiltrated by leukocytes (Fig. 2A). After 2 weeks of vehicle (control) treatment and an additional 5 weeks without treatment, the mice were severely hyperglycemic (FBG >30 mmol/l) and their islets were either devoid of β-cells or heavily infiltrated by leukocytes (Fig. 2B). In contrast, after 2 weeks of treatment with EGF plus gastrin and an additional 10 weeks without treatment, many islets with abundant β-cells were found, some with and some without surrounding leukocytes (Fig. 2C). Leukocytic infiltration of islets (insulitis score) was significantly less in pancreata of mice treated with EGF plus gastrin (0.72 ± 0.14; n = 5) than in mice before treatments were started (1.41 ± 0.15; n = 4; P < 0.02).

**Pancreatic insulin content.** Figure 3 shows that the pancreatic insulin content in diabetic NOD mice before treatments (basal, 0.21 ± 0.04 μg) was reduced even further after 2 weeks of vehicle (control) treatment plus 5 weeks off treatment (0.08 ± 0.01 μg). In contrast, pancreatic insulin content was significantly increased above the pretreatment level (0.21 ± 0.04 μg) in mice that survived for 10 weeks after being treated for 2 weeks with EGF (1.13 ± 0.07 μg; P < 0.05) or EGF plus gastrin (1.71 ± 0.18 μg; P < 0.01). Pancreatic insulin content in NOD mice treated with EGF plus gastrin (1.71 ± 0.18 μg) was 28% of that in normoglycemic NOD-scid mice (0.62 ± 0.33 μg), whereas the pancreatic insulin content in NOD mice before treatment (0.21 ± 0.04 μg) was only 3% of that in NOD-scid mice.

**Pancreatic β-cell mass.** Figure 4 shows that pancreatic
β-cell mass in diabetic NOD mice before treatment (basal, 0.27 ± 0.15 mg) was reduced even further after 2 weeks of vehicle (control) treatment plus 5 weeks off treatment (0.01 ± 0.01 mg). In contrast, pancreatic β-cell mass was significantly increased above the pretreatment level (0.27 ± 0.15 mg) in mice that survived for 10 weeks after being treated for 2 weeks with EGF plus gastrin (0.83 ± 0.17 mg; P < 0.05). Pancreatic β-cell mass in NOD mice treated with EGF plus gastrin (0.83 ± 0.17 mg) was 47% of that in normoglycemic NOD-scid mice (1.76 ± 0.20 mg), whereas the β-cell mass in NOD mice before treatment (0.27 ± 0.15 mg) was only 15% of that in NOD-scid mice. Correlations of blood glucose with pancreatic insulin and β-cell mass. Reductions in hyperglycemia by EGF, gastrin, and EGF plus gastrin correlated significantly with increases in pancreatic insulin content and β-cell mass induced by the respective treatments (Fig. 5).

Immunologic effects of EGF and gastrin. Our finding that a short course of combined EGF and gastrin therapy led to long-lasting control of hyperglycemia (Fig. 1) and reduced insulinitis (Fig. 2) suggested that EGF and gastrin had an effect against the autoimmune response in diabetic NOD mice. Therefore, we determined whether mice treated with EGF plus gastrin harbored cells capable of suppressing autoimmunity. Using a standard adoptive transfer model, we injected splenic cells prepared from acutely diabetic NOD female mice (diabetogenic cells) into NOD-scid mice together with or without splenic cells from NOD mice that underwent remission after treatment with EGF plus gastrin (putative immunoregulatory cells.) As shown in Fig. 6, cotransfer of cells from mice treated with EGF plus gastrin significantly delayed diabetes induction by two different doses of diabetogenic cells (Fig. 6A and B) in a dose-dependent fashion (Fig. 6B). These findings suggest that combination therapy with EGF and gastrin induces immunoregulatory cells that may inhibit autoimmunity, thereby contributing to the long-lasting survival of the increased β-cell mass induced by combined EGF plus gastrin therapy.

DISCUSSION
Recent studies have shown that the endocrine pancreas is capable of regeneration after injury in adult life (3,4). Furthermore, β-cell regenerative processes may be active even after diabetes onset in NOD mice with autoimmune diabetes (11–14). This report, to our knowledge, is the first to demonstrate an increase in pancreatic β-cell mass after diabetes onset in NOD mice where the increase was quantified. This increase was achieved by combination therapy with EGF and gastrin. Pancreatic β-cell mass was increased from 15 to 47% of normal, an increase that was sufficient to normalize blood glucose levels. We ascribe the glucose-lowering effects of EGF and gastrin to the abilities of these peptides to increase pancreatic β-cell mass and insulin content, because blood glucose levels were significantly correlated with both pancreatic β-cell mass and insulin content in the mice.

