A Fully Human, Allosteric Monoclonal Antibody That Activates the Insulin Receptor and Improves Glycemic Control


Many patients with diabetes mellitus (both type 1 and type 2) require therapy to maintain normal fasting glucose levels. To develop a novel treatment for these individuals, we used phage display technology to target the insulin receptor (INSR) complexed with insulin and identified a high affinity, allosteric, human monoclonal antibody, XMeta, which mimicked the glucoregulatory, but not the mitogenic, actions of insulin. Biophysical studies with cultured cells expressing human INSR demonstrated that XMeta acted allosterically and did not compete with insulin for binding to its receptor. XMeta was found to function as a specific partial agonist of INSR, eliciting tyrosine phosphorylation of INSR but not the IGF-IR. Although this antibody activated metabolic signaling, leading to enhanced glucose uptake, it neither activated Erk nor induced proliferation of cancer cells. In an insulin resistant, insulinoenic model of diabetes, XMeta markedly reduced elevated fasting blood glucose and normalized glucose tolerance. After 6 weeks, significant improvements in HbA1c, dyslipidemia, and other manifestations of diabetes were observed. It is noteworthy that hypoglycemia and weight gain were not observed during these studies. These studies indicate, therefore, that allosteric monoclonal antibodies have the potential to be novel, ultra-long acting, agents for the regulation of hyperglycemia in diabetes.
antibody, XMetA, for further characterization both in vitro and in vivo. In cultured cells, XMetA activated INSR signaling and promoted glucose uptake. In diabetic mice, XMetA normalized fasting blood glucose for 6 weeks without hypoglycemia and improved metabolic parameters of diabetes.

RESEARCH DESIGN AND METHODS

XMetA discovery. The extracellular domain of the human insulin receptor (hINSR; R&D Systems, Minneapolis, MN) was biotinylated with Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) according to the manufacturer’s protocol. To obtain allosteric antibodies to the INSR, panning and subsequent screening were performed with the biotinylated extracellular domain of the hINSR maintained in the presence of saturating insulin (10 μmol/L, Sigma-Aldrich, St. Louis, MO) to block the orthosteric binding site. This biotinylated receptor: ligand complex was immobilized on streptavidin-coated magnetic Dynabeads M-280 (Invitrogen Dynal AS, Oslo, Norway) and panned against two scFv antibody phage display libraries (XOMA [US], LLC, Berkeley, CA; BioInvent, Lund, Sweden), using standard methods (40,41). After each round of panning, phage were deselected against streptavidin-coated magnetic Dynabeads M-280 to remove nonspecific phage antibodies. After three rounds of panning and deselection, most phage were IL-4Rα (an IL-4 receptor) (27) and this binding is to an allosteric site, which is distinct from the orthosteric insulin binding site. Therefore, that XMetA binds to hINSR with high affinity in vitro and in vivo. In cultured cells, XMetA activated INSR signaling with an EC50 of 0.18 nmol/L (Fig. 1A). To determine the effect of XMetA on autophosphorylation of the INSR, CHO cells transfected with either the hINSR (CHO-hINSR) or mouse INSR (CHO-mINSR) were used. Both INSR transfected cell lines had ∼250,000 receptors per cell compared with the untransfected cells, which had less than 5,000 INSR per cell as determined by flow cytometry (similar numbers of IGF-1R were expressed on CHO-hIGF-1R cells; vide infra) (42). The single chain fragment (scFv) with the highest affinity was reformatted to a fully human IgG2a monoclonal antibody, XMetA (40,41).

In vivo. Middle-aged male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were studied. The Western diet was used to induce diabetes in C57BL/6 mice. After 8 weeks, mice were randomized into three groups administered either STZ or citrate buffer. The control group consisted of 11 mice and the experimental groups consisted of 22 mice. The mice were given XMetA or an anti-keyhole limpet hemocyanin isotype control antibody, as indicated. Blood glucose was measured using the Accu-Chek Performa (Roche Diagnostics) intraperitoneally and measured venous blood for glucose at 0, 30, 60, and 120 min after challenge and evaluated for blood glucose. Insulin tolerance tests were carried out at 10 weeks of age after a 4-h fast in animals by administering insulin (0.75 units/kg; Roche Diagnostics) intraperitoneally and measuring venous blood for glucose at 0, 30, 60, and 120 min after insulin challenge.

