

IgG Subclass Analysis of Insulin Antibodies in Patients With Type 1 Diabetes Treated With Inhaled Human Insulin (Exubera®) or Subcutaneous Insulin Regimens



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STUDY OVERVIEW

INTRODUCTION

- Inhaled human insulin (EXU; Exubera® (insulin human [rDNA origin]) Inhalation Powder) provides short-acting glycemic control comparable to that of subcutaneous (SC) insulin, and it provides better control than oral agents in patients with type 2 diabetes.^{1,7} It has been shown to be as effective a prandial therapy as regular SC insulin in patients with type 1 diabetes.^{1,2}
- EXU therapy is associated with a 22% increase (unadjusted 95% confidence interval [CI]: 19.5, 24.5) in median insulin antibody (IAb) binding compared to SC insulin in patients with type 1 diabetes, and a 3.5% increase (unadjusted 95% CI: 1.5, 4.5) in median IAb binding in insulin-using patients with type 2 diabetes.⁸
- The antibodies associated with EXU therapy are immunoglobulin (Ig) G in type. No clinical consequences of the IAb elevation have been identified.⁸
- This study evaluated the IgG subclass profile in patients with type 1 diabetes.

OBJECTIVE

- To compare IAb levels of the IgG1, IgG2, IgG3, and IgG4 subclasses in serum samples from patients with type 1 diabetes, formed in response to either EXU or SC insulin.

METHODS

STUDY DESIGN

- Serum samples were collected from adult patients with type 1 diabetes participating in a randomized controlled Phase 3 trial of EXU versus SC insulin.
- To ensure appropriate comparison of IgG subclass levels between treatment groups, samples with similar overall IAb levels (100-500 µU/mL; determined by polyethylene glycol precipitation) were selected for analysis.
- As duration of antigen exposure may influence IgG subclass profiles, samples were taken from all EXU-treated patients in the twelfth month of therapy, when IgG levels have been shown to plateau in patients with type 1 diabetes.⁸ Because of the limited number of SC insulin-treated patients with higher IAb levels, the sampling window for control patients was widened to 6 to 12 months. In summary, all Month 12 samples from the EXU group and last-drawn samples from the SC group collected in the 6- to 12-month treatment window with IAb levels of 100 to 500 µU/mL were analyzed in the IgG1-4 subclass assays.

ASSESSMENTS

- IgG subclass levels were measured using a validated radioligand binding method that uses sepharose beads conjugated to goat anti-mouse IgG antibodies and mouse anti-human IgG1-4 antibodies (Figure 1).

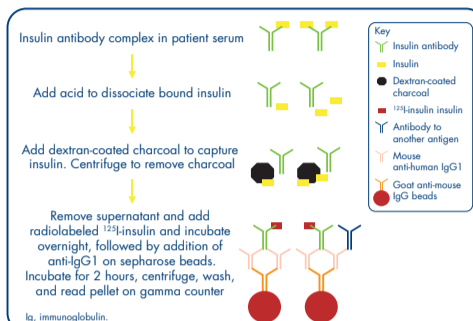


Figure 1. IgG subclass insulin antibody assays (example with IgG1).

ANALYSIS

- Data were expressed as insulin binding capacity in µU of insulin bound/mL of serum.
- To normalize the data, the binding capacity of each subclass of each sample was summed. The percent of sum of each IgG subclass to the overall subclass profile was then calculated.
- These values were used to determine the range, mean, and median of each subclass.

RESULTS

- Patients in the EXU group (n = 68) had a mean duration of diagnosis with type 1 diabetes of 19 years (range, 1-44 years) prior to study entry and switched their short-acting insulin to EXU for a mean duration of 11.9 months.
- The SC group (n = 21) had a mean duration of diagnosis with type 1 diabetes of 26 years (range, 1-48 years) prior to study entry and continued an exclusively SC insulin regimen for 9.7 months.
- The overall mean (±SD) IAb binding capacities were 251.9 ± 113.8 µU/mL and 170.7 ± 65.1 µU/mL for EXU and SC insulin samples, respectively (Table 1).

Table 2. Insulin Binding Capacities by IgG Subclass in Patients Treated With EXU or SC Insulin Regimens

	Insulin Binding Capacity (µU/mL)							
	EXU (n = 68)				SC Insulin (n = 21)			
	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
Minimum	19.0	6.0	2.8	6.0	19.0	6.0	2.8	6.0
25th percentile	61.5	13.0	13.0	14.5	28.5	16.0	13.5	16.5
Median	92.5	22.0	22.0	31.0	55.0	19.0	24.0	32.0
75th percentile	149.5	59.5	51.0	62.0	77.0	32.0	67.5	49.5
Maximum	355.0	208.0	220.0	238.0	136.0	73.0	168.0	309.0
Mean (±SD)	117.4	38.5	41.7	46.1	59.9	25.8	41.8	53.6
	±	±	±	±	±	±	±	±
	75.0	39.4	46.1	44.9	33.1	15.1	42.8	69.1

Ig, immunoglobulin; EXU, inhaled human insulin; SC, subcutaneous.

