Acute Sulfonylurea Therapy at Disease Onset Can Cause Permanent Remission of K_{ATP}-Induced Diabetes

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OBJECTIVE—Neonatal diabetes mellitus (NDM) can be caused by gain-of-function ATP-sensitive K^+ (K_{ATP}) channel mutations. This realization has led to sulfonylurea therapy replacing insulin injections in many patients. In a murine model of K_{ATP}-dependent NDM, hyperglycemia and consequent loss of β-cells are both avoided by chronic sulfonylurea treatment. Interestingly, K_{ATP} mutations may underlie remitting-relapsing, transient, or permanent forms of the disease in different patients, but the reason for the different outcomes is unknown.

RESEARCH DESIGN AND METHODS—To gain further insight into disease progression and outcome, we examined the effects of very early intervention by injecting NDM mice with high-dose glibenclamide for only 6 days, at the beginning of disease onset, then after the subsequent progression with measurements of blood glucose, islet function, and insulin sensitivity.

RESULTS—Although ~70% of mice developed severe diabetes after treatment cessation, ~30% were essentially cured, maintaining near-normal blood glucose until killed. Another group of NDM mice was initiated on oral glibenclamide (in the drinking water), and the dose was titrated daily, to maintain blood glucose <200 mg/dL. In this case, ~30% were also essentially cured; they were weaned from the drug after ~4 weeks and again subsequently maintained near-normal blood glucose. These cured mice maintain normal insulin content and were more sensitive to insulin than control mice, a compensatory mechanism that together with basal insulin secretion may be sufficient to maintain near-normal glucose levels.

CONCLUSIONS—At least in a subset of animals, early sulfonylurea treatment leads to permanent remission of NDM. These cured animals exhibit insulin-hypersensitivity. Although untreated NDM mice rapidly lose insulin content and progress to permanently extremely elevated blood glucose levels, early tight control of blood glucose may permit this insulin-hypersensitivity, in combination with maintained basal insulin secretion, to provide long-term remission. *Diabetes* **60**:2515–2522, 2011

I t is now clear that a large proportion of neonatal diabetes mellitus (NDM) can be accounted for by mutations in the *KCNJ11* and *ABCC8* genes that encode the Kir6.2 and sulfonylurea receptor 1 (SUR1) subunits of the ATP-sensitive K^+ (K_{ATP}) channel in pancreatic β-cells (1–9). Increased glucose metabolism leads to elevated intracellular [ATP]:[ADP], which normally closes K_{ATP} channels, leading to membrane depolarization, Ca^{2+}-entry,

and triggering of secretion (10,11). NDM mutations invariably result in reduced channel sensitivity to ATP inhibition, K_{ATP} channel overactivity, decreased membrane excitability, and reduced insulin secretion (12).

Interestingly, in most NDM cases, the disease is permanent NDM (PNDM), requiring lifelong therapy, but in the remainder, the disease is transient NDM (TNDM), spontaneously remitting within weeks or months of diagnosis, but typically relapsing around puberty or later in life (13–16). It is of note that either outcome can be obtained with the same K_{ATP} mutation. Members of the same family carrying the same K_{ATP} channel mutation show different disease outcomes ranging from TNDM to PNDM and even late-onset diabetes and gestational diabetes (15–19). This indicates variable penetrance of the molecular defect and leads us to speculate that the disease outcome might depend not only on additional genetic or epigenetic factors, but also on the timing of diagnosis and the nature of the therapeutic intervention.

Predicting the human disease, we previously developed transgenic mice constitutively expressing ATP-insensitive β-cell K_{ATP} channels (20). These mice developed profound neonatal diabetes and died shortly after birth, precluding detailed analysis of disease progression. We subsequently generated inducible K_{ATP} gain-of-function (GOF) transgenic mice by insertion of an ATP-insensitive Kir6.2 construct into the Rosa26 locus, under Cre-recombinase control (21). By crossing with tamoxifen-inducible Pdx1[PB]Cre[ER]TM (Pdx-Cre) (22) mice, we generate β-cell–specific (although PDX1 promoters could alter gene expression in the brain [23]) conditional Pdx-Cre/Rosa26-Kir6.2[K185Q,ΔN30] double transgenic (DTG) mice that develop severe glucose intolerance within 2 weeks after tamoxifen injection and progressively severe diabetes (21). Although these mice subsequently survive with uncontrolled blood glucose (>600 mg/dL), they secondarily develop profound loss of β-cell mass and dramatic reduction of insulin content (21). It is noteworthy that diabetes and the secondary progression of the disease are completely avoided by maintenance of normoglycemia, achieved either by syngeneic islet transplantation or by implantation of slow-release sulfonylurea (SU) pellets before disease onset (21). Diabetes can be completely avoided in each case, but in the first case this is because of insulin secretion from the transplanted islets and, in the second, because constitutive inhibition of K_{ATP} ensures persistent depolarization of endogenous β-cells (24), and hence endogenous insulin secretion (21).