In another recent study, the glucagon-like peptide-1 analog, exendin-4, was reported to reverse hyperglycemia in diabetic NOD mice; however, this required concurrent immunosuppressive treatment with antilymphocyte serum (29). In the present study, combination therapy with EGF and gastrin did not require additional immunotherapy to induce remission in diabetic NOD mice. Also, we achieved a long-lasting remission with EGF plus gastrin (blood glucose <11 mmol/l in 83% of mice) similar to that
EGF AND GASTRIN REVERSE DIABETES IN NOD MICE

FIG. 6. Development of diabetes after adoptive cell transfer. A: Splenic cells (1.5 × 10⁷) from diabetic NOD mice either alone (●; n = 5) or together with splenic cells (0.5 × 10⁷) from NOD mice treated with EGF plus gastrin (▲; n = 4) were injected intravenously into NOD-scid mice. B: Splenic cells (0.5 × 10⁷) from diabetic NOD mice, either alone (●; n = 5) or together with splenic cells (▲, 0.5 × 10⁷; ▪, n = 4; □, 3.0 × 10⁷, n = 6) from NOD mice treated with EGF plus gastrin were injected intravenously into NOD-scid mice.

achieved with exendin-4 plus antilymphocyte serum (blood glucose <11 mmol/l in 88% of mice) (29). Our finding that a short course (2 weeks) of EGF plus gastrin treatment induced a long-lasting (at least 10 weeks) remission from hyperglycemia in diabetic NOD mice without immunotherapy was unexpected. The finding of reduced islet inflammation (insulitis) in mice treated with EGF plus gastrin suggests that EGF and gastrin therapy may have interfered with the autoimmune response against β-cells. This interpretation is supported by the finding of immunoregulatory cell activity in mice treated with EGF plus gastrin; however, there may be other explanations for the decreased insulitis. For example, there could be nonimmunoregulatory cell–dependent effects of EGF and gastrin on the balance of proinflammatory (Th1) and anti-inflammatory (Th2 or Th3) cytokines. In fact, EGF receptor ligands have been reported to either increase or suppress inflammatory mediators (30).

Regarding possible mechanisms by which EGF plus gastrin therapy induced increases in β-cell mass in diabetic NOD mice in the present study, this therapy was recently reported to induce neogenesis of β-cells from pancreatic exocrine duct cells in mice with alloxan-induced diabetes (25). These findings are consonant with the different roles that EGF and gastrin have in islet development. EGF receptor ligands are expressed in the developing pancreas, and EGF receptor signaling stimulates proliferation and branching morphogenesis of fetal pancreatic ducts (31). This process is impaired and islet cell differentiation is delayed in mice lacking EGF receptors (32). Endogenous gastrin expression is activated in the developing pancreas during the secondary transition phase when proto-differentiated ducts develop into the fully differentiated exocrine and endocrine pancreas (33). Further study is needed to determine whether or not the increase in pancreatic β-cell mass in NOD mice induced by EGF plus gastrin in the present study resulted from β-cell neogenesis from duct cells, as reported when the combination of EGF and gastrin was used to treat mice with alloxan-induced diabetes (25). Similarly, we recently reported that combination therapy with EGF and gastrin significantly increased β-cell mass in adult human pancreatic islets in vitro and in vivo, an increase that appeared to result from the induction of β-cell neogenesis from pancreatic exocrine duct cells (34).

In summary, a short course of combined EGF and gastrin treatment of NOD mice after diabetes onset increased pancreatic β-cell mass and insulin content, decreased insulitis, and reversed hyperglycemia. These changes were accompanied by the induction of immunoregulatory cells. We conclude, therefore, that EGF plus gastrin combination therapy induces β-cell regeneration while inhibiting autoimmune β-cell destruction, and that this may dispense with the need for conventional immunosuppressive drug therapy for type 1 diabetes.

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REFERENCES
mune diabetes in nonobese diabetic mice by immunotherapy with anti-

12. Chatenoud L, Thervet E, Primo J, Bach JF: Anti-CD3 antibody induces
long-term remission of overt autoimmunity in nonobese diabetic mice.

Styche AJ, Trucco M: Recovery of the endogenous beta cell function in
the NOD model of autoimmune diabetes. Stem Cells 21:377–
388, 2003

regeneration during the reversal of autoimmune diabetes in NOD mice.

