

Resistance and allergy to recombinant human insulin

Michael A. Ganz, MD,^{***} Terry Unterman, MD,* Mary Roberts, RN,^{***}
Rogelio Uy, MD,* Sindarshan Sahgal, MD,** Max Samter, MD,* and
Leslie C. Grammer, MD^{***} Chicago, Ill.

Insulin allergy and antibody-mediated resistance may complicate therapy with animal insulins. We describe a 53-year-old man manifesting both resistance and persistent systemic allergy despite treatment with recombinant human insulin. Insulin resistance and symptoms of allergy appeared in this patient several months after initiating therapy with mixed beef-pork insulin, as is often the case. Symptoms initially improved, but persisted, and then worsened again, despite continuous human insulin therapy. Total insulin-binding capacity by Scatchard analysis, high plasma insulin-binding capacity, and specific anti-insulin antibody levels were consistent with an immunologic form of insulin resistance. Glucocorticoid therapy was required both to reduce allergic findings and to restore glycemic control. Although recently available human insulins may be less immunogenic than animal forms, immune responses to exogenous human insulin still may pose significant clinical problems. (J ALLERGY CLIN IMMUNOL 1990;86:45-52.)

Immunologic complications of insulin therapy have been evident since animal insulins became available for the treatment of diabetes mellitus. Insulin allergy has been particularly common, with local symptoms still occurring in approximately 5% to 10% of patients.¹ Wheal-and-flare reactions at the site of injection commonly occur early in the course of insulin therapy and are self-limited in patients receiving animal insulin preparations.^{2, 3} More significant is the finding that patients who are intermittently exposed to insulin because of irregular administration appear to be at risk for more persistent and severe allergic reactions, which may require specific intervention ranging from oral antihistamines to emergent treatment of systemic anaphylaxis.³ Other manifestations of the immune response to insulin may include antibody-mediated insulin resistance.^{2, 4} Furthermore, high levels of circulating anti-insulin antibodies may contribute to episodes of severe "spontaneous" or fasting hypoglycemia, both in patients receiving insulin

Abbreviations used

PEG: Polyethylene glycol
PBST: Phosphate-buffered saline Tween
OD: Optical density

therapy for diabetes mellitus^{5, 6} as well as subjects with no history of exposure to exogenous insulin manifesting insulin "autoimmunity."^{7, 10}

In view of the wide spectrum of immune-mediated complications of insulin therapy, much attention has been directed to the reduced immunogenicity of highly purified forms of animal insulins and the more recently available recombinant and semisynthetic forms of human insulin.¹¹⁻¹³ Only a minority of patients receiving therapy with purified porcine or human insulin develop anti-insulin antibodies,¹¹ whereas insulin antibodies can be demonstrated in nearly all patients after starting therapy with older "conventional" insulins.¹⁴ Moreover, patients allergic to animal insulins may at times be treated effectively with human insulin.¹⁵ Nevertheless, IgG and IgE antibodies can develop to exogenous human insulin,¹³ perhaps because of protein denaturation during preparation, including the formation of covalent aggregates that have prolonged half-lives in the circulation¹⁶ and may present novel antigenic structures to the host. Also, local and acute systemic responses to exogenous human insulin have been occasionally reported.¹⁷⁻²¹

We now relate a case of resistance to human insulin

From the Section of ^{***}Allergy-Immunology, Department of Medicine, Northwestern University Medical School, Chicago, Ill., and the Departments of ^{*}Medicine and ^{**}Pathology, The University of Illinois College of Medicine, and Veterans Administration West Side Medical Center, Chicago, Ill.

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Reprint requests: Leslie C. Grammer, MD, 303 East Chicago Ave., Chicago, IL 60611.

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associated with high levels of circulating antihuman insulin antibodies and sustained systemic allergic manifestations. Glucocorticoid therapy resulted in clinical improvement.