In human NDM, it is now quite clear that SU therapy can successfully control blood glucose levels and avoid (or reduce) insulin requirements in the majority of K_{ATP}-induced PNDM (15,25–27) and also in TNDM (28–31). As a preferable alternative to lifelong exogenous insulin injections, SU drugs circumvent the metabolic signal in the β-cell by directly targeting K_{ATP} overactivity to restore endogenous insulin secretion. This effect can also be

augmented in the presence of potentiating hormones (glucagon and glucagon-like peptide 1 [GLP-1]) and by other so-called K$_{ATP}$-independent mechanisms; thus insulin secretion becomes quasi-physiological and may maintain stable glycemia. SU requirements are quite variable. Successful therapeutic response in NDM patients frequently requires larger SU doses than the current recommended regimen for adult patients with type 2 diabetes (25,26), and in some cases SU have proved to be ineffective and the patients continue to require maintained insulin therapy (19,26,32). Paralleling these findings, and potentially explaining nonresponsivity to SU, we found that late-onset SU pellet treatment of mice with severe diabetes (glucose >300 mg/dL) was ineffective, presumably because the profound loss of β-cells had already occurred (21).

Controlled studies of drug therapy in human NDM are not practically feasible, and so, in the current study, we have explored the treatability of NDM by SU drugs after disease induction in DTG mice. The results indicate that there is a critical window early in the disease during which compensatory mechanisms may develop, switching the disease from a permanent, progressively worsening diabetes to a transient hyperglycemia with subsequent long-term remission. These striking results may have important implications for understanding human NDM progression and therapeutic possibilities.

RESEARCH DESIGN AND METHODS

Mouse model of K$_{ATP}$-induced NDM. Mice carrying the GOF Kir6.2[K185Q, ΔN30] mutant transgene were generated previously (21). By crossing these mice with Pdx-Cre (22), we generated pancreatic β-cell–specific DTG mice. Both wild-type and single transgenic mice were used as controls since these were previously shown to have normal blood glucose levels and insulin secretion (21). DTG mice 8 weeks of age received five consecutive daily doses of tamoxifen (50 mg/g body wt; experimental days 0–4). Blood glucose measurements were taken daily using a Glucometer Elite XL. The limit of detection was 600 mg/dL, and glucose at or above this level is recorded as 600 mg/dL but considered to be a lower limit of the true value. Cre-mediated recombination leads to the expression of the Kir6.2[K185Q,ΔN30] protein but also to the expression of green fluorescent protein (GFP) via internal ribosomal entry site; therefore GFP can be used to detect successful recombination and track transgene expression.

SU (glibenclamide) treatment. All experiments were performed in compliance with the relevant laws and institutional guidelines and were approved by the Washington University Animal Studies Committee. To test the effect of aggressive SU treatment in neonatal diabetes, mice were subjected to two independent experimental groups: *1*) by injecting high doses (0.5 g/kg i.p.) of glibenclamide for 6 consecutive days before the onset of diabetes and *2*) by adding glibenclamide (Sigma-Aldrich) to the drinking water (160 mg/L, or reduced accordingly) to titrate the dose required in each individual. Glibenclamide was prepared by dissolving 25 mg in 500 μL of DMSO, which was then diluted in 150 mL of water to a final concentration of 167 mg/L (337 μM) in 0.2% DMSO, with no visible precipitation. For the latter experiments, each animal was caged individually; the drug was freshly prepared every day. For SU injection, the drug was added to PBS buffer solution (Ambion). Similar numbers of males and females were used for these experiments.

Blood glucose and plasma insulin. Tail blood was assayed for glucose content using Glucometer Elite Xl meter (Bayer Corporation, Elkhart, IN). Plasma insulin was measured using a rat insulin enzyme-linked immunoabsorbent assay kit (ELISA kit).

Glucose and insulin tolerance tests. Intraperitoneal glucose tolerance test was performed in 12-h fasted mice by injection of a bolus of glucose (1.5 g/kg body wt) at day 70 after the first dose of tamoxifen for induction of the disease. Blood was taken at different times (as indicated in Fig. 2) and assayed for glucose content as above. Insulin tolerance test was done on mice after a 6-h fast. Animals were injected intraperitoneally with insulin (0.5 units/kg), and blood was isolated from the tail vein at times indicated in Fig. 3 and assayed for glucose as described above.

Hyperinsulinemic-euglycemic clamp. Weight-matched wild-type and TG mice were anesthetized with isoflurane and catheters (MRE 025; Braintree Scientific Inc., Braintree, MA) implanted into both the right internal jugular vein

and left femoral artery; mice were allowed to recover for 5–7 days. After a 5-h fast, catheters were flushed with saline, and heparin (20 units/kg) was administered to maintain catheter patency. Insulin (4 mU/kg/min) in saline containing 0.1% BSA was infused through the venous catheter (2 μL/min) using a Harvard-11 pump. At 5- to 10-min intervals, blood (100 μL) was removed from the arterial catheter for determination of blood glucose levels using Contour TS glucometer. Dead-space blood was then reinfused into the animal. Dextrose (25%) was infused through the venous catheter at a rate sufficient to maintain a plasma glucose level of 100 to 110 mg/dL.

