

Diagnostic Algorithm and Management of Immune-Mediated Complications Associated with Subcutaneous Insulin Therapy

C. Jaeger
M. Eckhard
M. D. Brendel
R. G. Bretzel

Abstract

Immune mediated complications associated with subcutaneous insulin therapy such as insulin neutralizing antibodies and/or skin reactions are rare conditions since human insulin is in general use. Nevertheless, if it occurs, a stepwise diagnostic approach is essential for differential diagnosis and consecutive treatment of these complications. Here we suggest a diagnostic algorithm to deal with e.g. insulin antibody formation of the IgG and/or IgE type and/or severe skin reactions resulting in poor metabolic control and often "brittle diabetes" in affected patients. This diagnostic algorithm includes step 1: Intradermal skin testing with positive and negative controls, additives and different insulin preparations; step 2: Quantification of insulin

specific IgG and IgE in the serum, and step 3: Analysis of the time dependent binding/dissociation curves of the insulin neutralizing antibodies in an *ex vivo/in vitro* assay to assess the clinical significance of these antibodies. Based on 158 insulin treated control subjects and four patients with typical symptoms and signs representing the spectrum of immune-mediated complications subsequent to subcutaneous insulin therapy we demonstrate that the proposed stepwise approach leads to a definite diagnosis as a prerequisite for individual and successful therapy.

Key words

Insulin antibodies · insulin allergy · insulin resistance · brittle diabetes · insulin therapy

Introduction

Refinements of manufacturing technology and finally the introduction of recombinant human insulin for the treatment of diabetes has significantly decreased the rate of immunological complications of insulin therapy (Federlin, 1985; Francis et al., 1985; Scherthaner, 1993). At present the use of insulin entails approx. 88% human recombinant insulin, 10% insulin analogues, and only 2% bovine and porcine insulin (von Kriegstein and Schatz, 2002). While this is of clear advantage to insulin-treated patients in general, the remaining low percentage of immune-mediated complications associated with subcutaneous insulin therapy

should not be underrated and therefore be "underdiagnosed". In affected individuals these complications are of great importance for diabetes management and prognosis. Naturally, such problems cluster at diabetes centres. At Giessen centre we observed in several diabetic patients on s.c. insulin therapy and regardless of the type of diabetes local skin reactions at the injection site and/or otherwise unexplained poor metabolic control both associated with extremely high titres of insulin antibodies. These clinical observations prompted us to develop a standardized and rational diagnostic approach to differentiate these immune-mediated complications (Fig. 1). Based on four typical cases representing the broad spectrum of immunological complications

associated with subcutaneous insulin therapy and 158 insulin-treated control subjects exclusively treated with human insulin we demonstrate a stepwise diagnostic approach for the differentiation and individual management of these complications.

Subjects and Methods

Subjects

Participants in this study were four consecutive patients with different types of diabetes mellitus, who were presented to the Diabetes Outpatient Department at Giessen Centre for routine clinical evaluation and blood sampling because of unexplained complications associated with subcutaneous insulin regimens: Two female patients, 24 and 21 years old, respectively, with type 1 diabetes, a 78-year-old female patient with type 2 diabetes and a 57-year-old male patient with type 3 c diabetes due to partial pancreatectomy. For establishing normal ranges of our assay systems we included sera of 158 diabetic patients on subcutaneous insulin therapy (human insulin) and without a history of any treatment related complications or allergic reactions.

Methods

The patients were subjected to extensive clinical evaluation and routine laboratory testing including fasting/post prandial c-peptide analysis, HbA1c etc. In addition we performed a detailed immunological evaluation. All immunological tests were performed in our Immune-Laboratory at Third Medical Department and Policlinic. The Laboratory was designated a DASP laboratory by Centers for Disease Control (CDC) and Immunology of Diabetes Society (IDS) on April 17, 2002 (Dr. C. Jaeger: CDC-0136). The immunological evaluation included:

1. **Intradermal skin testing** to assess allergic reactions of the immediate and/or late type. The test panel includes histamine (1: 0.01%, positive control), sodium chloride (2: 0.9%, negative control), the Aventis test kit (Insuman® Testlösungen) with additives of insulin preparations and galenic components (3: NaH₂PO₄, Glycerol, Phenol, m-Cresol; 4: Glycerol, Phenol, m-Cresol; 5: Phenol, m-Cresol; 6: Phenol; 7: m-Cresol; 8: Protaminsulfat, Phenol, m-Cresol, Glycerol; 9: Protaminsulfat). In addition we included different insulins (5 U/ml) depending on the individual patient. The test results were recorded separately after 15 minutes for immediate type of reaction (type 1 allergy) and after 12 hrs and 48 hrs for assessing type 3 vs. type 4 reactions, respectively. This procedure allows to differentiate insulin specific allergy from an allergy directed to additives or galenic components e.g. protamine, phenol, cresol, etc.
2. **Quantification of insulin specific IgG/E**: Insulin specific IgG-levels were determined using radioimmuno-electrophoresis according to the Christiansen method (Christiansen, 1973). The detection limit of this assay is 0.01 mU/ml. The "normal range" of insulin treated patients was established using sera of n = 158 diabetic patients, exclusively treated with s.c. human insulin. The mean titre was 0.038 mU/ml (95%-CI: 0.025–0.052 mU/ml). Data were cross-checked for plausibility with the commercially available CentAK®-IAA-assay (Medipan, Selchow, Germany), approved in the first DASP workshop (Lab # 0136). Insulin specific IgE-levels were determined us-

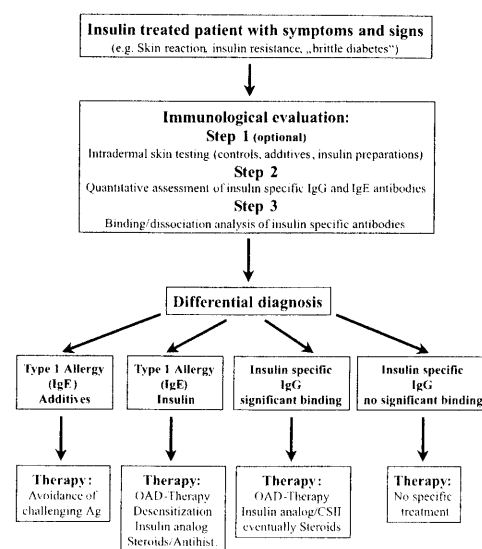


Fig. 1 Proposed stepwise algorithm for differential diagnosis and management of different immune-mediated complications associated with subcutaneous insulin therapy.

ing the CAP-SYSTEM, Pharmacia, Uppsala, Sweden (normal: <0.35 KU/l).

3. **Time dependent binding/dissociation analysis**: In case of evidence for insulin specific antibodies during step 2 the insulin binding capacity of these antibodies should be analyzed in order to assess the clinical relevance of this phenomenon. To differentiate this, we used a modified method according to Kerp et al. (1966); Dixon (1974) and Kuzuya et al. (1977) in order to detect the insulin binding capacity of these specific antibodies and the time dependent dissociation of the insulin/antibody complexes. Briefly, endogenous insulin was extracted by preincubation with acid charcoal. Aliquots are then incubated with J¹²⁵-labeled recombinant human insulin. Methyl-cellulose is added serving as free insulin adsorbent after acidification. Bound/free separation is performed at different time points (0, 30, 60, 90, 120, 240 minutes) by centrifugation (pellet/free insulin adsorbed to methyl-cellulose vs. supernatant/antibody bound insulin) followed by separate quantification in a gamma well counter. This procedure allows to determine the initial percentage antibody-bound/neutralized insulin (normal range of initial binding: 4.3%, CI: 2.9–5.7%; n = 158 diabetic patients exclusively treated with s.c. human insulin). Subsequent analysis of the other aliquots at later time points provide an indication of the time dependent dissociation of the insulin/antibody complexes. Thus, it is possible to characterize the individual insulin binding capacity of the antibodies over time in order to qualify the clinical relevance of these antibodies and to monitor the success of therapeutic strategies.