Table 3. Percent of the Sum of IgG Subclasses in Patients Treated With EXU or SC Insulin Regimens

	Percent of the Sum of IgG Subclasses							
	EXU (n = 68)				SC Insulin (n = 21)			
	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
Minimum	24.1	3.4	1.6	1.1	8.1	7.1	2.8	5.4
25th percentile	41.6	9.8	8.7	9.4	24.3	9.8	7.9	11.4
Median	52.5	12.9	13.5	18.4	32.4	14.5	16.0	20.7
75th percentile	59.6	17.8	20.6	27.5	46.0	21.4	34.7	36.9
Maximum	78.5	29.6	62.4	53.9	66.4	29.9	71.2	62.6
Mean (±SD)	51.2	14.2	15.7	18.8	35.0	15.3	24.2	25.6
	±	±	±	±	±	±	±	±
	13.4	6.5	10.3	11.5	15.3	6.5	21.1	17.1

Ig, immunoglobulin; EXU, inhaled human insulin; SC, subcutaneous.

Table 1. Total Insulin Binding Capacities in Patients Treated With EXU or SC Insulin Regimens

	Insulin Binding Capacity (µU/mL)	
	EXU (n = 68)	SC Insulin (n = 21)
Minimum	102	107
25th percentile	158	114.5
Median	213	143
75th percentile	353	214
Maximum	500	340
Mean (±SD)	251.9 ± 113.8	170.7 ± 65.1

EXU, inhaled human insulin; SC, subcutaneous.

- Serum analysis showed a similar pattern of distribution of IgG1-4 subclasses in EXU and SC patients (Table 2; Figure 2). The IgG1 and IgG4 antibodies were the dominant subclasses in both the EXU and SC insulin groups.
 - In the EXU group, mean insulin binding capacities (µU/mL) were IgG1, 117.4; IgG2, 38.5; IgG3, 41.7; and IgG4, 46.1.
 - In the SC group, they were IgG1, 59.9; IgG2, 25.8; IgG3, 41.8; and IgG4, 53.6.

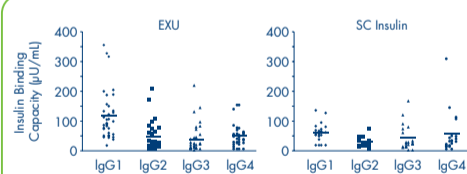


Figure 2. Insulin binding capacities by IgG subclass.

- The absolute mean IgG1 binding capacity appeared to be higher in the EXU group than in the SC insulin group.
- The mean percent of sum of each IgG subclass illustrates the relative distribution of subclasses in the EXU and SC samples (Table 3; Figure 3). In both treatment groups, IAb levels of the subclass IgG1 were greater than IgG4 levels, which were slightly greater than both IgG2 and IgG3.
 - In the EXU group, mean percents of sum of each IgG subclasses were IgG1, 51.2; IgG2, 14.2; IgG3, 15.7; and IgG4, 18.8.
 - In the SC group, they were IgG1, 35.0; IgG2, 15.3; IgG3, 24.2; and IgG4, 25.6.

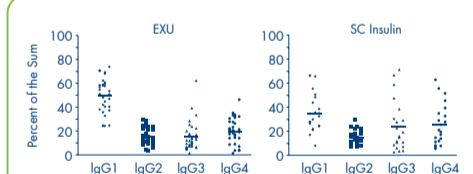


Figure 3. Percent of the sum of IgG subclasses.

- The relatively higher IgG1 level in the EXU group resulted in a slightly lower IgG4/IgG1 median ratio than found in the SC group (Figure 4).

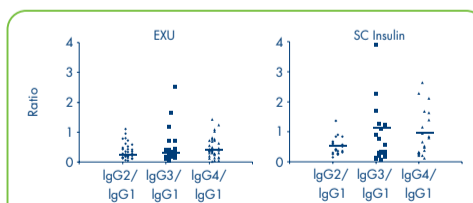


Figure 4. Ratios of insulin binding capacities.

CONCLUSIONS

- The EXU-associated IgG subclass profile is similar to that found in patients treated with SC insulin.
- After 12 months of treatment, the IgG1 and IgG4 antibodies were the dominant subclasses in both the EXU and the SC insulin patient groups.
- The higher mean IgG1 level observed in the EXU group compared with SC insulin-treated patients is of no known

clinical significance and likely reflects the mean difference in total IAb levels between the treatment groups and/or the greater duration of time the control group patients were treated exclusively with SC insulin (mean, 26 years) relative to treatment durations of the EXU group (mean, 11.9 months). Beyond 1 year of therapy, IAb response may shift from IgG1 to IgG4, as has been described with SC insulin exposure.⁹

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