Successful Treatment of Immune-mediated Insulin Resistance by Human Insulin (recombinant DNA)

FRANCO MANESCHI, S. EDWIN FINEBERG, AND EVA M. KOHNER

An 82-yr-old woman with type II diabetes developed antibody-mediated insulin resistance while on mixed pork-beef insulin concomitantly with a non-Hodgkin lymphoma. Insulin resistance was initially treated with highly purified pork insulin, but this was unsuccessful. Treatment with human insulin (recombinant DNA) was associated with marked decrease of both insulin requirement and high-affinity antibodies, increase of free insulin levels, and improvement of diabetic control. This patient's case shows that human insulin can be considered as an alternative treatment for immune-mediated insulin resistance. DIABETES CARE 5 (SUPPL. 2): 175–179, 1982.

Significant amounts of anti-insulin antibodies are detected in most diabetic individuals treated with conventional insulins, but they are only low or minimal in those treated with the more highly purified insulins.[1,2] Antibody levels greater than 20 U/L are commonly seen in patients who require more than 150 U/day.[3] The resulting syndrome, immunologic insulin resistance (IIR), may occur in association with many other diseases,[4] but its association with neoplasms of the immune system is very rare, and so far only eight such cases have been reported.[5] In the past IIR has been treated with adrenal steroids,[4] modified insulins,[6] immunosuppressors,[5] and plasma exchange.[5]

This study describes a patient with type II diabetes who, concomitantly with a lymphoma, developed IIR while she was treated with highly purified beef and pork insulins. IIR was unresponsive to highly purified (single-peak) pork insulin, but subsequent treatment with human insulin (recombinant DNA) resulted in a great reduction of both insulin requirement and antibody levels, which was associated with a marked improvement of diabetic control.

CASE REPORT

This 82-yr-old Caucasian woman (date of birth: August 24, 1900), was diagnosed as diabetic at the age of 39 yr. She was treated with diet alone until 1958; then tolbutamide treatment (2 g/day) was started. She received lente and then protamine zinc insulin (16 U/day) between April 1962 and March 1963; then tolbutamide treatment was recommenced. Between 1969 and 1979 she was treated with chlorpropamide (350 mg/day). Phenformin was added in 1970 but discontinued in 1978. Over those years, her fasting plasma glucose (FPG) ranged from 7.7 to 11.2 mmol/L and her weight varied between 115% and 135% of ideal body weight. She was initially referred to Hammersmith Hospital for bilateral cataracts and avascular fibrous retinitis proliferans. No specific treatment was ever required for the retinopathy. Cataract extraction in 1970 left her with a visual acuity of 6/36 on the right and 6/9 on the left, which has not changed since that time.

At the beginning of March 1979, the patient complained of polyuria, polydipsia, and a sore throat for a few weeks. The only physical abnormalities were enlarged tonsils and palpable lymph nodes at the angle of the jaw and upper neck. There was no other lymphadenopathy or hepatosplenomegaly, no lipodystrophy and acanthosis nigricans. Repeated cultures of throat swabs were negative, but a urine culture grew *E. coli*. The urinary tract infection was successfully treated with co-trimoxazole, but her control remained poor. With 500 mg/day of chlorpropamide her FPG remained elevated at 14–17 mmol/L; she had polyuria and polydipsia. Insulin therapy was started initially with highly purified beef insulin (Ultratard, Novo Industries, Wilton, Connecticut), which was later supplemented with highly purified pork insulin (Actrapid, Novo). Her diabetic control improved, but 2 mo later, in June 1979, she had 2% glycosuria and a postprandial plasma glucose (PPG) of 18 mmol/L. Records from the district nurse indicated that the glycosuria had increased gradually over the previous month. Cervical lymphadenopathy persisted, and an E.N.T. consultant found no evidence of malignancy in the upper air passages. The chest x-ray was normal. There was no hepatosplenomegaly.