15. Song SY, Gannon M, Washington MK, Scoggins CR, Meszoely IM,
Golden-ring JR, Marino CR, Sandgren EP, Coffey RJ Jr, Wright CV,
Leach SD: Expansion of PDX-1-expressing pancreatic epithelium and islet neogenesis
in transgenic mice overexpressing transforming growth factor alpha. Gastroenterology
117:1416–1426, 1999

 Miczalkowski B, Sarvetnick N: Transgenic expression of epidermal growth
factor and keratinocyte growth factor in beta-cells results in substantial

factor increases undifferentiated pancreatic embryonic cells in vitro: a
balance between proliferation and differentiation. Diabetes 50:1571–1579,
2001

18. Huotari MA, Palgi J, Otonkoski T: Growth factor-mediated proliferation
and differentiation of insulin-producing INS-1 and RINm5F cells: identification
of betacellulin as a novel beta-cell mitogen. Endocrinology 139:1494–
1498, 1999

, T, Moriwaki M, Imagawa A, Yanagata K, Nakajima H, Namba M, Tochino
Y, Hanafusa T, Matsuzawa Y: Recombinant human betacellulin promotes the
neogenesis of beta-cells and ameliorates glucose intolerance in mice with
diabetes induced by selective alloxan perfusion. Diabetes 49:2021–2027,
2000

20. Xu G, Stoffers DA, Habener JF, Bonner-Weir S: Exendin-4 stimulates both
beta-cell replication and neogenesis, resulting in increased beta-cell mass
and improved glucose tolerance in diabetic rats. Diabetes 49:2270–2276,
1999

peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-
treated newborn rats resulting in persistently improved glucose homeosta-

22. Rooman I, Lardon J, Bouwens L: Gastrin stimulates beta-cell neogenesis and
increases islet mass from transdifferentiated but not from normal exocrine
pancreatic tissue. Diabetes 51:686–690, 2002

23. Rooman I, Bouwens L: Islet neogenesis in the regeneration model of rat
pancreatic duct ligation requires endogenous gastrin action via CCK2
receptors. Diabetologia 45 (Suppl. 2):A26, 2002

Y: Pharmacological treatment of chronic diabetes by stimulating pancreatic
beta-cell regeneration with systemic co-administration of EGF and

25. Rooman I, Bouwens L: Combined gastrin and epidermal growth factor
treatment induces islet regeneration and restores normoglycemia in
C57Bl/6J mice treated with alloxan. Diabetologia 47:259–265, 2004

26. Wang TC, Bonner-Weir S, Oates PS, Chulak M, Simon B, Merlino GT,
Schmidt EV, Brand SJ: Pancreatic gastrin stimulates islet differentiation of
transforming growth factor alpha-induced ductal precursor cells. J Clin
Invest 92:1349–1356, 1993

K, Edwards AD, Playford RJ: Potency and stability of C terminal truncated

28. Petersen B, Christensen J, Rehfled JF: Acid potency and elimination of the
15-leucine gastrin-17 analogue in man. Scand J Gastroenterol 16:437–440,
1981

29. Ogawa N, List JF, Habener JF, Maki T: Cure of overt diabetes in NOD mice
by transient treatment with anti-lymphocyte serum and exendin-4. Diabete-
s 53:1700–1705, 2004

Inflamm Bowel Dis 5:44–60, 1999

31. Wang RN, Rehfled JF, Nielsen FC, Kloppe G: Expression of gastrin and
transforming growth factor-alpha during duct to islet cell differentiation in

32. Miettinen PJ, Huotari M-A, Koivisto T, Ustinov J, Palgi J, Rasilainen S,
Schmidt EV, Brand SJ: Pancreatic gastrin stimulates islet neogenesis and
differentiation of insulin-producing INS-1 and RINm5F cells: identifi-
cation at adult age. J Clin Endocrinol Metab 89:5341–5347, 2004

33. Brand SJ, Fuller PJ: Differential gastrin gene expression in rat gastroin-
testinal tract and pancreas during neonatal development. J Biol Chem
263:5341–5347, 1988

34. Suarez-Pinzon WL, Lakey JRT, Brand SJ, Rabinovitch A: Combination
therapy with epidermal growth factor and gastrin induces neogenesis of
human islet beta-cells from pancreatic duct cells and an increase in functional
beta-cell mass. J Clin Endocrinol Metab 90:3401–3409, 2005