

The Immunogenicity of Insulin Preparation. Antibody Levels before and after Transfer to Highly Purified Porcine Insulin

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Summary. Ninety-two insulin-dependent diabetics (aged 4–20 years, mean \pm SD: 13 ± 4) with a duration of diabetes from 2 to 17 years (7 ± 3) were transferred from Lente or NPH ($5 \times$ crystallised insulin) to Monotard insulin (highly purified insulin). Total serum immunoreactive insulin levels and concentrations of antibodies against insulin, porcine proinsulin, a-component and pancreatic polypeptide were determined prior to [I] and at a mean of 220 [II], 460 [III], 830 [IV], and 1170 [V] days after the change. All but two subjects had insulin antibodies (IgG) at the start, with a mean value of 2864 μ U/ml. There was a significant fall in the mean insulin antibody level between [I] and [II] to 2165 μ U/ml ($p < 10^{-7}$), followed by an increase between [II] and [III] whereafter a slight decrease was observed being significant between [III] and [IV], as well as between [IV] and [V] ($p < 0.05$); some patients showed a constant fall over the entire period, while others showed fluctuations. Total serum insulin showed a similar pattern, with a mean value of 1141 μ U/ml at [I] declining to 522 μ U/ml at [V]. The percentage fall between [I] and [V] was greater (54%) than that in the insulin antibodies (30%). Antibodies against a-component, proinsulin and pancreatic polypeptide were present in 96%, 72% and 41% of the patients respectively before the change in therapy. There was a decline in these antibodies between each sampling (p values between $< 10^{-3}$ and 10^{-8}) and, at the end of the investigation antibodies against a-component were above the detection limit in only 4 patients, and none of the patients showed antibodies against proinsulin or pancreatic polypeptide. Thus, removal of the impurities, including the hormonal contaminants of insulin, leads to a slow fall in antibodies to insulin and a much faster disappearance of antibodies against a-component, proinsulin and pancreatic polypeptide.

Key words: Insulin antibodies, proinsulin antibodies, pancreatic polypeptide antibodies, a-component

antibodies, total serum immunoreactive insulin, insulin immunogenicity, insulin-dependent diabetes.

It is well known that treatment of diabetes with insulin preparations purified only by crystallization leads to the formation of considerable amounts of insulin antibodies in nearly all patients [1–4].

In addition, the impurities present in some insulin preparations may lead to the formation of other types of antibodies, such as antibodies against the high-molecular-weight proteins a-component [5, 6], proinsulin [6–10], pancreatic polypeptide (PP) [10–12], VIP (vasoactive intestinal peptide) and glucagon [11, 12]. However, reports so far published are not very extensive. Only one study reported antibodies against the impurities after transfer of the patients from Lente ($5 \times$ crystallised) to Monotard (Monocomponent = MC) insulin, but provided no information concerning the antibody levels prior to the transfer [6].

In the present study, a group of diabetic children and adolescents was transferred from Lente or NPH insulins to Monotard insulin. This paper describes the circulating levels of IgG against insulin, a-component, proinsulin and PP and the total amount of immunoreactive insulin (IRI) before and up to 3 years after the transfer.

Materials and Methods

Patients

Ninety-two insulin-dependent diabetics, aged 4–20 years at the beginning of the study, were included in the study. No patient had any other chronic disease or was taking steroids during the course of the study. The duration of diabetes varied from 2 to 17 years. Therapy prior to transfer comprised one (57%) or two (43%)

Table 1. Number of patients from whom serum was available included in the assays at the different sampling times

Sampling no.	I	II	III	IV	V
Days, mean	0	223	463	834	1170
Days, range		102–364	316–588	604–944	1024–1267
Insulin					
antibodies	92	89	89	69	62
Total IRI	91	89	89	69	61
a-component					
antibodies	54	54	54	39	33
Proinsulin					
antibodies	54	54	54	39	33
PP					
antibodies	40	40	40	27	25

daily injections of Lente (70% of crystalline bovine and 30% of amorphous porcine insulin) or NPH. The $5 \times$ crystalline insulin in Lente contained approximately 15000 ppm (parts per million by weight) of proinsulin, 1 ppm of PP, whereas the contents of proinsulin in the highly purified insulin used in this study was ≤ 1 ppm and that of PP ≤ 0.01 ppm. All patients were transferred to Monotard insulin from one day to another. Blood samples were drawn in the fasting state prior to the morning insulin injection, and serum was stored at -18°C until use. Samples were taken before the transfer (sample [I]) and then at 223 days (range 102–364 days), sample [II]; at 463 days (316–588), sample [III]; at 834 days (604–944), sample [IV]; and at 1170 days (1024–1267), sample [V].