In October 1979 she presented to the clinic with generalized lymphadenopathy; a lymph node biopsy showed a well-differentiated non-Hodgkin lymphoma. Because bone marrow function was normal (hemoglobin 13.2 g/dl, normal red cell indices, normal white cell and platelet count), no treatment for the lymphoma was advised. The insulin was changed to twice-daily Actrapid and Rapitard insulin (highly purified pork and beef insulin, Novo), at a total dose of 148 U/day. Over the following weeks diabetic control progressively deteriorated and in March 1980, when on 186 U/day, she was readmitted with diabetic ketosis. She was rehydrated and intravenous Actrapid insulin was given as a continuous infusion for 12 days (up to 80 U/day of insulin) before the diabetes was better controlled. Since her bone marrow function remained normal no specific treatment for the lymphoma was given. The following tests were negative: protein electrophoresis, immunoglobulins, complement screen, immune complexes, renal and liver function tests, proteinuria and Bence-Jones proteinuria, antinuclear antibodies, antimitochondrial, anti-smooth muscle, anti-skeletal muscle, anti-gastric parietal cell, anti-reticulin antibodies, and monoclonal insulin-binding gammaglobulins. An aliquot of the patient's serum bound 85% of ^{125}I-pork insulin and 89% of ^{125}I-beef insulin. She was started on highly purified pork insulin only, which resulted in a transient reduction of insulin requirement; however, within 3 mo this had returned to 196 U/day and diabetic control was poor. She refused increased dose because of the discomfort caused at the site of injection by the large volume given. Her progress was further marred by development of angina pectoris in December 1980, requiring 30 mg/day of isosorbide-dinitrate.

At the end of May 1981, human insulin (Eli Lilly and Company, Indianapolis, Indiana) became available and the patient was offered that treatment. After negative intradermal skin tests, on January 6, 1981 she was started on twice-daily regular (total 120 U) and NPH (total 64 U) human insulin. At the end of June 1981 a permanent heart pacemaker was inserted because of Stokes-Adams attacks. During this admission her control improved and the total daily insulin was reduced to 148 U. Since that time she showed a marked reduction of insulin requirement with improvement of diabetic control with all blood glucoses in the physiologic range. In April 1982, her postprandial plasma glucose was 9.8 mmol/L and her HbA_1, 9.4%. Her insulin requirement was reduced to 18 U of soluble and 24 U of NPH human insulin in the morning and 12 U soluble insulin at night. During the last 12 mo the lymphoma has remained untreated and has progressed slowly, as indicated by enlarged mediastinal lymph glands on the chest x-ray taken at the end of 1981.

MATERIALS AND METHODS

Blood for glucose, free and total insulin, and antibodies was sampled 3 h after the morning insulin injection. Plasma glucose was measured by the glucose-oxidase method.[7] Hemoglobin A_1 (HbA_1) was measured by Bio-Rad kit (Bio-Rad, Richmond, California). Insulin-binding capacity and binding constants were measured by formal antibody titration using the method of Goldman et al.[8] Free and total insulin were measured according to Nakagawa et al.[9] and Heding.[10] Species-specific insulins used for antibody titration were labeled with ^{125}I by lactoperoxidase, and tyr-A_{14}-insulin was separated by high-performance liquid chromatography.[11]

To look for monoclonal insulin-binding antibodies, immunoglobulins were separated by isoelectric focussing, both on polyacrylamide and agarose gel.[12] The patient's serum and that of two normal subjects were processed at the same time. Plates were then overlaid with radioactive insulin (mono ^{125}I-tyr-A_{14} porcine insulin) and after appropriate incubation and washing the radioactivity was measured by autoradiography. All other tests were performed by standard laboratory techniques.