RESULTS

XMetA binds to INSR at an allosteric site. Allosteric antibodies to the INSR were identified by screening phage display libraries for human scFv fragments that bound the insulin-INSR complex. The highest affinity scFv fragment was then reformatted into a fully human IgG2 monoclonal antibody, XMetA. By flow cytometry, we determined that XMetA, but not an isotype control monoclonal antibody, specifically bound to CHO cells expressing the human INSR (CHO-hINSR) with an half-maximal effective concentration (EC50) of 0.10 nmol/L (Fig. 1A). With the use of KinExA methodology to determine the binding affinity of XMetA to the hINSR (43), XMetA bound to the hINSR with a Kd of 0.040 nmol/L (data not shown). Neither XMetA nor the control antibody altered the affinity of insulin binding to the hINSR in the absence of antibodies (Kd of 0.17 nmol/L) (Fig. 1B). XMetA also had no effect on the binding of labeled insulin to the INSR on these same cells by flow cytometry (data not shown). These data demonstrate, therefore, that XMetA binds to hINSR with high affinity, and this binding is to an allosteric site, which is distinct from the orthosteric insulin binding site.

Mouse model of diabetes. Animal experiments were approved by the XOMA (US) Institutional Animal Care and Use Committee (IACUC) and performed in accordance with IACUC guidelines. All animals were maintained in a pathogen-free environment and allowed free access to food and water. Six- to eight-week-old male ICR mice (Harlan, Indianapolis, IN) were randomly divided into groups administered streptozotocin (STZ; Sigma) or treated with citrate buffer. Buffer-treated (control) mice were fed a standard chow diet (Harlan), and diabetic animals were fed a 40 kcal% fat, 35 kcal% sucrose diet (Research Diets, New Brunswick, NJ) on arrival (44). STZ was dissolved in citrate buffer and injected intraperitoneally (40 mg/kg) for 5 consecutive days during the third week after arrival. After an additional week, mice were divided into groups (n = 8) and treated twice weekly, intraperitoneally, with XMetA or an anti-keyhole limpet hemocyanin isotype control antibody, as indicated. Blood glucose was measured using the OneTouch Select Plus Glucose Monitoring System (LifeScan) at 10 weeks of age after a 4-h fast in animals by administering insulin (0.75 units/kg; Roche Diagnostics) intraperitoneally and measuring venous blood for glucose at 0, 30, 60, and 120 min after insulin challenge.

Insulin and IGF-I receptor signaling in cultured cells. CHO cells at 37°C expressing mouse or human INSR or IGF-IR were incubated in serum-free culture media and stimulated with increasing concentrations of insulin, control antibody, or XMetA for 10 min. Total Akt and Akt phosphorylated at Ser473 and total Erk1/2 and Erk1/2 phosphorylated at Thr202/Tyr204 were measured by enzyme-linked immunosorbent assay (ELISA; Meso Scale Discovery, Gaithersburg, MD). To evaluate the effect of XMetA on the hINSR and mINSR autophosphorylation, CHO cells were transfected with increasing concentrations of CHO-hINSR or CHO-mINSR plasmid DNA. Cells were pelleted by centrifugation, and the amount of free insulin in solution was measured by immunofluorescence using a KinExA instrument (45). Insulin concentration data were curve-fit using KinExA software to yield the relative change in insulin binding affinity.

Insulin and IGF-I receptor signaling in cultured cells. CHO cells at 37°C expressing human INSR or IGF-IR were incubated in serum-free culture media and stimulated with increasing concentrations of insulin, control antibody, or XMetA for 10 min. Total Akt and Akt phosphorylated at Ser473 and total Erk1/2 and Erk1/2 phosphorylated at Thr202/Tyr204 were measured by enzyme-linked immunosorbent assay (ELISA; Meso Scale Discovery, Gaithersburg, MD). To evaluate the effect of XMetA on the hINSR and mINSR autophosphorylation, CHO cells were transfected with increasing concentrations of insulin, control antibody, or XMetA for 10 min and phosphotyrosine content of the INSR was evaluated by ELISA (Millipore, Billerica, MA). To determine the effect of XMetA on autophosphorylation of the IGF-IR, CHO cells transfected with the human IGF-IR that had ∼250,000 receptors per cell were used (28). Cells were preincubated at 37°C in serum-free medium with a saturating concentration of XMetA, isotype control antibody, or insulin (100 nmol/L). Tyr308-phosphorylated INSR or IGF-IR was determined by Western blotting.