Affiliation

Third Medical Department and Policlinic, Justus-Liebig University of Giessen, Germany

In honour of Prof. Dr. Drs. h.c. K. Federlin on the occasion of his 75th birthday

Correspondence

Dr. med. C. Jaeger · Third Medical Department and Policlinic · Justus-Liebig University of Giessen · Rodtbohl 6 · 35392 Giessen · Germany · T + 49 064 1994 28 23 · F + 49 064 1994 27 59 · E-mail: clemens.jaeger@innere.med.uni-giessen.de

Received: August 29, 2003 · First decision: November 27, 2003 · Accepted: February 26, 2004

Bibliography

Exp Clin Endocrinol Diabetes 2004; 112: 416–421 © J. A. Barth Verlag in Georg Thieme Verlag KG · Stuttgart · New York · DOI 10.1055/s-2004-821186 · ISSN 0947-7349

Table 1 Immunological key findings using a stepwise diagnostic approach

	Type of diabetes	Skin testing positive reaction	Insulin specific antibodies		Binding/dissociation analysis	
			IgG (mU/ml)	IgE (KU/l)	initial (%)	dissociation
Pat. # 1	Type 1	–	0.37	–	68	delayed
Pat. # 2	Type 1	–	0.32	–	24	rapid
Pat. # 3	Type 2	protamine	–	–	–	–
Pat. # 4	Type 3c	insulin	0.43	86	70	delayed

Normal values (n = 158 diabetic patients exclusively treated with human insulin): insulin specific IgG: 0.038 mU/ml (95% CI: 0.025–0.052 mU/ml); IgE < 0.35 KU/l; initial binding: 4.3% (95% CI: 2.9–5.7%)

Results

Patients were evaluated according to the proposed stepwise approach. For summary of the key findings we refer to Table 1.

Patient #1

A 24-year-old female patient with type 1 diabetes for 7 years. No diabetic secondary complications, mild hypertension, normal body weight (169 cm, 60 kg, BMI: 21), no residual insulin secretion (c-peptide fasting/post prandial < 0.1 ng/ml), no skin reactions at the injection sites. Intensified insulin therapy with Protaphane®, Semilente®, and Actrapid® since the time of diabetes onset. Presently, the leading problem is poor metabolic control (HbA1c 8.9%) due to hyperglycemia, several hospitalizations with diabetic ketoacidosis, and frequent episodes of predominantly nocturnal hypoglycemia despite repeated and extensive patient education. The insulin requirements temporarily reached more than 150 units a day. The immunological evaluation revealed: 1. Negative skin testing for additives and several insulin preparations. 2. High titres of insulin specific IgG (0.37 mU/ml) but no elevated insulin specific IgE which is in accordance with the absence of skin reactions. 3. The binding/dissociation analysis showed a significant initial insulin binding of 68% with delayed dissociation over time. Even after 2 hours 40% of the insulin is bound/neutralized (Fig. 2). In summary, the diagnosis in this patient is best described as "brittle diabetes" caused by neutralizing insulin specific IgG antibodies with delayed dissociation.

Patient #2

A 21-year-old female patient with type 1 diabetes for 12 years on intensified insulin therapy (Insuman basal® and Insuman rapid® at present). During antibody screening programs for potential diabetes associated autoimmune disorders this patient was incidentally diagnosed as being insulin antibody positive with significant titres but presented with good metabolic control (HbA1c 6.9%) and without any treatment related complications, especially no dermal reactions or increased rate of hypoglycemia. The immunological evaluation revealed: 1. Negative skin testing for additives and different insulin preparations. 2. High titres of insulin specific IgG (0.32 mU/ml) in the absence of insulin specific IgE. 3. By contrast, the binding/dissociation analysis showed an initial insulin binding of only 24% with rapid dissociation over time. After 2 hours only 4% of the insulin is bound/neutralized (Fig. 2). In this case, the insulin antibodies are clinically not relevant

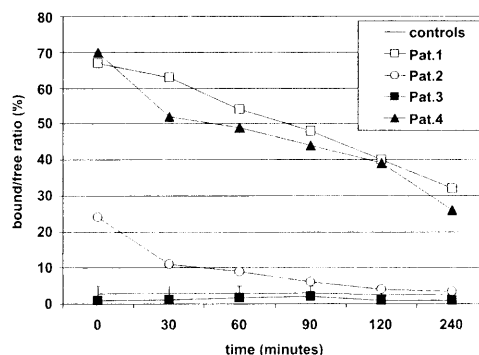


Fig. 2 Bound/free ratio in percent (%) of insulin binding IgG antibodies and dissociation over time assessed in sera of patients with immune-mediated complications associated with s.c. insulin therapy. Patient #1 and #4 exhibit very high initial insulin binding and subsequently delayed dissociation (normal value: 4.3%; 95% CI: 2.9–5.7%; n = 158 control sera of diabetic patients exclusively treated with s.c. human insulin). After 2 hours approximately 40% of insulin is still antibody bound/neutralized. By contrast, patient #2 presents with only moderate initial insulin binding and rapid dissociation. Patient #3 shows no significant insulin binding.

and were classified as epiphenomenon. As a consequence, so far the insulin therapeutic regimen was not changed.