RESULTS

Figure 1 shows the daily insulin dose and the indices used to assess diabetic control, i.e., glycosuria, postprandial plasma glucose, and HbA_1. From March 1979 to March 1980, the insulin dose increased to nearly 200 U/day and diabetic control remained poor; the mean HbA_1 over that period was

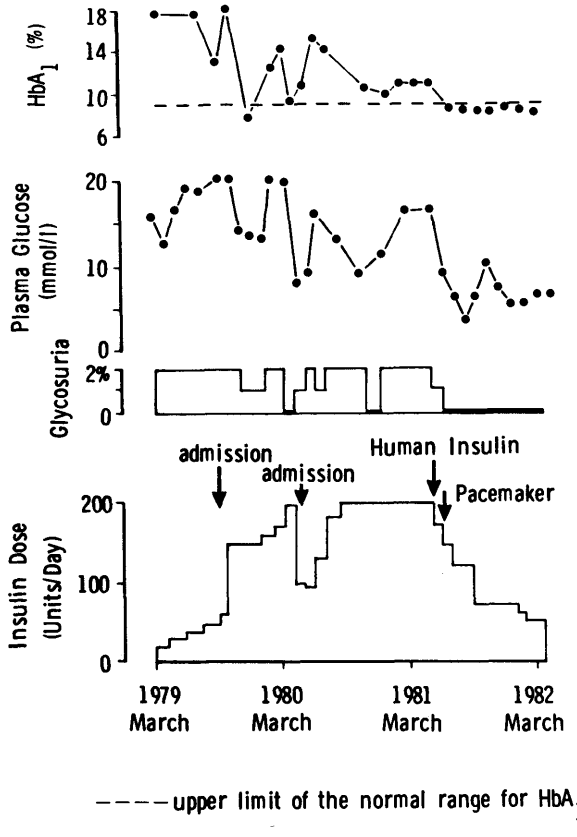

FIG. 1. The daily insulin dose and the indices used to assess diabetic control; glycosuria, postprandial plasma glucose, and HbA_1.

13.6%, the mean postprandial plasma glucose was 17.3 mmol/L, and glycosuria was constantly 2%. After the change from mixed beef and pork insulin to pork insulin only in April 1980, there was a temporary drop of the insulin dose to 90 U/day and an improvement of diabetic control as shown by HbA_1 level of 9.6%, a postprandial plasma glucose of 7.3 mmol/L, and absence of glycosuria at the end of April. However, this lasted only 3 mo; by the end of August 1980 the insulin dose was again 196 U/day and diabetic control was poor, with HbA_1 levels above 13%, plasma glucose above 13 mmol/L, and 2% glycosuria.

After beginning human insulin in June 1981 there was a progressive decrease of the insulin dose associated with disappearance of glycosuria and normalization of both glucose and HbA_1 levels.

The test for monoclonal insulin-binding immunoglobulins performed on a serum sample of March 1980 showed that the patient's serum bound radioactive insulin all over the IgG area without any predominant band. The normal sera did not bind any radioactive insulin.

Table 1 shows the results of the formal antibody titration tests. In April 1980, after 1 yr of therapy with a mixture of highly purified beef and pork insulin, the high-affinity (N_1) and low-affinity (N_2) insulin-binding capacities of this patient were approximately 1000 times greater than those found in insulin-treated diabetic individuals. In May 1981, after 1 yr of therapy with highly purified pork insulin only, there was a decrease of insulin-binding capacities, particularly of the low-affinity ones, for beef and pork insulin. However, both N_1 and N_2 were still much greater than the reference values. Interestingly, over the same period, both N_1 and N_2 for human insulin had increased. One year after the introduction of human insulin, in April 1982, there was a further drop in the insulin-binding capacities, but this time the N_1 values were within the reference range. N_2 values, although decreased, were still above the reference values.

The affinity constants, K_1 and K_2 of both families of antibodies, did not change significantly over the follow-up period.