Glucose transport and proliferation assays. To measure 2-deoxy-glucose uptake, 3T3 cells expressing hINSR (3T3-HIR cells; ∼105 hINSR/cell) were incubated in serum-free medium for 1 h with increasing concentrations of XMetA, isotype control antibody, or insulin. [3H]-2-deoxy-glucose was then added for 20 min, and its uptake was measured (28). MCF-7 cells (ATCC, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium containing glucose at 4.5 g/L supplemented with 10% FBS and 2 mmol/L glutamine (Invitrogen) for normal maintenance. For the proliferation assay, cells (1 × 104) were seeded in 96-well white opaque microtiter plates and allowed to reattach for 24 h. After 24 h, cells were washed twice with prewarmed PBS and incubated in Dulbecco’s modified Eagle’s medium containing glucose at 1 g/L and insulin (1 μmol/L) for 72 h (10% FBS and 2 mmol/L glutamine (Invitrogen; “starvation media”). XMetA, isotype control antibody, or insulin (Sigma-Aldrich) was added to the cells for a further 24 h and cell proliferation was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI).

Glucose and insulin tolerance tests. For glucose tolerance tests, mice were fasted overnight for 14 h followed by a glucose challenge (1 g/kg; Mediatech, Manassas, VA) by intraperitoneal injection or oral gavage, as indicated. Whole venous blood was obtained from the tail vein at 0, 15, 30, 60, and 120 min after challenge and evaluated for blood glucose. Insulin tolerance tests were carried out at 10 weeks of age after a 4-h fast in animals by administering insulin (0.75 units/kg; Roche Diagnostics) intraperitoneally and measuring venous blood for glucose at 0, 30, 60, and 120 min after insulin challenge.

Statistical procedures. All values, unless otherwise indicated, are expressed as mean ± SEM. Statistical analyses were carried out using a two-tailed Student unpaired t test using Prism 5.0 graphing software (GraphPad, La Jolla, CA).
XMetA improved fasting blood glucose levels in diabetic mice. To evaluate the in vivo activity of XMetA, we used an animal model of insulinopenic, insulin-resistant diabetes, the multi-low dose STZ, high-fat diet (MLDS/HFD) mouse (44). Ten days after the last dose of STZ, blood glucose levels after a 14-h fast were elevated to ~200 mg/dL, and these levels increased over the course of the 6-week study (Fig. 5B). In contrast, nondiabetic control animals maintained fasting glucose levels of ~100 mg/dL. XMetA was administered by intraperitoneal injection twice weekly to the diabetic mice. Seven days after treatment, fasting blood glucose levels in the XMetA-treated diabetic mice were near normal and remained near normal for up to 28 days, but were slightly elevated at 42 days. At this time, anti-human IgG antibodies were detected in the treated mice (data not shown). These antibodies likely
enhanced XMetA clearance, causing this elevation in blood glucose. An effect of XMetA was detected at doses as low as 0.1 mg/kg (Fig. 5C). XMetA maximally improved fasting blood glucose levels at a dose of 1.0 mg/kg. At this and higher doses of XMetA, there was no evidence of hypoglycemia.

**XMetA improves nonfasting blood glucose levels in diabetic mice.** Glucose levels in diabetic mice allowed free access to food and water were also measured. Non-diabetic control animals maintained glucose levels in the range of 150 mg/dL (Fig. 5D). Diabetic animals had glucose values in the range of 600 mg/dL. Treatment with XMetA lowered nonfasted glucose values in diabetic mice, but in contrast with the results observed in fasted diabetic mice, XMetA did not normalize nonfasted glucose values.