Table 1 shows the number of patients included in each examination. Fewer patients were examined for IgG against a-component, proinsulin and PP due to shortage of serum from the first drawing and the study of these antibodies included only those patients where serum was available from the first three examinations. Total IRI and insulin binding IgG were measured throughout the study while the other antibodies were determined together at the end of the study. All parents and children (or adolescents) were informed of and gave their consent to the change of insulin preparation.

Antibody Determinations

Total IRI was determined according to [13] and IgG binding of insulin by radioimmuno-electrophoresis [14].

Determination of IgG with Affinity to Proinsulin and a-component

Prior to the determination of proinsulin and a-component binding to IgG, all insulin binding IgG must be removed: 200 μl of serum was added to 600 μl of a Sepharose-highly purified bovine insulin suspension containing 1 mg of Sepharose-bound insulin per ml. After 20 h shaking at 4°C the bound insulin antibodies were removed by centrifugation at 2000 rpm (Mistral 6L) and the supernatant used for proinsulin and a-component antibody determinations.

Determination of Porcine Proinsulin Binding IgG

Fifty μl of sepharose-insulin supernatant (see above) was mixed with 50 μl of barbital buffer and 50 μl of porcine ^{125}I -proinsulin

(125 ng/ml, 25 $\mu\text{Ci}/\mu\text{g}$) in barbital buffer. After 20 h at 4°C the ^{125}I -proinsulin bound to IgG was determined by radioimmuno-electrophoresis as described previously [14]. The amount bound in ng/ml supernatant was then calculated. In normal serum a mean nonspecific binding of 0.65 ng/ml (SD = 0.28) has been recorded. The results are corrected for this nonspecific binding and only values >1 ng/ml (~ 3 SD) were considered positive.

Determination of Porcine a-component Binding to IgG

Fifty μl of the sepharose-insulin supernatant was mixed with 50 μl of barbital buffer and 50 μl of ^{125}I -porcine a-component (1250 ng/ml) in barbital buffer, pH 8.6. The procedure was thereafter the same as that described above. In normal serum the mean nonspecific binding was 32 ng/ml (SD = 7). The results were corrected for the nonspecific binding in normal serum and values >25 ng/ml (~ 3 SD) were considered positive.

Determination of Porcine Pancreatic Polypeptide (PP) Binding to IgG

Fifty μl of serum was mixed with 50 μl of barbital buffer and 50 μl of ^{125}I -PP (diluted with barbital buffer to 125 ng/ml). After incubation as described above, the amount bound to IgG was determined by radioimmuno-electrophoresis. In normal serum the mean nonspecific binding was 0.79 ng/ml (SD = 0.14). The results were corrected for this nonspecific binding and only values >0.5 ng/ml were considered positive.

The statistical evaluation of the differences between the various results on samples at I, and II to V was performed using the Pearson Rank Test.

Results

Total IRI and Insulin Antibodies

Serum total IRI and insulin antibody levels are shown in Figures 1 and 2. Only two patients were free of insulin antibodies at the start. Table 2 shows the mean values and the statistical evaluation (ranges are shown in Figure 1 and 2). The majority (45) of patients had significantly lower, 11 significantly higher and 6 unchanged levels of insulin antibodies 1170 days after transfer. The major fall of both IgG binding and serum total IRI took place within the first interval, after which there was an increase in IgG which fell slightly again later. Total IRI was virtually unchanged between periods [II] and [V].