METHODS

Plasma insulin and C peptide levels

Disodium ethylenediaminetetraacetate plasma was obtained from the patient and two healthy male volunteers with no history of insulin treatment. Disodium ethylenediaminetetraacetate-treated blood was immediately centrifuged; then aliquots of plasma were treated within 7 minutes of blood drawing with 30% PEG at 4° C to precipitate anti-insulin antibodies and allow measurement of circulating free-insulin levels.²² Whole plasma and PEG supernatants were stored at -20° C for assay. Total plasma insulin was measured after acid treatment and PEG precipitation of antibodies.²³ For C peptide measurement, plasma was collected with Trasylol (Bayer Chemical Co., Leverkusen, West Germany) 1000 KIU/ml. Immunoassay of C peptide and insulin was performed in the Diabetes Research and Training Center at the Indiana University Medical Center, Indianapolis, Ind.

Insulin-binding studies

Purified porcine insulin, provided by Dr. M. Root (Eli Lilly & Co., Indianapolis, Ind.), was monoiodinated with chloramine-T to a specific activity of 150 to 250 $\mu\text{Ci}/\mu\text{g}$.²⁴ To test the effects of plasma on binding of iodinsulin to human insulin receptors, human placental plasma membranes were prepared by differential centrifugation.²⁵ Binding incubations were performed in duplicate for 20 hours at 4° C in 1.5 ml microfuge tubes containing 400 μg of membrane protein with 0.1 to 50 μl of plasma and 10,000 cpm of iodinsulin in a total volume of 0.5 ml of binding buffer (100 mmol/L of NaCl, 0.25% (wt/vol) of bovine serum albumin (BSA), and 0.02% of Na azide, pH 7.4). Incubations were terminated by centrifugation at 11,000 g, for 10 minutes, at 4° C. Nonspecific binding was estimated in the presence of 1 μg of unlabeled insulin and subtracted from total membrane-associated radioactivity to determine specific binding. To estimate circulating binding activity, 20 hours at 4° C binding incubations were conducted without placental membranes and were terminated by addition of 0.625 ml of ice-cold 5% (wt/vol) albumin-treated Norit (Matheson, Coleman, and Bell, Norwood, Ohio) charcoal, which adsorbs free insulin.²⁶ After 30 minutes, tubes were centrifuged 10 minutes at 4° C, 10,000 g, and radioactivity in the supernatants was determined. Nonspecific binding in the presence of 1 $\mu\text{g}/\text{ml}$ of unlabeled insulin was determined for each concentration of plasma tested. Control plasma demonstrated no specific insulin binding in this assay.

Equilibrium binding assay for insulin antibodies

Total insulin-binding capacity of the patient's serum via Scatchard analysis was performed according to the method

described by Goldman et al.²⁶ Deinsulinization of the patient's serum was accomplished by combining one part of the serum with one and one-fourth parts (vol/vol) of 0.12 N HCl and five-tenths parts of dextran-coated charcoal suspension; this was mixed intermittently for 5 minutes. Next, one and one-fourth parts of 0.12 N NaOH were added, and the mixture was centrifuged twice at 2500 rpm for 30 minutes at 4° C, resulting in removal of charcoal particles with the adsorbed insulin.

The supernatant, representing deinsulinized serum, was studied at a final dilution of 1:10. ¹²⁵I-labeled human insulin at a concentration of 300 ng/100 μl , (1750 Ci/mmol) (Amersham Corp, Arlington Heights, Ill.) was dissolved in a barbital-albumin buffer, pH 7.5 (7 mmol/L of sodium barbital, 12 mmol/L of sodium acetate, 130 mmol/L of sodium chloride, and 0.5% bovine serum albumin). Binding assays were performed by the addition of cold human (rDNA) insulin (Eli Lilly & Co.) to the ¹²⁵I-labeled human insulin, with the total insulin concentration varying between 10^{-10} and 10^{-5} mol/L. Incubations were performed at 37° C for 2 hours in a water bath with duplicates run at each insulin concentration. After incubation, 300 μl of ice-cold carrier protein (0.36% bovine gamma globulin) and 600 μl of ice-cold 25% (wt/vol) PEG (PEG 8000, Sigma Chemical Co., St. Louis, Mo.) in water was added to the final incubation volume of 300 μl per tube. The precipitate that formed was centrifuged at 500 g for 1 hour at 4° C. The pellets were washed with 1.0 ml of 12.5% PEG, centrifuged as before, and counted in an automatic gamma counter (PRIAS, Packard, Downer's Grove, Ill.).