Pancreatic islet isolation. Mice were anesthetized with isoflurane (0.2 mL) and killed by cervical dislocation. The bile duct was cannulated and perfused with Hanks' balanced salt solution (Sigma-Aldrich) containing collagenase (0.3 mg/mL, Collagenase Type XI; Sigma-Aldrich). Pancreata were removed and digested for 7 min at 37°C, hand shaken, and washed three times in cold Hanks' solution. Islets were isolated by hand under a dissecting microscope, and pooled islets were maintained overnight in CMRL-1066 (5.6 mmol/L glucose) culture medium (GIBCO) supplemented with FCS (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL).

Insulin release from isolated islets. After overnight incubation in CMRL-1066 medium containing 5.6 mmol/L glucose, islets (10/well in 12-well plates) were preincubated in glucose-free CMRL-1066 plus 3 mmol/L glucose for half an hour and then incubated for 60 min at 37°C in CMRL-1066 plus varying glucose, 1 μM glibenclamide, or 30 mmol/L KCl, as indicated. After the incubation period, the medium was removed and assayed for insulin release. Islets were disrupted using ethanol-HCl extraction and sonicated on ice before estimation of insulin content. Experiments were repeated in triplicate. Rat insulin radioimmunoassay according to manufacturer's procedure (Millipore, St. Charles, MO) was used to determine insulin secretion and content.

Statistics. Data are presented as mean ± SEM. Differences among groups were tested using ANOVA and post hoc Duncan test. When only two groups were compared, unpaired t tests were used to assess significance. Differences were assumed to be significant in each case if $P < 0.05$, and nonsignificant (ns) differences are indicated.

RESULTS

Early aggressive SU therapy in K$_{ATP}$-induced NDM mice can cause remission of otherwise permanent diabetes. To induce transgene expression, 2-month-old Pdx-Cre/Kir6.2[K185Q,ΔN30] DTG and littermate control mice were injected daily for 5 consecutive days with tamoxifen (50 μg/g body wt). Without SU treatment, DTG mice develop severe diabetes within 2 weeks after induction, whereas single transgenics and wild-type littermates are unaffected (21) (Fig. 1A). The clinical observation that SU dose requirement typically declines with time in NDM patients provides a hint that some secondary compensatory changes may be occurring. We demonstrated previously two key relevant features of disease progression in DTG (21). First, chronic SU treatment (implanted slow-release glibenclamide pellets) provides long-term protection against hyperglycemia, as in the human disease. Second, protection against hyperglycemia either by this treatment or by transplantation of exogenous islets preserves insulin content of endogenous islets, but, without protection, insulin content is lost, and subsequent administration of SU is ineffective. This leads us to hypothesize that part of the drug sensitivity variability in human NDM is a result of long-term poor control of the diabetes and that aggressive early therapy is critical for maintaining islet insulin.