Patient #3

A 78-year-old female patient with type 2 diabetes for 18 years (172 cm, 69 kg, BMI: 23), oral antidiabetic drug treatment (OAD) for 16 years, subsequently insulin therapy with Insuman comb 25® twice daily. Hypertension, mild nephropathy, no signs for neuropathy or retinopathy. Interestingly, she presented with a preserved residual insulin secretory capacity (c-peptide fasting/post prandial 1.8 vs. 3.7 ng/ml). The leading problems were severe local reactions at the injection site, 10–15 min. after the insulin injection (itching, erythema, and swelling) and consequently poor metabolic control with HbA1c of 8.6%. The immunological evaluations revealed: 1. Positive intradermal skin testing for protamine, negative skin testing for other additives or protamine free insulin preparations (Fig. 3). 2. No elevation of insulin specific IgG or IgE. 3. No significant insulin binding in the binding/dissociation analysis (Fig. 2). In this patient, an allergy

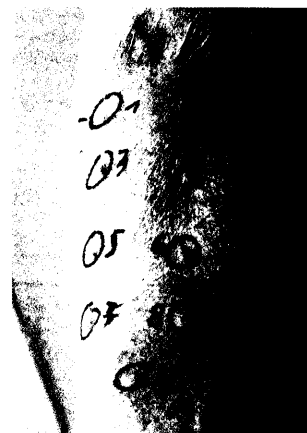


Fig. 3 Intradermal skin testing in patient #3: Negative control (1), positive control (2), positive reaction directed to protamine (8,9), whereas other additives tested negative (3–7). On the other forearm positive reactions against all protamine based formulations were present with negative reactions at all sites with protamine free insulin preparations (not shown).

against protamine was diagnosed as the cause of the local skin reaction with presumably affected insulin absorption. This complication resolved completely by avoidance of the challenging antigen, i.e. protamine in NPH-insulins.

Patient #4

A 57-year-old male patient with presumed "type 3c diabetes" due to partial pancreatectomy because of a pancreatic mass suspicious for malignancy (not confirmed on histopathology). He was presented to our outpatient department with significant loss of pancreatic exocrine function and weight loss (82 → 64 kg, 170 cm, BMI: 22) despite enzyme substitution. Interestingly, c-peptide analysis revealed high plateau levels of fasting c-peptide (4.1 ng/ml) with no significant post prandial stimulation (4.5 ng/ml) indicating residual beta cell function but a high "peripheral insulin demand" even in the fasting situation (here caused by insulin neutralizing IgG antibodies, see below). The insulin regimen had been changed several times, finally Berlinsulin® 30/70 twice daily. At the time of presentation, his leading problem was a very severe local reaction 10–15 min. at all injection sites in use with itching, erythema, and swelling. In addition, he presented with very poor metabolic control (HbA1c 9.3%) due to frequent hyperglycemia. Since the surgery he had already suffered two hospitalizations because of diabetic ketoacidosis and several, again predominantly nocturnal, hypoglycemic episodes. The immunological evaluations revealed: 1. Positive skin testing for all types of insulin while intradermal testing for additives was negative. 2. Quantification of insulin specific antibodies showed very high titres of insulin specific IgG (0.43 mU/ml). Additionally, insulin specific IgE were extremely high (86 KU/ml), which is well in accordance with the positive dermal reaction. 3. The binding analysis demonstrated a significant initial binding of approx. 70% with delayed dissociation over time (Fig. 2). In this patient the diagnosis therefore comprises a combination of immunological complications: IgE mediated classical "insulin allergy" and additionally insulin neutralizing IgG antibodies both contributing to the significant problems in the daily management of the diabetes.

Discussion

The immune response to insulin can elicit various reactions. Two types of antibodies to insulin are well defined. Insulin autoantibodies that appear in insulin-naïve subjects through prediabetic stages of type 1 diabetes. These insulin autoantibodies can be observed very early in life (Ziegler et al., 1999) and are used in algorithms for the prediction of type 1 diabetes (Jaeger et al., 1999). In the world literature few cases are reported of the "insulin autoimmune syndrome" caused by insulin autoantibodies in the absence of prior exposure to exogenous insulin and presenting with recurrent and severe hypoglycemia, hyperinsulinemia, and impaired glucose tolerance (Discher et al., 1990; Lohmann et al., 2001).