Counts precipitated represented the total bound insulin (BT). Bound ¹²⁵I-labeled insulin not displaced by micromolar concentrations of cold insulin was defined as insulin bound nonspecifically (BNS). Subtraction of BNS from BT yielded specific bound insulin (BSP). Human insulin has a molecular weight of 6000, and the concentration of bound insulin in moles per liter was calculated from the following equation:

$$\frac{B \text{ (moles)}}{\text{liter}} = \frac{BSP \text{ (cpm)}}{T \text{ (cpm)}} \frac{T \text{ (moles)}}{\text{liter}}$$

where $\frac{T \text{ (moles)}}{\text{liter}}$ represents the sum of the ¹²⁵I-labeled insulin and cold insulin concentrations added to each tube. Binding data were then plotted with Scatchard equation $BSP/F = -k(B) + kR$ with R representing the binding capacity of the insulin antibodies, and k the association constant. Regression analysis of the binding data was then performed, and the binding capacity of the insulin antibodies was calculated from the slope and intercept of the corresponding Scatchard plot.

Cutaneous end point titration

Intradermal testing for insulin allergy was performed with serial tenfold dilutions of purified porcine or human insulin (Eli Lilly) at an initial concentration of 0.001 U/ml. The last dilution that resulted in a 5 mm wheal and 10 mm flare constituted the positive end point.

ELISA

Serum was obtained at baseline and after 1, 2, and 7 months of therapy for measurement of anti-insulin antibodies. Immunoglobulins directed against bovine, porcine, and human insulins were measured by ELISA, as previously described.²⁷ Briefly, Immulon micro-ELISA plates (Greiner & Sons, Nürtingen, West Germany) were coated with 100 µg/ml of purified insulin in carbonate buffer, pH 9.6, for 2 hours at 37° C and then washed with PBST solution. Next, 200 µl aliquots of plasma diluted in PBST were placed in individual wells and incubated for 2 hours at 37° C. Plates were washed three times and then 200 µl of rabbit antihuman IgG, IgE, or IgA diluted 1:1000 in PBST was added to each well and incubated at 37° C for 2 hours. After plates were washed, they were developed by addition of 200 µl 1:1000 diluted alkaline phosphatase-conjugated goat anti-rabbit globulin and p-nitrophenyl phosphate, as described.²⁷ OD at 405 nm (model 210 reader, Artek Systems Corp., Farmingdale, N.Y.) was measured in triplicate, and average values were used. ELISA titers were taken as the lowest dilution that had an OD of 200% that of the negative control. To compare plasma obtained at different time points, all samples were tested in a single assay with 1:80 dilutions of sera to measure IgE and IgA antibody and a 1:1000 dilution of sera to measure IgG, and results were expressed relative to normal values by calculation of an insulin antibody index:

$$\text{Insulin antibody index} = \frac{\text{OD of test serum}}{\text{OD of control serum}}$$

Case presentation

The patient is a 53-year-old black man who was initially observed at the Veterans Administration West Side Medical Center, Chicago, in March 1986 with a 3-month history of polyuria, polydipsia, and weight loss (25 pounds during 3 months), with recent onset of nausea, vomiting, and intermittently blurred vision. He denied fever, chills, sweats, dysuria, or productive cough. He had consumed three pints of liquor/week for many years, and his last drink was 3 days before admission. There was no history of liver disease. He had no history of pancreatitis. His mother and one of five siblings acquired diabetes mellitus as adults. He had a history of "hay fever" and a positive serology for syphilis was treated in the 1950s. Initial examination revealed height 6 feet and 1 inch; weight, 129½ pounds; respiratory rate, 12; and supine blood pressure, 150/90 with a pulse of 96, which increased to 116 on standing. Fundoscopic, skin, lymph node, chest, heart, lung, abdominal, and neurologic examinations were normal. Laboratory evaluation included hemoglobin of 17.9 gm/dl, which fell with hydration. Liver function tests were within normal limits. White blood cell count was 10,700, and no eosinophils were noted. Chemistries included serum sodium, 120 mEq/L; potassium, 6.9 mEq/L; chloride, 78 mmol/L; CO₂, 7.4 mmol/L; blood urea nitrogen, 65 mg/dl; creatinine, 4.2 mg/dl; and glucose, 720 mg/dl. Urinalysis revealed 3+ glucose, 2+ ketones, and no protein. Arterial blood gas had pH 7.21; PCO₂, 14.3;