Antibodies to a-component and Porcine Proinsulin

The distributions of the a-component and proinsulin antibodies at samplings [I] to [V] are shown in Table 3. Only two patients were free of a-component antibodies, while proinsulin antibodies were found in

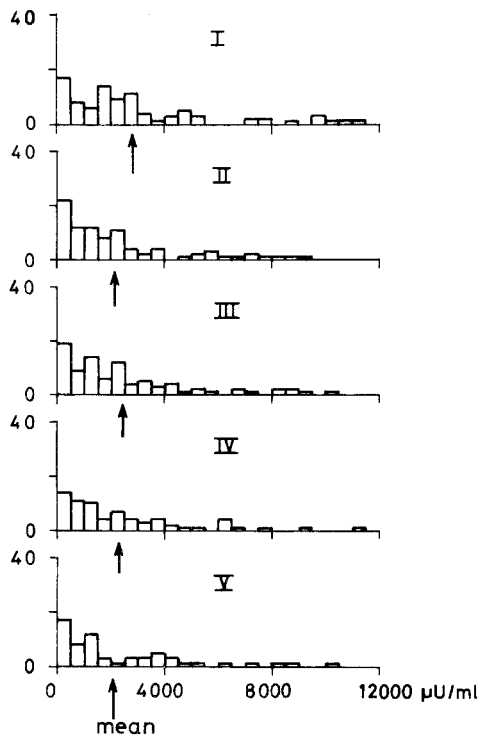


Fig. 1. Insulin binding IgG in the serum of diabetics before [I] and at intervals after [II-V] transfer to Monotard insulin from Lente and NPH. Arrows = mean values, the ordinate shows number of patients in each group. Values are given as binding capacity per ml serum. For definition of sampling times see Table 1

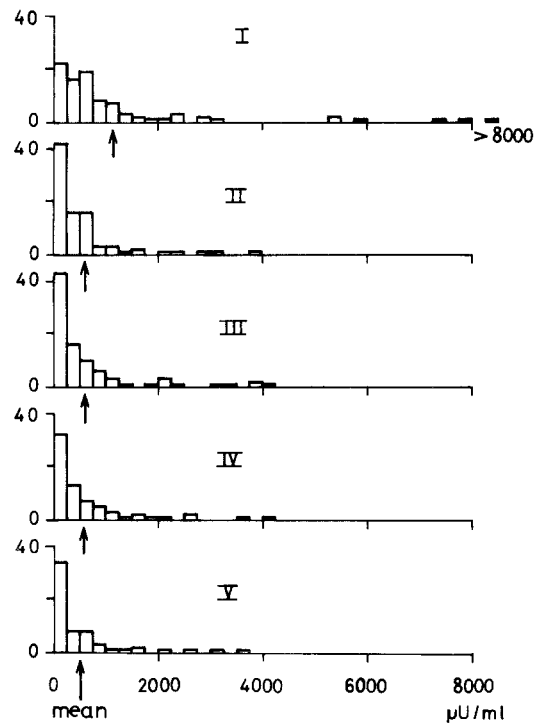


Fig. 2. Total serum IRI in diabetics before [I] and after [II-V] transfer to Monotard insulin from Lente and NPH. Arrows = mean values. The ordinate shows number of patients in each group. For definition of sampling times see Table 1

Table 2. Serum insulin IgG and total IRI at different sampling times

Sampling	Serum insulin IgG µU/ml			Total serum IRI µU/ml		
	mean	range	p-value	mean	range	p-value
I	2864 (3004) ^a	2-11029	<10 ⁻⁷	1141 (1139) ^a	10-9120	10 ⁻⁵
II	2165	6-9039		598	6-8000	
III	2440	20-10275	<0.001	626	16-4160	NS
IV	2330	0-11265	<0.05	598	10-4160	NS
V	2109	1-10212	<0.005	522	6-3520	NS

^a Values within brackets indicate mean for the 62 patients who were examined at [V]. For details of sampling times see Table 1. Statistical analysis by Pearson Rank test

72% of the 54 patients examined prior to transfer to Monotard. There was a significant decrease in these types of antibodies from one sampling to the next until levels became undetectable. The only exception was 4 patients who still had a-component antibodies at the final sampling.