**XMetA improves metabolic markers of diabetes.** Various parameters in the mice were studied at the end of the 6 weeks of treatment. In addition to improving fasting blood glucose to near normal levels and reducing nonfasted glucose levels, treatment with XMetA improved other metabolic indices in the diabetic animals. Consistent with the decrease in glucose levels, XMetA had a major effect on the metabolic markers.
on hemoglobin A1c (Fig. 5E). The diabetic animals were markedly ketotic as measured by β-hydroxybutyrate; XMetA normalized this value (Fig. 5F). XMetA treatment increased insulin levels (295 ± 37 vs. 158 ± 38 pg/mL, P < 0.05) without increasing the level of C-peptide (334 ± 40 vs. 353 ± 29 pmol/L) (Table 1). Diabetes reduced weight gain in diabetic animals, and XMetA treatment of diabetic animals did not change this parameter. However, other manifestations of diabetes were improved by XMetA. Food intake was decreased (4.8 ± 0.6 vs. 7.7 ± 0.3 g/day, P < 0.05), and water intake was decreased (11.1 ± 2.2 vs. 21.3 ± 0.9 g/day, P < 0.05). In addition, XMetA also improved non-HDL cholesterol (78 ± 4 vs. 107 ± 6 mg/dL, P < 0.05) and free fatty acids (21.2 ± 2.4 vs. 32.9 ± 1.3 mg/dL, P < 0.05).

### XMetA improves glucose tolerance in diabetic mice.

After 3 weeks of XMetA treatment, fasted diabetic animals underwent glucose tolerance tests, with either intraperitoneal (Fig. 6A) or oral (Fig. 6B) glucose. Diabetic animals developed markedly elevated glucose levels, reaching 600 mg/dL or greater. In contrast, during both types of glucose tolerance tests, animals treated with XMetA maintained near normal blood glucose concentrations.

XMetA improves insulin tolerance in diabetic mice.

We next studied the effect of XMetA administration on the glucose response to exogenous insulin administration. After 5 weeks of treatment, animals were given intraperitoneal exogenous insulin, and the fall in blood glucose was measured for up to 120 min (Fig. 7A). Control, diabetic, and diabetic XMetA-treated animals all responded to insulin with a fall in blood glucose. When expressed as the percent change from initial glucose levels, the response to insulin in the diabetic animals was blunted compared with normal mice. This blunted response was corrected by XMetA treatment, suggesting that XMetA effects in this context are additive (Fig. 7B).

### DISCUSSION

In the current study, we characterized XMetA, a novel fully human monoclonal antibody that is a high-affinity allosteric agonist of the INSR. To identify this type of antibody, we used the insulin:INSR complex to screen phage libraries. This approach reduced the potential for obtaining antibodies that bind to the orthosteric site of the INSR because the latter site was occupied by its ligand, insulin. Two lines of evidence indicated that the binding of XMetA to the INSR was allosteric and not orthosteric. First, although XMetA bound directly to the INSR, it did not compete with insulin for receptor binding. Second, functional studies in cells and metabolic studies in animals demonstrated that XMetA did not interfere with the actions of insulin. A major finding was that XMetA activated the insulin receptor in a manner that was different than insulin, an effect likely a result of a distinct structural state of the INSR induced by XMetA binding to its allosteric site.

After insulin binds to the INSR, it stimulates receptor autophosphorylation. XMetA also stimulated this function, but compared with insulin, it acted as a partial agonist. In contrast with its effects on the INSR, and unlike insulin itself (46), XMetA did not activate the IGF-IR. The metabolic effects of the INSR are known to occur primarily through the PI3K/Akt pathway (17). XMetA was also a partial agonist of the PI3K/Akt pathway as measured by Akt phosphorylation. XMetA, like insulin, activated glucose transport but, unlike insulin, did not stimulate the growth of cancer cells. Accordingly, XMetA did not activate Erk, which mediates insulin-dependent mitogenic

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**FIG. 3.** XMetA does not activate the IGF-IR. CHO-hIGF-IR cells were incubated with either 33 nmol/L XMetA or isotype control antibody in the presence or absence of 100 nmol/L IGF-I. IGF-IR autophosphorylation was measured by ELISA (n = 3). p, phosphorylation.

**FIG. 4.** XMetA promotes glucose uptake, but not cell growth. A: 3T3 cells expressing hINSR were incubated with increasing concentrations of either XMetA ( ), isotype control antibody (○), or insulin (▲), and 2-deoxy-D-glucose uptake was measured (n = 5). B: MCF-7 cells were incubated with increasing concentrations of XMetA ( ), isotype control antibody (○), or insulin (▲), and cell proliferation was determined by CellTiter Glo assay (n = 6). C: MCF-7 cells were incubated with either 33 nmol/L XMetA ( ) or isotype control antibody (○) with increasing concentrations of insulin. Cell proliferation was then measured (n = 3). mAb, monoclonal antibody.
signaling (47,48). Therefore, XMetA, which bound allosterically to the INSR, recapitulated glucoregulatory functions of insulin, without mimicking insulin-promoted cell proliferation.