To gain further insight into SU sensitivity in the disease, DTG animals were initially treated daily with a high dose intraperitoneal injection of glibenclamide (0.5 g/kg) for 6 consecutive days, during and immediately after tamoxifen induction (Fig. 1B). For the period of glibenclamide injection the rise in blood glucose was similarly suppressed in all treated animals (Fig. 1C). However, the subsequent response of DTG animals fell into two clear groups. In the first, blood glucose was maintained normal during SU treatment, but rose and did not fall once SU treatment was terminated, as expected for persistent expression of the
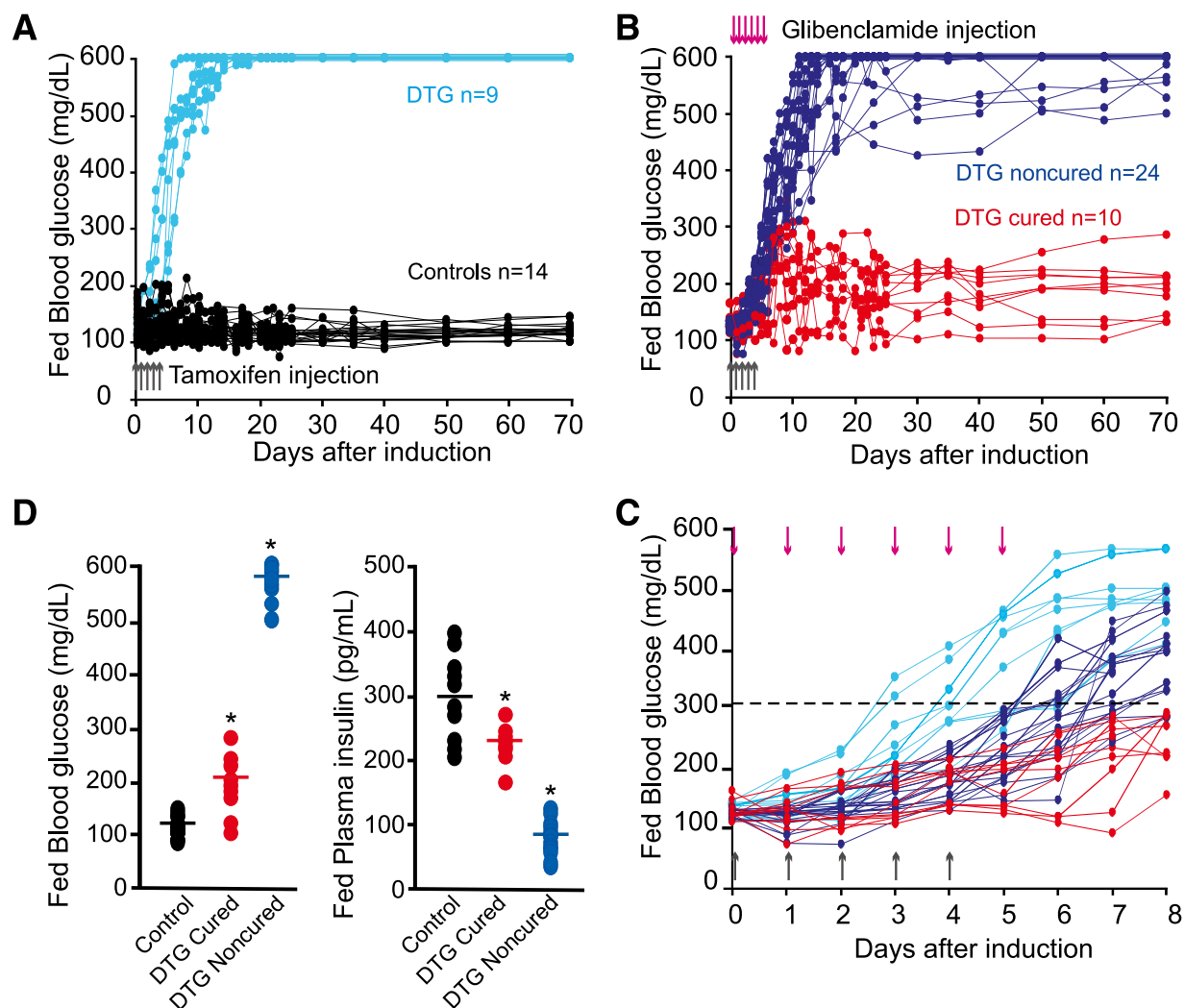
FIG. 1. Short period of intraperitoneal glibenclamide treatment of DTG mice early on can cause permanent remission of diabetes. *A* and *B*: Individual traces of fed blood glucose vs. time. Control (black) and SU untreated DTG mice (light blue; *A*) and DTG mice treated with the SU glibenclamide (0.5 g/kg; *B*) were injected intraperitoneally on days 0–5 during tamoxifen induction. Cured mice are shown in red, and noncured mice are shown in dark blue. *C*: The same data as in *A* (untreated DTG only; light blue), and cured (red) and noncured (dark blue) glibenclamide-injected mice, for the first 8 days after tamoxifen induction. *D*: Individual values of fed blood glucose and plasma insulin (day 60 after tamoxifen induction) from controls (black; *n* = 27 mice) and DTG animals (cured: red, *n* = 10 mice; and noncured: dark blue, *n* = 24 mice) acutely treated with glibenclamide. Significant differences: *$P < 0.05$ with respect to control.

GOF mutation (i.e., PNDM). In this noncured group, 24/34 (~70%) animals developed severe diabetes; blood glucose was >500 mg/dL by 14 days and stayed high until approximately day 70 when the animals were killed (Fig. 1*B*, blue), comparable with untreated DTG animals (Fig. 1*A*). It is noteworthy that, however, ~30% (10/34) were apparently cured by this early treatment, maintaining blood glucose <200 mg/dL for ~70 days after treatment was terminated until being killed, without any further intervention (Fig. 1*B*, red); i.e., this group exhibited a chronic remission, essentially a cured phenotype.

To further examine the implications of this important finding (that early aggressive SU treatment can essentially cure the disease and provide subsequent prolonged normoglycemia without further therapy), an additional group of DTG animals was treated orally with glibenclamide from disease onset, and then the drug dose was titrated daily to attempt to maintain stable blood glucose levels (Fig. 2*A*). Glibenclamide was initially dissolved in DMSO and then added to the drinking water at the highest concentration

used for these experiments (160 mg/L). Glucose was measured daily, and the drinking water dose for each animal was then adjusted according to the following regimen: glibenclamide was initiated at the highest dose (160 mg/L) as soon as the blood glucose was >200 mg/dL and kept at this concentration for 4 days, independently of the blood glucose level. Subsequently, starting on day 5, if blood glucose was >250 mg/dL, the dose was restored to the preceding dose; if blood glucose was 200–250 mg/dL, the dose was reduced by half; if glucose was <200 mg/dL, glibenclamide was removed from the water.