and PO₂, 113.8. Serum acetone was 45 mg/dl. Glycohemoglobin was 13.2%. Chest x-ray film was clear. Electrocardiogram revealed sinus tachycardia with peaked T waves. The patient responded initially to therapy with intravenous fluid and infusion of mixed beef-pork insulin (Eli Lilly Co.). Subcutaneous insulin therapy was successful, although the patient did note swelling and pruritus at injection sites. Twelve days after admission he was discharged on 35 units of mixed beef-pork insulin per day with fasting plasma glucose levels ranging from 100 to 180 mg/dl.

During the next 4 months, the patient became refractory to subcutaneous insulin therapy and was switched to recombinant human insulin. Subsequently, insulin injections induced formation of firm 1 by 1 cm subcutaneous nodules at injection sites and occasional episodes of generalized pruritus with urticaria. Despite human insulin doses of >200 units and 20 mg of glyburide per day, he required hospitalization for blood glucose levels >300 mg/dl associated with polyuria, weight loss, and orthostatic hypotension. Physical examination revealed axillary, cervical, and inguinal lymphadenopathy and parotid gland enlargement. Liver and spleen were normal, and acanthosis nigricans was absent. Blood glucose was 430 mg/dl with normal electrolytes. Glycohemoglobin was 19.5%. White blood cell count was 9000 with 14% eosinophils noted. Total eosinophil count was 880. Rheumatoid factor, antinuclear antibody, anti-Smith antibody, antiribonuclear protein, anti-RO (salicylsalicylic acid), and anti-La (stereospecific binding) were negative. Serum protein electrophoresis demonstrated a monoclonal protein. Serum immunoelectrophoresis confirmed the presence of a monoclonal IgA kappa protein, but bone survey and 24-hour urine for Bence Jones protein and immunoelectrophoresis were negative. Quantitative immunoglobulins included IgA, 1340 mg/dl (N = 70 to 310); IgG, 1160 mg/dl (N = 639 to 1349); IgM, 218 mg/dl (N = 56 to 352); IgD, <1 mg/dl (N = 0 to 32); and IgE, 7 U/ml (N = 0 to 48). Biopsy specimen of a subcutaneous nodule revealed chronic inflammatory infiltrates, including many eosinophils and a few multinucleated giant cells, predominantly involving the deep dermis and subcutaneous fat, and associated with fibrosis. Axillary lymph node biopsy specimen revealed reactive hyperplasia with dense eosinophil and plasma cell infiltration. Lymph node immunocytochemistry staining was negative for IgA. Anti-islet cell antibody was negative (Scripps Institute, La Jolla, Calif.). HLA-DR3 and HLA-DR4 typing were negative. During hospitalization, blood glucose levels were controlled and then maintained by intravenous infusion of 5 U/hr of recombinant human insulin (Eli Lilly and Co.).

To evaluate the possibility that circulating anti-insulin or anti-insulin receptor antibodies were contributing to insulin resistance in this patient, plasma was tested for ability to block binding of radioactively labeled porcine insulin to human placental plasma membrane insulin receptors (Fig. 1, A). Binding of insulin to receptors was reduced by 50% in the presence of only 0.2% (vol/vol) patient plasma, whereas up to 5% control plasma had no effect on receptor binding. This result could not be explained by the amount of insulin present in the patient's plasma. (Actual plasma