After starting human insulin, the total and free insulin levels were measured monthly. Figure 2 shows the ratio of free insulin and total insulin levels (F/T) in relation to the daily insulin dose. During the period June–September 1982, the F/T ratio remained more or less constant. Over the period September 1981–April 1982, there was a progressive increase of the free insulin levels from 3.0 to 11.0 μU/ml and a decrease of the total insulin levels from 67.7 to 12.4 mU/ml. As a result, the F/T index increased progressively, up to 80 times of that found before the beginning of human insulin.

DISCUSSION

Although diabetes is rarely associated with neoplasms of the immune system,[13] in this subgroup of diabetic subjects IIR is more frequent (18/1000 patients) than in the general diabetic population, in which it is less than 1/1000. This has led to the hypothesis that these tumors (lymphomas, leukemias, and plasma cell myelomas) produce monoclonal immunoglobulins that specifically bind insulin, thus causing insulin

TABLE 1
Results of the formal antibody titration tests

	April 1980	May 1981	April 1982
Pork ^{125}I-insulin			
N_1 (U/L)	59.7	108.0	0.6
N_2 (U/L)	1066.5	431.5	93.2
Total	1126.2	539.5	93.8
K_1	5.8×10^7	2.2×10^7	1.5×10^8
K_2	4.3×10^5	7.3×10^5	1.1×10^7
Beef ^{125}I-insulin			
N_1 (U/L)	38.0	23.1	0.01
N_2 (U/L)	373.9	276.9	145.6
Total	411.9	300.0	145.6
K_1	2.6×10^9	2.3×10^8	1.2×10^9
K_2	1.7×10^7	1.6×10^7	2.2×10^7
Human ^{125}I-insulin			
N_1 (U/L)	1.9	49.1	0.2
N_2 (U/L)	133.0	280.1	140.0
Total	134.9	329.1	140.2
K_1	6.0×10^9	3.2×10^7	1.2×10^9
K_2	3.3×10^7	3.3×10^6	6.4×10^6

The reference values of insulin-binding capacities were obtained from 33 insulin-treated diabetic subjects. For beef insulin, N_1 ranged from 0.002 to 4.56 U/L and N_2 from 0.008 to 37.16 U/L. The N_1 and N_2 values for pork insulin were lower than those for beef insulin.
N_1 = high-affinity insulin-binding capacities; N_2 = low-affinity insulin-binding capacities; Total = $N_1 + N_2$; K_1 = high-affinity capacities binding constant; and K_2 = low-affinity capacities binding constant.

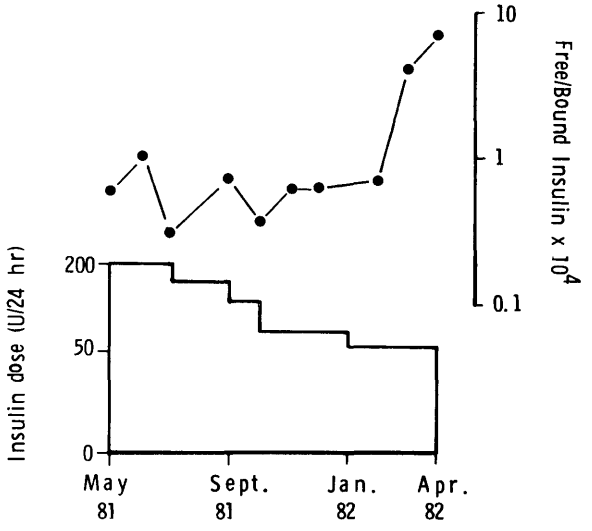

FIG. 2. The ratio of free insulin (FREE) and total (BOUND) insulin levels in relation to the daily insulin dose.

resistance.[5] Such immunoglobulins were demonstrated in two of the eight patients so far reported.[5] In this patient monoclonal insulin-binding immunoglobulins were not demonstrated; therefore, the causal relationship between the lymphoma and IIR could not be validated. The patient had received insulin for 17 yr before the development of the lymphoma and her immune system was primed by insulin. It is therefore possible that the relationship between IIR and the lymphoma was only coincidental, in the sense that the lymphoma worsened her diabetes and, when insulin was restarted to improve diabetic control, the production of such a high titer of insulin antibodies represented an exaggerated anamnestic response. In this respect it has to be noted that a history of intermittent insulin therapy was observed in 80% of the patients with IIR reported by Davidson and Debra.[6]