Conversely, insulin antibodies occur in patients who have been exposed to exogenous insulin treatment. Stimulation of the T- and B-cells can lead to the production of anti-insulin antibodies of the IgE and IgG class, and less frequently of the IgA, IgM, and IgD class (Velcovsky and Federlin, 1982; Federlin, 1985; Hobler et al., 1991; Scherthner, 1993).

Two different mechanisms are considered to be the cause of immune-mediated complications in insulin treated individuals. Classically, insulin allergy presenting as type 1 allergy being mediated by insulin specific IgE antibodies and histamine release typically associated with severe dermal reactions at the injection site and consecutively deterioration of insulin absorption and metabolic control. The incidence of classical insulin allergy has decreased with the use of human recombinant insulin and is now reported in less than 1% of diabetic patients treated with insulin (Nagai et al., 1997; Eapen et al., 2000). Classical insulin allergy has to be separated from an allergy directed to additives, e.g. protamine. This was the case in our patient #3 but the diagnosis could be easily established through skin testing being only positive for protamine and confirmed by the negative results with regard to insulin specific IgE and IgG antibodies. This complication could be controlled simply by avoidance of NPH insulins. In view of the sufficient residual insulin secretion in this patient (c-peptide fast/pp 1.8 vs. 3.7 ng/ml) we restarted her on a combination of OAD therapy resulting in a good metabolic state (HbA1c 7.6%). Optionally, it would be possible to set her on prandial insulin therapy with regular insulin or analog if necessary in the future.

In contrast, generation of IgG antibodies to exogenous insulin is more frequent compared to insulin allergy even in the era of human recombinant insulins. The presence of insulin specific IgG antibodies is not necessarily associated with classical immunological insulin resistance of that type, that was observed in the last century while using impure insulin preparations or bovine/porcine insulins resulting in insulin requirements of several thousand units a day (Scherthner, 1993; Van Haeften et al., 1987). Even lower levels of insulin specific IgG antibodies against human recombinant insulin can influence insulin profiles by temporarily neutralizing insulin causing significant metabolic consequences e.g. poor metabolic control and severe hypoglycemia (Van Haeften, 1989). This was the case in our patient #1. The diagnostic approach revealed high titres of insulin specific IgG antibodies (0.37 mU/ml) with very high initial insulin binding

(68%) and delayed dissociation in the absence of dermal reactions or insulin specific IgE. Pharmacokinetics of insulin is unpredictable in the presence of such antibodies potentially causing delayed insulin peaks, prolonged duration of activity, increased risk for hypoglycemia, and varying bioavailability (Van Haeften, 1989). In order to adjust the insulin delivery to near optimal steady state conditions, we changed the therapeutic regimen in this patient and started her on continuous subcutaneous insulin infusion (CSII) using insulin aspart. Potentially, the immune reactivity against human insulin can be bypassed, at least partially, by using an analog. This has been shown for generalized insulin allergy by the use of lispro, insulin aspart, and glargine (Näf et al., 2002; Kumar, 1997; Eapen et al., 2000; Airaghi et al., 2001; Moriyama et al., 2001). Although the main immunogenic insulin epitopes remain unchanged in the lispro or insulin aspart molecule, it has been suggested that these analogs have reduced immunogenicity because of the rapid dissociation in monomers (Airaghi et al., 2001; Kumar, 1997). In our patient the combination of the beneficial effects of insulin pump therapy together with the use of an insulin analog (insulin aspart) improved metabolic control significantly (HbA1c 6.1%) and decreased the rate of hypoglycemic episodes. In the world literature there is only one case report using lispro in an insulin pump for treatment of immunological insulin resistance (Lahtela et al., 1997). To our knowledge we report here for the first time successful treatment of metabolic complications caused by insulin neutralizing IgG-antibodies by using insulin aspart in combination with continuous subcutaneous insulin infusion (CSII). Meanwhile, we have confirmed the beneficial effects of this regimen in six other type 1 diabetic patients presenting with the same phenotype of immunological complication.