Antibodies to PP

Table 3 shows the results of assays for antibodies to PP. Forty-one percent of the 40 patients had anti-

bodies against PP, but these disappeared quickly and were found in only 2 patients at the fourth sampling.

Discussion

Extensive studies have been published concerning the development of antibodies to insulin after treatment with insulin [1-4], hence the immunogenicity of insulin preparations has often been considered as a matter of induction of insulin antibodies. In fact,

Table 3. Serum a-component, porcine proinsulin and PP-antibodies at different sampling times. See Table 1 for details of sampling times

Sampling	Serum a-component antibodies ng/ml			Serum porcine proinsulin antibodies ng/ml			Serum antibodies PP ng/ml		
	mean	range	p-value	mean	range	p-value	mean	range	p-value
I	172	0-326	<10 ⁻⁸	9.38	0-103.8	<10 ⁻⁵	1.59	0-8.1	<10 ⁻⁴
II	69	0-159	<10 ⁻⁸	1.77	0-36.5	<0.05	0.36	0-2.2	<0.01
III	45	0-130	<10 ⁻⁸	0.70	0-13.8	NS	0.23	0-1.1	NS
IV	12	0-70	NS	0.40	0-4.8	<0.001	0.19	0-0.8	NS
V	12	0-59	NS	0.14	0-0.4		0.12	0-0.4	NS

insulin antibodies are only one of many different types of antibodies, e. g. antibodies against glucagon, VIP, PP, somatostatin, proinsulin, and a-component [5-12] formed following treatment with insulin not purified by extensive chromatographic procedure.

The removal of the hormonal contaminants has been found to abolish the formation of antibodies against VIP, PP, glucagon, and somatostatin [12], as well as a-component and proinsulin [5, 6] in patients treated exclusively with MC-insulins. Bruni et al. [6] found a-component antibodies in significantly fewer patients after 5 years of transfer to MC-insulin as compared to the group continued on Lente, but initial values were lacking which make evaluation of the difference impossible. Furthermore, the quality of the MC-insulin used in the studies was inferior to the one used in this study, the latter containing less than 1 ppm of proinsulin, whereas previously the content was between 100-400 ppm [15]. The finding of a-component antibodies in 52 of the 54 patients treated confirms the strong immunogenic nature of the a-component present in 5 × cryst. insulin. Treatment with highly purified insulin of a quality known not to induce antibodies against a-component [5] led to a quick fall in the levels, but at the third sampling (463 days after transfer) 43 patients still had detectable a-component antibodies. After approximately 2 and 3 years 5 out of 39 and 4 out of 33 had low, but detectable, levels of a-component antibodies.

Proinsulin antibodies were present in 38 of 54 patients prior to transfer. However, these antibodies disappeared quickly as 6 patients only had low levels after 463 days of treatment. PP antibodies were present in about 40% of the patients and the disappearance of these antibodies was similar to that of proinsulin antibodies. The time required before the levels of antibodies against a-component, proinsulin and PP became non-detectable was between 1 and 3 years, indicating a continued synthesis of these antibodies at a decreasing rate after transfer to MC-insulin. It can-

not be determined whether the continued formation of antibodies is due to a weak continued stimulation of the organism or simply reflects the time it takes before the synthesis stops.

The levels of insulin antibodies follow the patterns described by others, such as Bruni et al. [16]. Most patients showed a decrease after 220 days followed by a small rise and then a slow decline. It is likely that the highly purified insulin used in the present study, despite its low immunogenicity as regards insulin antibodies, is capable of maintaining the stimulation of an organism sensitized by prior treatment to continued antibody production, although the mean rate of production is declining.

The increase in insulin antibody levels observed between samplings [II] and [III] remains obscure. It was not due to problems in the methodology as the daily controls were unchanged. Others have observed the same intriguing finding [16].

The fairly large decrease in total IRI observed between the first and second sampling probably reflects a species change (porcine insulin being bound to a lower degree than bovine insulin), as well as the observed decrease in the level of insulin antibodies. The relation between insulin antibody levels and clinical parameters in these patients is described elsewhere [17].

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