Essentially the outcome in the orally treated animals was the same as observed in animals injected with glibenclamide; ~60% (6/9) developed severe diabetes by day 14 (noncured), with blood glucose levels of >500 mg/dL, even though glibenclamide dose was not stopped or lowered. In the orally treated group (cured; 3/9), glibenclamide dose requirement declined, and in each case the drug was eventually removed permanently (between days 20 and 40) (Fig. 2*A*). Most strikingly, after glibenclamide
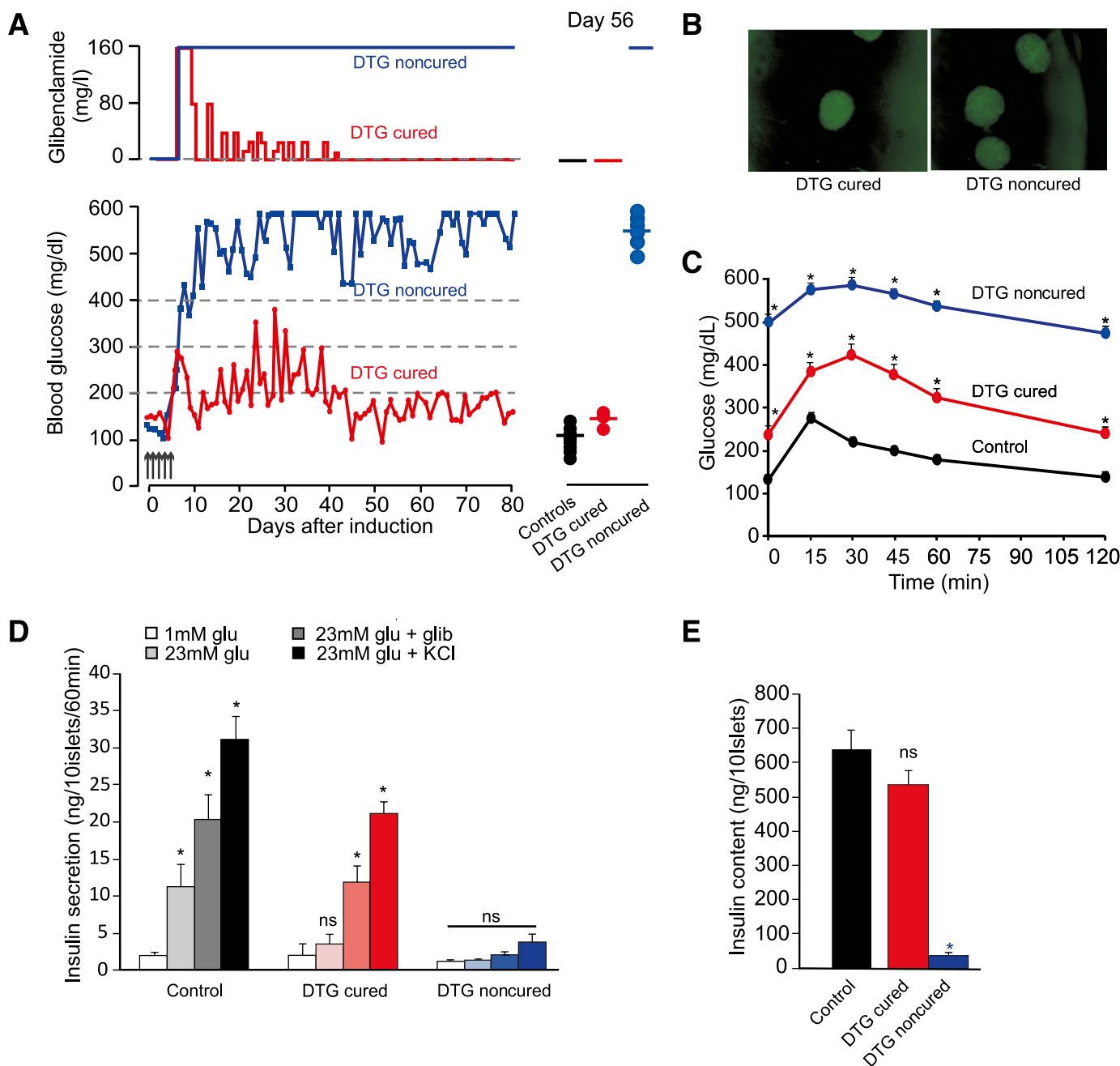
FIG. 2. Early oral glibenclamide treatment can prevent the development of diabetes and preserve insulin content. *A, left*: Representative daily glibenclamide dose (*top*) and corresponding fed blood glucose (*bottom*) from a single (DTG cured [red] and noncured [blue]) mouse. *A, right*: Average glibenclamide dose and individual fed glucose at day 56 in controls (black; *n* = 10) and in cured (red; *n* = 3) and noncured DTG mice (blue; *n* = 6). Glibenclamide was added to the drinking water, with an initial dose of 160 mg/L. The dose was reduced by half each day that blood glucose fell. All cured mice were eventually weaned from the drug, whereas noncured mice, even at the highest dose, did not improve blood glucose. *B*: GFP fluorescence in pancreatic islets from DTG cured and noncured mice. *C*: Blood glucose vs. time after injection of 1.5 g/kg glucose after overnight fast. Glucose tolerance test in control (black) and noncured DTG (blue) and cured DTG (red) mice treated with glibenclamide (day 60 after tamoxifen induction; *n* = 10–34 mice per group) is shown. *Significant difference of *P* < 0.05 with respect to control at each time point. *D*: Insulin secretion from isolated islets in response to glucose (white and light color), glibenclamide (medium color), and KCl (dark color). *E*: Islet insulin content from control mice (black) and DTG mice treated with glibenclamide (cured: red; and noncured: blue). Data in *D* and *E* are shown as mean ± SEM (*n* = 8–10 mice in each group). Significant difference: *P* < 0.05 with respect to 1 mmol/L glucose or to control islets, respectively; glib, glibenclamide; glu, glucose; ns, nonsignificant differences are indicated. (A high-quality digital representation of this figure is available in the online issue.)

removal, these cured animals all maintained blood glucose <200 mg/dL for more than 60 days (Fig. 2A). All mice drank ~10 mL daily, implying a maximum oral dose of ~1.67 mg/25 g/day. This is considerably lower than the daily dose received by injection in Fig. 1 and may have

been insufficient to stop the development of hyperglycemia in the noncured subset, even during treatment.