XMetA bound to and activated both the human and mouse INSR with similar dynamics, thus allowing in vivo studies in the MLDS/HFD mouse. The MLDS/HFD mouse is a commonly used model of insulin-resistant, insulinopenic diabetes (44,49). In these animals, XMetA normalized both fasting glucose levels and glucose tolerance. Nonfasted glucose levels, however, were improved, but not fully normalized. The reasons for this are currently unclear. One possibility is that, as a partial agonist that does not activate Erk, XMetA may not activate insulin-dependent gene expression in the same manner as insulin itself. Additional studies are needed to fully understand this phenomenon. Nevertheless, the improvements in glucose resulted in a significant improvement in hemoglobin A1c. XMetA also improved other indices of diabetes including hyperphagia and polydipsia, without significantly impacting body weight in this model. Efficacy, as measured by fasting blood glucose levels, was observed at an intraperitoneal dose as low as 0.1 mg/kg and was maximal at 1.0 mg/kg. Preliminary studies suggest that subcutaneous administration of XMetA for up to 3 weeks is equally as effective as intraperitoneal dosing in MLDS/HFD mice (V.B. and A.L., unpublished data).

Of major interest was the observation that XMetA did not cause hypoglycemia. XMetA was not associated with hypoglycemia even when administered at doses that were

**FIG. 5.** XMetA improves hyperglycemia and other metabolic markers of disease in diabetic mice. **A**: CHO-mINSR cells were incubated with increasing concentrations of XMetA (■), isotype control antibody (○), or insulin (▲), and Akt phosphorylation was measured by ELISA (n = 3). **B**: Fasting blood glucose measurements were obtained weekly for 6 weeks from control mice treated with 10 mg/kg isotype control antibody (○) and diabetic mice treated with either 10 mg/kg XMetA (■) or isotype control antibody ( ●) and diabetic mice treated with either 10 mg/kg XMetA (■) or isotype control antibody ( ●). **C**: After 3 weeks of treatment, fasting blood glucose was measured in control mice treated with 10 mg/kg isotype control antibody (white bar), diabetic mice treated with 10 mg/kg isotype control antibody (gray bar), and diabetic mice treated with the indicated doses of XMetA (black bars). **D**: Nonfasted blood glucose measurements were obtained weekly for 6 weeks from control mice treated with 10 mg/kg isotype control antibody (○) and diabetic mice treated with either 10 mg/kg XMetA (■) or isotype control antibody ( ●). **E**: After 6 weeks of treatment, blood hemoglobin A1c (E) and nonfasted plasma β-hydroxybutyrate (F) were measured in control mice treated with 10 mg/kg isotype control antibody (white bar) and diabetic mice treated with either 10 mg/kg isotype control antibody (gray bar) or XMetA (black bar). Values shown are mean ± SEM. *P < 0.05 for diabetic mice treated with XMetA compared with isotype control; n = 8 mice/group. mAb, monoclonal antibody.

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<th>Nondiabetic control mAb</th>
<th>Diabetic control mAb</th>
<th>Diabetic XMetA</th>
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<td>Body weight (g)</td>
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<td>37.6 ± 0.6</td>
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<td>C-peptide (pmol/L)</td>
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Data are represented as mean ± SEM (n = 8). mAb, monoclonal antibody. *P < 0.05 vs. MLDS-HFD/control.
10-fold above the minimum dose required for complete efficacy. The reasons for this positive outcome are not fully understood at this time. One possibility is that, as a partial agonist of the INSR, the hypoglycemic potential of XMetA is lower than that of insulin and does not overwhelm the endogenous compensatory mechanisms that prevent hypoglycemia. Another possibility is that XMetA tissue-specific distribution and/or activity differs from that of insulin. Further studies may help fully understand this phenomenon.