Berson and Yalow found a correlation between the antibody levels and insulin requirement[3] and in 1970 classified insulin resistance into mild (80–125 U/day), moderate (125–200 U/day), and severe (more than 200 U/day).[13] In the two more recent studies, which assessed a total of 54 patients with IIR, the total insulin-binding capacities ranged from 13 to 4700 U/L.[6,14] In the patient reported here the total insulin-binding capacity, while on 196 U/day of highly purified insulins, was 1126 U/L for pork insulin, 412 U/L for beef insulin, and 135 U/L for human insulin. The finding of antibodies against human insulin in a patient who had never previously received it is probably explained with the cross-reactivity of this type of insulin with antibodies raised against beef and pork insulins.[15] Indeed, with a method similar to ours, Goldman et al.[8] reported total binding capacities for human insulin ranging from 0.4 to 66.6 U/L in diabetic patients who had never had human insulin.

In this patient, IIR appeared during treatment with highly purified beef and pork insulins. The more immunogenic beef insulin was used during the period March 1979–March 1980, but the highly purified pork insulin is less immunogenic and it is one of the standard treatments for IIR, resulting in at least a decrease of insulin requirement in 50% of the patients.[16] Apart from a short initial period, pork insulin did not produce a decrease of insulin requirement in this woman. Although it resulted in a decrease of total insulin-binding capacities, the high- and low-affinity capacities on pork insulin were far above the control range, and diabetic control remained poor. This patient is the first to be reported in whom IIR appeared during treatment with highly purified insulins; her reaction shows that highly purified pork insulin can be ineffective in IIR. Other forms of treatment, such as high doses of adrenal steroids,[4] immunosuppressors,[4] and exchange[4] were not advised because of her age, and modified insulins[6] were not available. Human insulin (recombinant DNA) was chosen to treat IIR in this woman because it has the same primary structure as naturally occurring human insulin—which should make it less immunogenic. In addition, human insulin is now readily available and can be used for long-term treatment. Human insulin has been very effective for this patient's IIR. Only 4 mo after beginning the treatment with human insulin, the insulin requirement was less than one-third that on highly purified pork insulin. One year later, the insulin requirement was 54 U/day, which is below the range of patients with mild insulin resistance. The decrease of insulin requirement was associated with a marked decrease of the total insulin-binding capacities and with the return of high-affinity antibodies to within the control range. In keeping with the hypothesis that the high affinity antibodies are directly related to the levels of free insulin and its hypoglycemic action,[3,17] the "normalization" of high-affinity antibodies in this patient was associated with increased free-insulin levels and improvement of diabetic control.

Interestingly, the changes in antibody levels and diabetic control occurred while this patient's lymphoma somewhat deteriorated, as indicated by the presence of mediastinal lymphadenopathy in the chest X-ray taken in December 1981, while the chest x-ray taken in 1980 was normal. This excludes the possibility that the improvement of IIR was due to an improvement of the lymphoma.

In conclusion, we have reported the progress of the first patient in whom IIR was treated with human insulin. Its effectiveness in reducing insulin requirement, normalizing antibody levels, increasing free insulin levels, and improving diabetic control shows that human insulin can be considered a valid alternative treatment for IIR.

ACKNOWLEDGMENTS: We thank M. Boltz, Department of Medicine, Hammersmith Hospital, for monoclonal insulin-binding immunoglobulin studies; J. Rathbun, Department of Medicine, Indiana University, for antibody titration studies; and Dr. J. Scotton, Eli Lilly and Company, Basingstoke, Hants., United Kingdom, for the generous supply of human insulin.