These two drug trials reveal that in a subset of animals (cured) the initial pathology that follows onset of K_{ATP} overactivity can actually remit, since affected islets must

subsequently release sufficient insulin to maintain normal blood glucose levels. Why only a subset of animals remits is unclear. Retrospective analysis reveals no difference in weight (20.4 ± 0.82 g, n = 10; vs. 20.1 ± 0.41 g, n = 24; cured vs. noncured) or blood glucose (129 ± 4.68 mg/dL vs. 127 ± 1.14 mg/dL, cured vs. noncured) at the time of induction. As shown in Fig. 2B, the level of transgene expression (assessed by GFP fluorescence) in islets from cured DTG mice is at least as high as in islets from noncured DTG mice, indicating that glibenclamide did not reduce the efficiency of Cre-recombination and transgene expression, nor survival/replication of nonrecombinant β-cells.

Cured DTG mice secrete sufficient insulin to maintain near-normal glycemia long term after SU treatment is terminated. Consistent with severe diabetes, plasma insulin is dramatically reduced in untreated DTG mice, but, strikingly, cured DTG mice treated for only a short term with SU maintained nearly normal glycemia reflecting a sufficient, albeit reduced, circulating level of insulin to halt the development of diabetes (Fig. 1D). All DTG mice showed lower plasma insulin after SU treatment was terminated, but this was more dramatic in the noncured mice (Fig. 1D).

Mice underwent glucose tolerance testing at days 57 and 60 after tamoxifen induction, ~6 weeks after SU treatment was terminated. Mice were intraperitoneally injected with glucose after a 12-h fast. Noncured DTG mice show a dramatic impairment in glucose tolerance, with fasting blood glucose of >500 mg/dL. It is noteworthy that, however, cured mice at days 57 and 60 show fasting blood glucose of ~220 mg/dL and are only mildly glucose-intolerant compared with control littermates (Fig. 2C).

Insulin content is maintained in islets from cured DTG mice, but glucose-dependent insulin secretion remains markedly suppressed. Isolated islets from noncured DTG mice that develop severe diabetes show negligible insulin secretion not only in response to glucose, but also to glibenclamide, or high [K^+] (Fig. 2D), consistent with the drastic reduction of insulin content (Fig. 2E). However, even though they still show no significant glucose-dependent secretion (predicted given the constitutive expression of overactive β-cell K_{ATP} channels), islets from cured DTG animals show enhanced insulin secretion in response to glibenclamide, and to high K^+ depolarization, reflecting preservation of insulin content long after SU treatment is terminated (Fig. 2D and E).

DTG cured mice are more insulin sensitive than control mice. Because there is still very little glucose stimulation of insulin secretion in cured mice, we examined the possibility that normoglycemia results from greater insulin responsiveness. We determined the glucose-lowering effect of intraperitoneal insulin (0.5 units/kg) on 6-h fasted mice. Noncured mice do not show any obvious lowering of blood glucose, even though absolute levels of blood glucose are much higher than in control mice. Conversely, cured mice show a prolonged drop in blood glucose levels, indicating a relative increase in insulin sensitivity with respect to control littermates (Fig. 3A).