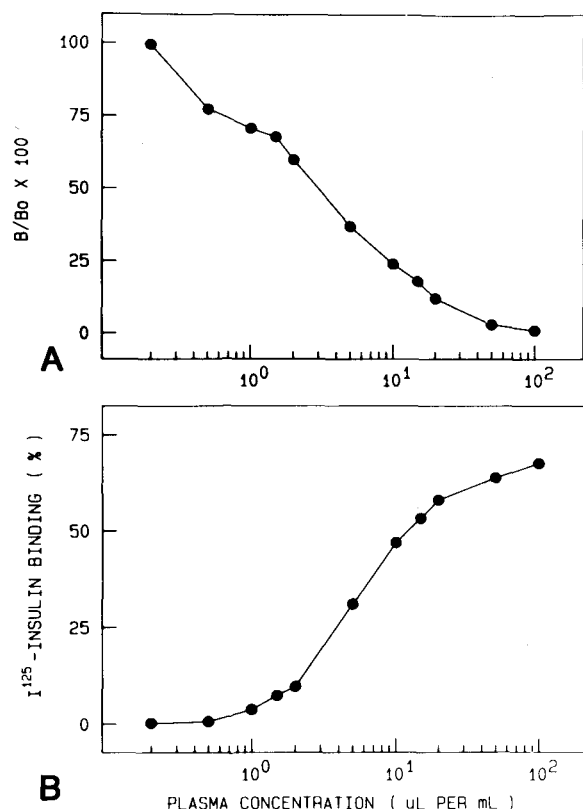


FIG. 1. A, Effect of plasma from insulin-resistant patient on binding of ^{125}I -labeled insulin to insulin receptors. Monoiodinated porcine insulin was incubated with human placental plasma membranes in the presence of increasing amounts of plasma, as described in METHODS. Maximal specific binding (B_o) in this assay was 33.8% total ^{125}I -labeled insulin counts per minute. The percentage of maximal binding ($[B/B_o] \times 100$) is illustrated versus the concentration of added plasma (microliters per milliliter). Each point is the average of duplicate determinations. Nonspecific binding in the presence of 1 $\mu\text{g}/\text{mL}$ unlabeled insulin is subtracted. **B,** Plasma ^{125}I -labeled insulin-binding activity. Labeled insulin was incubated with increasing amounts of patients' plasma; then free insulin was adsorbed to Norit charcoal, as described in METHODS. Percent ^{125}I -labeled insulin bound to plasma proteins is illustrated versus the concentration of added plasma. Nonspecific binding is subtracted, and average value is illustrated as stated previously.

insulin was 432 $\mu\text{U}/\text{mL}$ versus 8600 $\mu\text{U}/\text{mL}$ required to achieve a comparable effect.) Next, circulating anti-insulin antibody-binding activity was assessed by incubation of plasma with iodinsulin followed by adsorption of free insulin by activated charcoal. Significant plasma insulin-binding activity was detected in the presence of 0.1% (1 $\mu\text{L}/\text{mL}$) of patient plasma (Fig. 1, B). Total insulin-binding capacity of the patient's serum with Scatchard analysis was performed with the maximum binding capacity determined to be 1.0×10^{-5} mol/L (Fig. 2). Measurement of specific anti-insulin antibody titers by ELISA revealed high IgG

($\geq 1:40,000$) and detectable IgE and IgA ($\geq 1:80$ dilution) antibodies against human, bovine, and porcine insulin. Based on skin testing results (10^{-2} U/mL titer to porcine insulin, and 10^{-1} to human insulin), human insulin was selected for further therapy.

Insulin infusion was stopped overnight in anticipation of reinitiation of subcutaneous insulin therapy. The following morning the patient was fasting in preparation for other tests. Surprisingly, blood glucose levels declined without any insulin injection, and he became lethargic with a blood glucose of 39 mg/dL. Total insulin, free insulin, and C peptide levels were 259 $\mu\text{U}/\text{mL}$, 12.2 $\mu\text{U}/\text{mL}$, and <0.1 ng/mL, respectively. Symptoms resolved after intravenous administration of 50 mL of 50% (wt/vol) dextrose, and then blood glucose levels continued to rise.