However, an increased titre of insulin specific IgG does not mean automatically, that these immunoglobulins significantly neutralize insulin. Even significantly elevated antibody titres can be clinically irrelevant and staged as epiphenomenon in case of low-avid insulin antibodies and consecutively low insulin binding capacity. IgG-insulin antibodies of low levels have been reported in approximately half of diabetic patients (n = 578) after exclusive treatment with human insulin for 2 years (Scherthaner, 1993). We confirmed these findings of low level insulin specific IgG in our control population of n = 158 diabetic patients who were treated exclusively with s.c. human insulin demonstrating a mean IgG-level of 0.038 mU/ml (CI: 0.025 – 0.052 mU/ml). To differentiate significant from non significant insulin binding, the binding/dissociation analysis (step 3 in our diagnostic approach) is essential. Patient #2 represents an example of significantly increased insulin specific IgG (0.32 mU/ml) that contribute only to a very low level of insulin binding (24%) with rapid dissociation (Fig. 2, patient #2). In view of the stable metabolic control in this patient these antibodies can be staged as clinically not relevant and are therefore without therapeutic consequence.

The findings in patient #4 represent a very rare case. The diagnostic algorithm had confirmed both mechanisms of immunological complications being present. Classical insulin allergy with dermal reactions and high titres of insulin specific IgE and additionally significant titres of insulin binding and neutralizing IgG antibodies. First, we tried a desensitization protocol accord-

ing to Galloway (Galloway and deShazo, 1990) but the patient did not respond. Despite partial pancreatectomy there was initially a residual endogenous insulin secretion. We therefore decided to try a maximum of OAD therapy (Biguanide, Sensitizer, Glinide) in order to avoid exogenous insulin therapy. This attempt was successful for four months only, then followed by complete deterioration of the residual insulin secretion (c-peptide decrease from 4.1 ng/ml to <0.6 ng/ml) and hospitalization because of ketoacidosis. The patient was now ultimately dependant on exogenous insulin supply. In the literature there is evidence for a combined therapy using corticosteroids and antihistamines in such situations (Ganz et al., 1990). We introduced corticosteroids (1 mg/kg) in combination with antihistamines (H1/2-antagonists) and started the patient on prandial therapy with lispro insulin. The steroid dosage was tapered according to the level of dermal reaction which was monitored carefully. This regimen was successful, his present medication comprises 12.5 mg Decortin-H in combination with antihistamines and prandial lispro therapy only. The metabolic situation is stabilized (HbA1c 7.4%) with a decreased rate of hypoglycemic episodes in the absence of dermal reactions. During follow-up we observed a significant decrease of insulin specific IgG and IgE levels.

In summary, we have demonstrated a diagnostic algorithm for the differential diagnosis as prerequisite for individual management of different forms of immune-mediated complications associated with subcutaneous insulin therapy (Fig. 1). An essential part of this diagnostic approach is the binding/dissociation analysis (step 3) in case of positive insulin specific IgG (step 2) giving information about the clinical significance of these antibodies. As shown, the proposed stepwise approach leads to a definite diagnosis providing a basis for differential and adjusted therapy.

Acknowledgements

This paper is to acknowledge and honour the pioneering work of Professor emeritus Konrad Federlin diagnosing and treating insulin allergy. He was chairman and head of our department from 1976 to 1996 when he retired. He has celebrated his 75th birthday on August 15, 2003. The authors would like to express their gratitude to the local preceaders in this research and clinical field, Hans-Georg Velcovsky, Hubert Hobler, and Thomas Discher. We would like to thank Jutta Schmidt and Michael Stein, technicians, for expert technical assistance. We are grateful to Dr. D. Leihener, Aventis Pharma, for providing us with radiolabelled human recombinant insulin.