As an allosteric activator of the INSR, XMetA allowed the binding of insulin and subsequent insulin signaling. When an insulin bolus was given to diabetic mice, the effect of insulin, as measured by kinetics of glucose lowering, was diminished relative to that observed in normal mice. It is noteworthy that XMetA normalized these kinetics, most likely by its ability to correct many of the metabolic abnormalities associated with the diabetic state. These studies demonstrate, therefore, that XMetA improves the response to an insulin bolus in diabetic mice. This outcome raises the interesting possibility that XMetA may be used in combination with short-acting insulins in diabetes.

In addition to positive effects on glucose metabolism in diabetic mice, XMetA improved other metabolic parameters. XMetA lowered plasma non-HDL cholesterol and free fatty acids and completely normalized hyperketonemia. The observed improvement in β-hydroxybutyrate was greater than the improvement in hemoglobin A1c, consistent with the observation that lower levels of INSR signaling are required to prevent diabetic ketoacidosis compared with that required for complete glycemic control (50). XMetA increased circulating insulin levels without a concomitant increase in C-peptide, suggesting that XMetA may affect insulin clearance by binding the INSR in the liver and other organs. Thus it is possible that the observed increase in circulating insulin contributes to the metabolic improvements.

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**FIG. 6.** XMetA improves glucose tolerance in diabetic mice. A: After 3 weeks of treatment, glucose was administered intraperitoneally (IP) at 1 g/kg to fasted mice. Blood glucose levels were measured for 120 min in control mice treated with 10 mg/kg (○) and diabetic mice treated with either 10 mg/kg XMetA (■) or isotype control antibody (●). B: After 3 weeks of treatment, glucose was administered orally at 1 g/kg to fasted mice. Blood glucose levels were measured for 120 min in control mice treated with 10 mg/kg isotype control antibody (○) and diabetic mice treated with either 10 mg/kg XMetA (■) or isotype control antibody (●). Values shown are mean ± SEM. *P < 0.05 for diabetic mice treated with XMetA compared with isotype control antibody; n = 8 mice/group.

**FIG. 7.** XMetA improves insulin tolerance in diabetic mice. A: After 5 weeks of treatment, insulin was administered intraperitoneally at 0.75 units/kg. Blood glucose levels were measured for 120 min in control mice treated with 10 mg/kg isotype control antibody (○) and diabetic mice treated with either 10 mg/kg XMetA (■) or isotype control antibody (●). B: Data normalized to preinsulin glucose values. Values shown are mean ± SEM. *P < 0.05 for diabetic mice treated with XMetA compared with isotype control antibody; n = 8 mice/group.
described herein, in addition to the direct glycoregulatory activity of XMetA. Again, further studies may help fully understand the mechanisms whereby XMetA controls glucose and insulin metabolism.

In the treatment of diabetes, novel and improved therapeutic modalities for those individuals with impaired insulin secretory function would be helpful. Currently, long-acting basal insulins are given daily, or more often, and are associated with both hypoglycemia and weight gain (20). To more effectively control basal hyperglycemia, either very long acting insulins or other novel agents that stimulate the INSR could be of benefit. Therefore, a highly specific, ultra-long acting activator of INSR, such as a monoclonal antibody, would represent a new paradigm in diabetes therapy. Herein, we demonstrate that XMetA, an allosteric activator of INSR both in vitro and in vivo, has the potential to normalize glycemic control in a model of insulinopenic diabetes without causing hypoglycemia or promoting weight gain. To our knowledge, XMetA is the first fully human monoclonal antibody to the INSR reported to correct hyperglycemia and improve diabetes in vivo. Fully human antibodies typically have a half-life greater than 1 week and relatively low immunogenicity in humans. Thus, a fully human monoclonal antibody that activates the INSR has the potential for dosing in humans at once weekly intervals or less. These observations suggest, therefore, that antibodies like XMetA, either alone or in combination with short acting insulins/oral agents, may represent a new strategy for controlling blood glucose levels in diabetes.

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V.B. designed the in vivo experiments, researched the data, and wrote the manuscript. I.D.G. wrote and edited the manuscript and contributed to the discussion. D.H.B., H.F.K., M.H., B.A.M., S.R.W., S.Z., L.W., S.D.W., N.L., X.W., D.C.-V.C.T., S.R.L., and S.W. researched the in vitro data. A.L., L.M.G., A.J.N., and S.R.L. researched the in vivo data. D.W. and M.L.W. edited the manuscript and contributed to the discussion. J.A.C. led the research team, designed the in vivo experiments, and edited the manuscript. V.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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