From the Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England (F.M. and

E.M.K.); and the Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana (S.E.F.).

Address reprint requests to E. M. Kohner, Department of Medicine, Royal Postgraduate Medical School, DuCane Road, London, W12 OHS, England.

REFERENCES

[1] Berson, A. S., and Yalow, R. S.: Quantitative aspects of the reaction between insulin and insulin binding antibodies. J. Clin. Invest. *38*: 1996–2016, 1959.

[2] Czycic, A., Lawecki, J., Rogala, H., Miedzinska, E., and Popik-Hankiewicz, A.: Serum levels of insulin-binding antibodies in diabetics treated with monocomponent insulin. Diabetologia *10*: 233–36, 1974.

[3] Berson, S. A., and Yalow, R. S.: Insulin "antagonists" and insulin resistance. *In* Diabetes Mellitus: Theory and Practice. Ellenberg, M., and Rifkin, H., Eds. New York, McGraw-Hill, 1970, pp. 338–422.

[4] Shipp, J. C., Cunningham, R. W., Russell, R. D., and Marble, A.: Insulin resistance: clinical features, natural course and effects of adrenal steroids treatment. Medicine *44*: 165–86, 1965.

[5] Rhie, F. H., Ganda, O. P., Bern, M. M., Soeldner, J. S., Gerson, B., and Azizi, F.: Insulin resistance and monoclonal gammopathy. Metabolism *30*: 41–45, 1981.

[6] Davidson, J. K., and Debra, D. W.: Immunologic insulin resistance. Diabetes *27*: 307–18, 1978.

[7] Robin, M., and Saifer, A.: Determination of glucose in biologic fluids with an automated enzymatic procedure. Clin. Chem. *11*: 840–45, 1965.

[8] Goldman, J., Baldwin, D., Pugh, W., and Rubinstein, A. H.: Equilibrium binding assay and kinetic characterization of insulin antibodies. Diabetes *27*: 653–60, 1978.

[9] Nakagawa, S., Nakayama, H., Sasaki, T., Voshimo, K., Yu, U. U., Shinozaki, K., and Mashimo, K.: A simple method for the determination of serum free insulin levels in insulin-treated diabetic patients. Diabetes *22*: 590–600, 1973.

[10] Heding, L. G.: Determination of total serum insulin in insulin treated diabetic patients. Diabetologia *8*: 260–66, 1971.

[11] Frank, B. H.: HPLC preparation of high specific activity of ^{125}I-labels of insulin, proinsulin and other polypeptide hormones. Diabetes 29 (Suppl. 2): 106A, 1980.

[12] Williamson, A. R.: Isoelectric focussing of immunoglobulins. *In* Handbook of Experimental Immunology, Weir, D. M., Ed. 3rd edit. Oxford, Blackwell Scientific Publications, 1978, pp. 1–31.

[13] Goldstein, H. H.: Disorders of the blood. *In* Joslin's Diabetes Mellitus. Marble, A., White, P., Bradley, R. F., et al., Eds. Philadelphia, Lea and Febiger, 1971, pp. 647–48.

[14] Berson, S. A., and Yalow, R. S.: Insulin inhibitors and insulin resistance. NY State J. Med. *60*: 3658–67, 1960.

[15] Chance, R. E., Kroeff, E. P., Hoffmann, J. A., and Frank, B. H.: Chemical, physical, and biological properties of biosynthetic human insulin. Diabetes Care *4*: 147–54, 1981.

[16] Kahn, R. C., and Rosenthal, A. S.: Immunologic reactions to insulin: insulin allergy, insulin resistance, and the autoimmune insulin syndrome. Diabetes Care *2*: 283–95, 1979.

[17] Kerp, L., and Kasemiv, H.: High and low affinity insulin antibodies. Proceedings of the International Symposium: Immunological Aspects of Diabetes Mellitus. Anderson, O. O., Deckert, T., and Nerup, J., Eds. Gentofte, 1975, pp. 211–22.