Dexamethasone mixed with subcutaneous insulin was administered initially to permit continued treatment; however, systemic glucocorticoids (prednisone, 40 mg every day) and antihistamines (Benadryl; Parke-Davis, Morris Plains, N.J., 50 mg every day) were eventually required. Four weeks later, total eosinophil count was down to 334 cells per cubic millimeter, and antibody levels were reduced (Table I). Also, blood glucose levels were improved (80 to 240 mg/dL) on 55 units of neutral protamine Hagedorn and 10 units of regular human insulin per day. Skin reactions also were diminished, and lymph nodes were no longer enlarged.

DISCUSSION

Only a small proportion of diabetic patients will develop the complications of systemic insulin allergy or insulin-mediated resistance.³ In most patients, the presence of anti-insulin antibodies results in increased total serum insulin concentration but does not necessarily affect serum-free insulin level and has very little effect, if any, on glycemic control.^{27, 28} In contrast high anti-insulin antibody titers have been associated with insulin resistance, reflected by an increase in insulin requirement.^{3, 29} Patients with markedly elevated serum insulin-binding capacity may have altered insulin metabolism and reduced biologic responses to administered insulin.^{30, 31} After subcutaneous injection, initial rates of increase of free insulin may be reduced, time to peak free insulin level may be retarded, and return to baseline of plasma-free insulin levels may be delayed.³⁰ In such patients, the half-life of free insulin also may be prolonged^{30, 31} and may affect the availability of administered insulin for interaction with tissue insulin receptors.³²

Several features of the present case reflect an immunologic basis for insulin resistance. The patient developed marked insulin resistance as evidenced by persistent hyperglycemia, weight loss, and orthostasis despite receiving >200 units of human insulin per day. Anti-insulin antibody levels were demonstrated to be high, and the patient's serum interfered with binding of labeled insulin to human insulin receptors

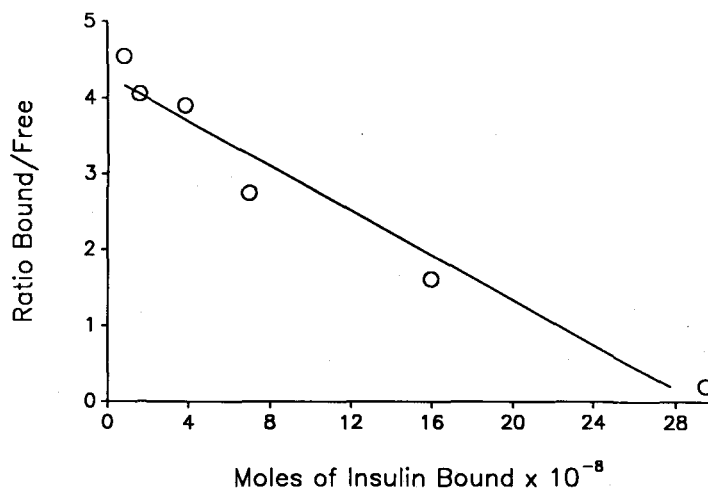


FIG. 2. Plot of Scatchard analysis of patient's serum reflecting total insulin-binding capacity. Moles of insulin bound by 0.03 ml of serum is illustrated versus ratio of bound to free insulin.

TABLE I. Effect of therapy on serum anti-insulin antibody level

Months of therapy	IgG antibody index			IgE antibody index			IgA antibody index		
	Human	Pork	Beef	Human	Pork	Beef	Human	Pork	Beef
Baseline	109.0	36.0	51.5	27.0	6.5	10.5	11.0	5.6	5.3
1	79.5	20.0	27.0	18.0	4.5	7.5	4.3	3.6	3.6
2	52.0	15.3	19.5	17.0	5.5	7.0	11.0	8.0	4.6
8	42.5	11.6	18.0	11.0	4.0	7.0	8.0	4.0	4.0

in vitro. Further, with Scatchard analysis, the patient's serum exhibited a high total insulin-binding capacity. More specific testing demonstrated high titers of anti-insulin antibodies in each of several immunoglobulin classes. During glucocorticoid therapy, the patient was able to gain weight and achieve improved glycemic control while he was receiving <100 units of human insulin per day.