To more directly determine peripheral insulin sensitivity in cured DTG mice, hyperinsulinemic-euglycemic clamps (33) were performed in control and in cured DTG mice. The lack of glucose-stimulated insulin secretion is reflected in the higher overshoot of blood glucose in response to onset of infusion during the establishment of the clamp (Fig. 3B), but once comparable, stable glucose levels are attained (~60 min), the rate of glucose disposal is markedly higher
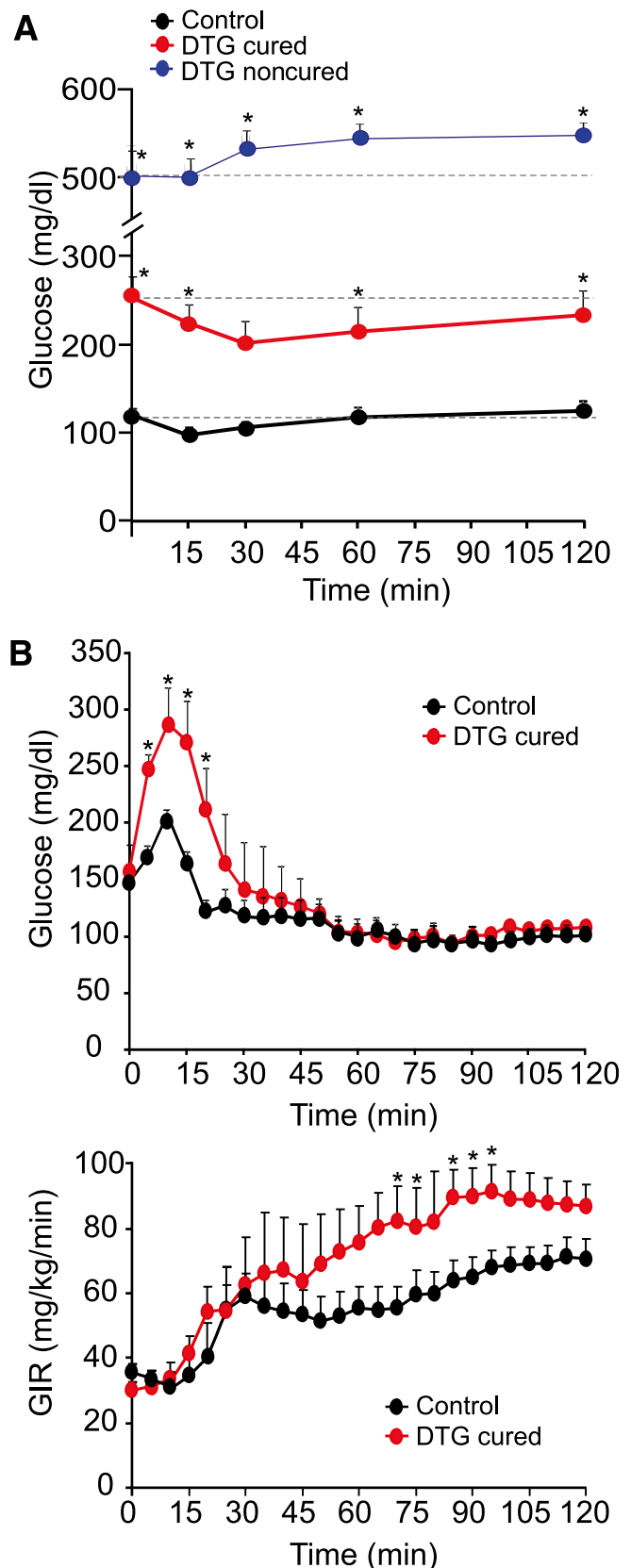

FIG. 3. DTG cured mice are more insulin sensitive. *A*: Insulin tolerance test was performed at day 60 after tamoxifen induction in controls and DTG mice treated early with glibenclamide (*n* = 8–10 mice per group). *B*: Glucose (mg/dL; *top*) and glucose infusion rate (GIR; mg/kg/min; *bottom*) over time during hyperinsulinemic-euglycemic clamp on control (black) and cured DTG (red) mice. *Significant difference of *P* < 0.05 with respect to control mice at each time point.

(by ~30%) in cured DTG mice compared with control littermates.

DISCUSSION

K$_{ATP}$ channel overactivity causes both PNDM and TNDM in humans. GOF mutations in the pore-forming Kir6.2 and the regulatory SUR1 subunits of the K$_{ATP}$ channel are major causes of both PNDM and remitting-relapsing TNDM in humans (4,8,13,15,25,34,35). SU directly inhibit the K$_{ATP}$ channel, thereby promoting insulin secretion independently of the metabolic state of the cell. The identification of K$_{ATP}$ mutations as causal in NDM led straight to the realization that SU could be an effective treatment. Now many PNDM and TNDM patients have been successfully transferred from insulin injections to oral SU therapy, frequently demonstrating improved glycemic control (26,32,36–38). However, successful transfer has not proved to be possible in all PNDM cases; in general, transferability correlates negatively with the age of the patient and the severity of the disease (12,31,39). There are several examples of successful transfer of an infant to relatively low SU doses, with the parent afflicted with the same mutation being less responsive (19,26,38). It is also clear that either PNDM or TNDM can result from the same K$_{ATP}$ mutation (9). These disparities imply that additional genetic or nongenetic factors must contribute to the determination of disease severity and treatability.

Mouse model of K$_{ATP}$-induced NDM reveals the development of secondary consequences and their avoidance by chronic SU treatment. We previously demonstrated development of severe diabetes in inducible mouse NDM, in which we express GOF K$_{ATP}$ channels specifically in pancreatic β-cells (21). Similarly, Girard et al. (40) demonstrated development of diabetes in mice expressing the NDM-associated GOF Kir6.2 V59M mutation using the same approach. It is noteworthy that we identified unpredicted and previously unrecognized secondary consequences of systemic diabetes in these mice: deterioration of pancreatic architecture, with drastic loss of islet insulin content and β-cell mass (21,40). These consequences underlie the lack of insulin secretion in response to glibenclamide, or to high K$^+$ depolarization from these islets, at late stages of the disease (21). It is equally noteworthy that maintenance of normoglycemia, achieved either by syngeneic islet transplantation or by chronic SU therapy (glibenclamide pellet implantation, drug present for 90 days), not only completely avoided hyperglycemia, but also prevented the development of the secondary consequences (21). Isolated islets from chronically SU-treated animals retained normal insulin secretion in response to high K$^+$, and near-normal response to glibenclamide, yet glucose-dependent secretion above basal was essentially absent, consistent with the expected consequences of GOF K$_{ATP}$ channels (21). It is noteworthy that, however, if SU therapy was initiated after the disease had developed (blood glucose >300 mg/dL), this treatment was ineffective, a result of the systemic diabetes leading to loss of β-cell mass and insulin content (21). We speculate that exposure to episodic hyperglycemia in human NDM may lead to similar secondary consequences, reflected in the demonstration that ability to transfer to SU in members of a single family carrying the same identified K$_{ATP}$ mutation declines with duration of the disease, and in some cases this has proved to be completely ineffective (14,26). Thus, our studies in mice provide an immediate potential explanation for why older NDM patients typically show reduced SU responsivity (26,38).