References

- Airaghi L, Lorini M, Tedeschi A. The insulin analog aspart: a safe alternative in insulin allergy. *Diabetes Care* 2001; 24: 2000
- Christiansen AH. Radioimmuno-electrophoresis in the determination of insulin binding to IgG. *Methodological studies. Horm Metabol Res* 1973; 5: 147 – 154
- Discher T, Seipke G, Friedmann E, Velcovsky HG, Federlin K. Recurrent hypoglycemia in the insulin autoimmune syndrome. *Dtsch Med Wochenschr* 1990; 115: 1950 – 1955
- Dixon K. Measurement of antibodies to insulin in serum. *Clin Chem* 1974; 20: 1275 – 1281
- Eapen SS, Connor EL, Gern JE. Insulin desensitization with insulin lispro and an insulin pump in a 5-year-old child. *Ann Allergy Asthma Immunol* 2000; 18: 395 – 397
- Federlin K. Diabetes mellitus and immunology – a manifold interrelation. *Immun Infekt* 1985; 13: 1893 – 1899
- Francis AJ, Hanning I, Alberti KGM. The influence of insulin antibody levels on the plasma profiles and action of subcutaneously injected human and bovine short acting insulins. *Diabetologia* 1985; 28: 330 – 334
- Galloway JA, deShazo RD. Complications of insulin treatment. In: Ellenberg M, Rifkin H (Eds). *Diabetes Mellitus: Theory and Practice*. New Hyde Park: NY Med Exam, 1990: 506 – 508
- Ganz MG, Untermann T, Roberts M, Uy R, Sahgal S, Samter M, Grammer LC. Resistance and allergy to recombinant human insulin. *J Allergy Clin Immunol* 1990; 86: 45 – 52
- Hobler H, Zoltobrocki M, Scherthaner G, Schnack C, Federlin K. Formation of protamine, insulin, and native NPH-human insulin antibodies in diabetic patients treated with NPH-human insulin. One year observation study. *Diab Nutr Metab* 1991; 4: 275 – 281
- Jaeger C, Hatzigeorgaki E, Stroedter A, Becker F, Scherer S, Petzoldt R, Federlin K, Bretzel RG. The Giessen-Bad Oeynhausen family study: improved prediction of type 1 diabetes in a low incidence population of relatives using combinations of islet autoantibodies in a dual step model. *Exp Clin Endocrinol Diabetes* 1999; 107: 496 – 505
- Kerp L, Steinhilber S, Kasemir H. Ein Verfahren zum Nachweis insulin-bindender Antikörper durch Differentialadsorption. *Klin Wschr* 1966; 90: 806 – 814
- Kumar D. Lispro analog for the treatment of generalized allergy to human insulin. *Diabetes Care* 1997; 20: 1337 – 1359
- Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH. Determination of free and total insulin and c-peptide in insulin treated diabetics. *Diabetes* 1977; 26: 22 – 29
- Lahtela JT, Knip M, Paul R, Anttonen J, Salmi J. Severe antibody-mediated human insulin resistance: successful treatment with the insulin analog lispro. A case report. *Diabetes Care* 1997; 20: 71 – 73
- Lohmann T, Kratzsch J, Kellner K, Witzigmann H, Hauss J, Paschke R. Severe hypoglycemia due to insulin autoimmune syndrome with insulin autoantibodies crossreactive to proinsulin. *Exp Clin Endocrinol Diabetes* 2001; 109: 245 – 248
- Moriyama H, Nagata M, Fujihira K, Yamada K, Chowdhury SA, Chakrabarty S, Zhenji J, Yasuda H, Ueda H, Yokono K. Treatment with human analog insulin glargine resolves a generalized allergy to human insulin in type 1 diabetes. *Diabetes Care* 2001; 24: 411 – 412
- Näf S, Esmatjes E, Recasens M, Valero A, Halperin I, Levy I, Gomis R. Continuous subcutaneous insulin infusion to resolve an allergy to human insulin. *Diabetes Care* 2002; 25: 634 – 635
- Nagai T, Nagai Y, Tomizawa T, Mori M. Immediate-type human insulin allergy successfully treated by continuous subcutaneous insulin infusion. *Intern Med* 1997; 36: 575 – 578
- Scherthaner G. Immunogenicity and allergic potential of animal and human insulins. *Diabetes Care* 1993; 16: 155 – 165
- Van Haeften TW, Heiling VJ, Gerich JE. Adverse effects of insulin antibodies on postprandial plasma glucose and insulin profiles in diabetic patients without immune insulin resistance. Implications for intensive insulin regimens. *Diabetes* 1987; 36: 305 – 309
- Van Haeften TW. Clinical significance of insulin antibodies in insulin-treated diabetic patients. *Diabetes Care* 1989; 12: 641 – 648
- Velcovsky HG, Federlin K. Insulin-specific IgG and IgE antibody response in type 1 diabetic subjects exclusively treated with human insulin (recombinant DNA). *Diabetes Care* 1982; 5: 126 – 128
- Von Kriegstein E, Schatz H. Insulin. In: Schatz H (ed). *Diabetologie kompakt. Grundlagen und Praxis*. 2nd ed. Berlin, Wien: Blackwell, 2002: 86 – 101
- Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 1999; 48: 460 – 468