It is possible that the patient's allergic reactions to human insulin represent a primary immunologic response to mixed beef-pork insulin in an atopic individual characterized by cross-reaction with human insulin. However, there was no history of previous insulin exposure before initial observation of the patient. Since that time, there has been no interruption of insulin therapy.

In this patient, restoration of insulin sensitivity was accompanied by reduction in circulating levels of anti-insulin antibodies (IgE and IgG). Paradoxically, in patients with high titers of anti-insulin antibodies, insulin resistance may be associated with a propensity for "spontaneous" or fasting hypoglycemia.^{6-10, 31} Our patient developed fasting hypoglycemia more than 12 hours after intravenous insulin therapy was stopped.

At that time, serum-free insulin was inappropriately high (12.2 μ U/ml),⁴² whereas C peptide level was suppressed, indicating that free insulin was of exogenous origin and probably reflected continued release of insulin from a large pool of circulating insulin-antibody complexes.^{6, 30, 32} Other investigators have suggested that insulin antibodies may behave not only as simple carrier proteins, with a stable equilibrium between free and bound states, but also may cause unexpected, erratic patterns of insulin release.⁵ Such episodes of unexpected hypoglycemia may further complicate the therapy of both diabetic patients with high anti-insulin antibody titers^{5, 6, 32} and patients who spontaneously develop antibodies to human insulin without prior exposure to exogenous insulin.⁷⁻¹⁰

The response of the patient to intravenous insulin administration during hospitalization suggests that the insulin resistance may have been mediated by a failure of absorption rather than antibody-mediated resistance. However, the dose of insulin received (0.1 U/kg/hr) is sufficient to cause hypoglycemia in normal subjects and is similar to the rate of insulin infusion in patients with diabetic ketoacidosis, a highly insulin-resistant state.³³ Additionally, in numerous pa-

tients with diabetes mellitus, it had been suspected that a defect in absorption of subcutaneously injected insulin might be an explanation for diabetic instability. Schade and Duckworth³⁴ evaluated a series of patients with a presumptive diagnosis of resistance to subcutaneous insulin and found no abnormalities in absorption. They concluded that the syndrome of subcutaneous insulin resistance is extremely rare and that misdiagnosis is common.

Previous studies of allergy to human insulin have included one patient with transient local responses shortly after initiation of human insulin therapy¹⁸ and several patients who demonstrated allergic responses during reinitiation of therapy with animal insulins when they either responded positively to human insulin on skin testing^{19, 21} or developed acute systemic reactions to human insulin.³⁵⁻³⁹ Positive skin tests to human insulin do not necessarily have any clinical significance, since many patients receiving insulin therapy will demonstrate wheal-and-flare responses on intradermal testing but do not have symptoms of insulin allergy.⁴⁰ In the present study, symptoms of insulin allergy and resistance emerged during continuous insulin therapy and persisted for many months after switching to human insulin. Also, this patient exhibited both local and systemic manifestations of insulin allergy, including immediate swelling and subsequent development of persistent subcutaneous nodules at injection sites, as well as episodes of generalized urticaria with pruritus, significant eosinophilia, and diffuse lymphadenopathy reminiscent of the serum sickness-like response noted in earlier patients receiving impure preparations of heterologous insulin.⁴¹ This patient did not require desensitization because insulin therapy was continuous and was not accompanied by acute life-threatening symptoms. Instead, glucocorticoids provided an effective therapeutic intervention for both insulin resistance and insulin-allergic symptoms in this patient.

In summary, it is well-known that occasional reactions to human insulin occur, often in those patients previously treated with mixed beef-pork insulin. A more important consideration is the frequency of such reactions, which is not known but probably remains low. Both resistance and allergy to recombinant human insulin in an individual patient has not been previously reported. Although it might be expected that recombinant human insulin would offer a safe alternative for these patients who are already allergic to other forms of insulin,^{15, 16} this case illustrates that a wide array of clinically significant immunologic responses to human insulin still may occur when it is used in the insulin-allergic patient.

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