Early aggressive SU therapy can cause chronic remission of PNDM. The variable disease outcome in our experiments and dependence of efficacy of SU treatment on the time of therapeutic intervention in human NDM open the possibility that remission of NDM might actually be possible with early aggressive SU treatment. We demonstrate the striking finding that a short period (6 days) of intraperitoneal glibenclamide treatment at disease onset can indeed provide sustained remission from what is otherwise permanent worsening diabetes in mice. Plasma insulin levels remain below normal after glibenclamide therapy is terminated, but they are sufficient to maintain near normoglycemia and to preserve islet insulin content. Maintained islet insulin secretion in response to SU and KCl indicates that the secretory machinery itself is not affected in cured DTG mice. Oral glibenclamide treatment, with the drug added to the drinking water, also prevented diabetes in a responding subset of animals, which were weaned from glibenclamide in ~4 weeks and subsequently maintained near normoglycemia for an indefinite period without further treatment. Thus aggressive control with glibenclamide only at the onset of disease can lead to subsequently sustained, drug-free recovery from hyperglycemia in these NDM mice.

Pancreatectomy in rats leads to development of uncontrolled diabetes with a similar time course to that we observe after induction of GOF K$_{ATP}$ expression (41). With partial (90%) pancreatectomy, rats demonstrate a marked dichotomy of response; although glucose levels are quite variable in early stages after pancreatectomy, by 10 weeks all rats either show nearly normal glycemia or are overtly diabetic (41). This progression is strikingly similar to the bifurcated progression that we observe in response to early glibenclamide treatment. In both cases, the divergent outcomes indicate an apparent threshold effect at glucose levels of ~300 mg/dL; if glucose rises above this level, β-cell deterioration and loss of insulin content ensues, but if not, compensation and remission can occur (21,41).

In human NDM, mutant K$_{ATP}$ channels will also be expressed in muscle, brain, and other tissues, which may complicate the outcome, but it is conceivable that early aggressive SU treatment might also lead to stable remittance in human PNDM. We have recently reported a family case of NDM as a result of Kir6.2[R201H] mutation in which the carrier patient was treated from the time of diagnosis (day 6) with the SU glyburide (0.2 mg/kg/day) (38). By day 270, the drug dose had been reduced to 0.017 mg/kg/day with HbA$_{1c}$ in the normal range. Strikingly, the proband is currently receiving a 75-fold lower dose of SU than his affected mother and sister, who had been treated with insulin for 26 and 6 years, respectively (38). It is noteworthy that the proband dose is lower than that reported for any of 44 NDM patients who successfully switched from insulin to SU therapy (26) and considerably lower than the standard dose used to treat patients with type 2 diabetes. Potentially the dose is actually subtherapeutic at this juncture, and the disease has essentially entered remission as a result of the early aggressive treatment.

The mechanism of compensation. What is the compensatory mechanism behind an SU dose requirement and disease outcome? In TNDM patients, the mechanism of remittance is unknown but has been proposed to be a result of either a reduced insulin requirement at the time of remission or to some compensation at the level of the β-cell, pancreas, or whole body that can overcome the

secretory defect (13). It may reflect an increase in insulin sensitivity, a compensatory increase in β-cell function, or both. Here, we demonstrate that cured DTG mice do have higher insulin sensitivity relative to control mice. An unanswered question is why do we only see ~30% of the animals being cured? It is possible that individual-specific variation in other genes is involved in the compensation, but this will require extensive in-breeding experiments to test.

In humans, the Kir6.2 polymorphism E23K is highly associated with type 2 diabetes (42,43). Like NDM mutations, the E23K mutation induces a weak K_{ATP} GOF phenotype and is therefore predicted to reduce insulin secretion in vivo. Nondiabetic human subjects homozygous for E23K demonstrate ~40% lower insulin secretion in response to oral and intravenous glucose (44). However, glucose tolerance is normal, and hyperinsulinemic-euglycemic clamps reveal that these individuals are more insulin sensitive than control subjects. Thus, greater insulin sensitivity in individuals with E23K or other K_{ATP} GOF mutations may provide a general compensatory mechanism for reduced insulin secretion. Conceivably, this may underlie the remission that characterizes K_{ATP}-dependent TNDM, which, in the face of environmental or dietary challenges, becomes insufficient. Reduced insulin secretion may then drive development of type 2 diabetes in E23K carriers (44) or subsequent relapse of TNDM patients.

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