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Insulin antibodies are prevalent in adults with Type 1 diabetes referred for islet cell transplantation and are modified by islet transplantation and immunosuppression: An Australian experience.

Running title: Insulin antibodies post islet cell transplantation in Type 1 diabetes

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Word count: 1125 **Figure:** 1

Abstract

We have analysed insulin antibodies in 149 adults with Type 1 diabetes and 2859 people without diabetes. We have determined that insulin antibody levels are higher in adults with vs. without diabetes and that the levels are falling, and more patients are becoming antibody-negative post islet cell transplantation.

People with diabetes can develop antibodies to endogenous and exogenous insulin, which can temporarily bind insulin, leading to hyperglycaemia, followed by delayed insulin release, causing hypoglycaemia [1, 2]. A minority of people with Type 1 diabetes (T1D) have severe hypoglycaemia unawareness and life-threatening, rapidly fluctuating glucose levels which may require islet-cell transplantation (ICT) [3, 4].

We determined if insulin antibody positivity is more common and antibody levels are higher in adults with vs. without T1D and in people with T1D referred for ICT than in those not referred for ICT. We also determined whether ICT, post-ICT immunosuppression and insulin-independence reduced insulin antibody positivity and levels.

Insulin antibody levels were determined (St. Vincent's Hospital Melbourne, Department of Endocrinology and Diabetes Laboratory) during 1995-2018 in 2859 subjects without T1D, 103 adults with T1D (not referred for ICT), and (during 2005-2016) 46 ICT candidates. The study

was approved by St Vincent's Hospital Ethics Committee (HREC number: 062-06). No control subjects (n=2859) had known Type 2 diabetes, pre-diabetes or other forms of diabetes, but as glucose and HbA1c data were incomplete and undiagnosed Type 2 diabetes is common, we refer to this group as non-T1D rather than non-diabetic. Plasma antibody levels were quantified using a dextran coated charcoal radioimmunoassay [5, 6] and expressed in % specific binding, with a positive test taken as >1.4% specific binding, with intra- and inter-assay CVs of 2.1% at 2.6% and 6.2% at 3.7% respectively. There is a related National Association of Testing Authorities QC program implemented in our laboratory. The 103 T1D subjects were usually participants in clinical research, such as hyperinsulinaemic euglycaemic clamp studies, which required knowledge of insulin antibody status to accurately quantify circulating insulin. Serial post-ICT antibodies were measured in 17 (of 19) ICT recipients 2-37 occasions over median (Interquartile range) 4.0 (1.0, 7.2) years follow-up. Mean values of results from every 100 days for each subject were used for trend analyses. The mean(range) age at screening for the ICT recipients was 52 (35-66) years, with a mean(range) diabetes duration of 36 (14-62) years.

Between group (non-T1D, T1D not referred for ICT, and T1D referred for ICT) differences were assessed using Kruskal-Wallis ANOVA with Games-Howell post-hoc tests. Specific binding level differences over time were analysed with repeated measures ANOVA using mixed models. Differences in antibody positive/negative status in groups were assessed using Pearson Chi-square test with simulates p-value (based on 2000 replicates). Trends in antibody positive/negative status over time were assessed using Cochrane-Armitage test. Significance was at $p<0.05$.

Insulin antibodies were positive in 5.3% of 2859 non-T1D subjects, in 42.7% of 103 T1D subjects not referred for ICT, in 63.0% of 46 T1D adults referred for ICT, and in 73.7% of 19

ICT recipients. Median (LQ, UQ) specific binding was higher in both T1D groups (not referred to ICT: 1.23(0.50, 4.43)% and referred to ICT: 4.43 (0.74, 9.96)%) vs. controls (0.41 (0.21, 0.69)%; both p<0.001) but did not differ between people with T1D referred vs. not referred to ICT (p=0.26). Of 46 ICT candidates, 19 received ≥1 ICT, which partially restored C-peptide (endogenous insulin) and mitigated glucose instability, with nine subjects no longer requiring exogenous insulin. Of these nine insulin independent subjects, pre-transplant seven were insulin antibody positive and two were negative, with antibody levels of 10.11 (7.70, 17.29)% vs. 0.89 (0.69, 1.10)%, p=0.06.

Figure 1 shows specific binding pre-and post-ICT. Specific binding decreased in 12 of 17 subjects with post-ICT data within 200-300 days post-transplant. Over time the percentage of patients with undetected antibodies increased from 17.6% pre-ICT (3/17) to 66.7% 3.8 years later (6/9), p for trend=0.002 (Panel A). Mean specific binding decreased over time from 10.7% to 3.2% (p<0.001) (Panel B). The frequency of antibody negativity in ICT recipients who achieved insulin independence increased over time (p for trend 0.001), whilst that in ICT recipients not achieving insulin independence was unchanged (p for trend=0.90) (Panel C). Antibody level changes over time did not differ in those who did vs. did not achieve insulin independence (p=0.94).

Discussion

Insulin antibodies are ≈8-times more common in people with T1D (not referred for ICT) vs. non-T1D subjects. This is consistent with prior research [7] and likely reflects both exposure to exogenous insulin and propensity to develop antibodies. Not unexpectedly some non-T1D subjects had insulin antibodies, which may relate to increased humoral immune responsiveness to endogenous insulin related to organ and non-organ specific autoimmunity or transient insulin treatment for e.g. "stress hyperglycaemia" [8]. Such antibodies have been identified in over one

third of Graves' disease or chronic hepatitis patients [8]. The antibodies analysed in our study are insulin-binding immunoglobulins to endogenous or exogenous insulin, which can develop to bovine, porcine, human or human analogue insulins [9, 10]. These differ from autoantibodies to insulin-producing cells, such as anti-GAD, -IA2 and -ZnT8, which are associated with propensity to develop T1D and often decline with increasing T1D duration.

Transplantation of matched cadaver donor islets is approved for clinical use in Australia for the very small subset of adults with T1D with good renal function and life-impacting recurrent severe hypoglycaemia, which cannot be resolved with other therapies, such as an insulin pump and/or continuous glucose monitoring [1, 3]. Insulin antibody positivity (but not levels) were higher in T1D patients receiving ICT relative to T1D subjects who were not ICT candidates (73.7% vs. 42.7%, p=0.01).

Post-ICT and with (corticosteroid-free) immunosuppression insulin antibody levels decreased over time and the frequency of negative antibody status increased (Figure 1). In ICT recipients antibody levels did not differ over time between those who did vs. did not gain insulin independence, but there was a significant trend in those who achieved insulin independence becoming antibody negative. This is concordant with other groups reporting that insulin antibody positivity is associated with lower rates of post-ICT insulin independence [11].

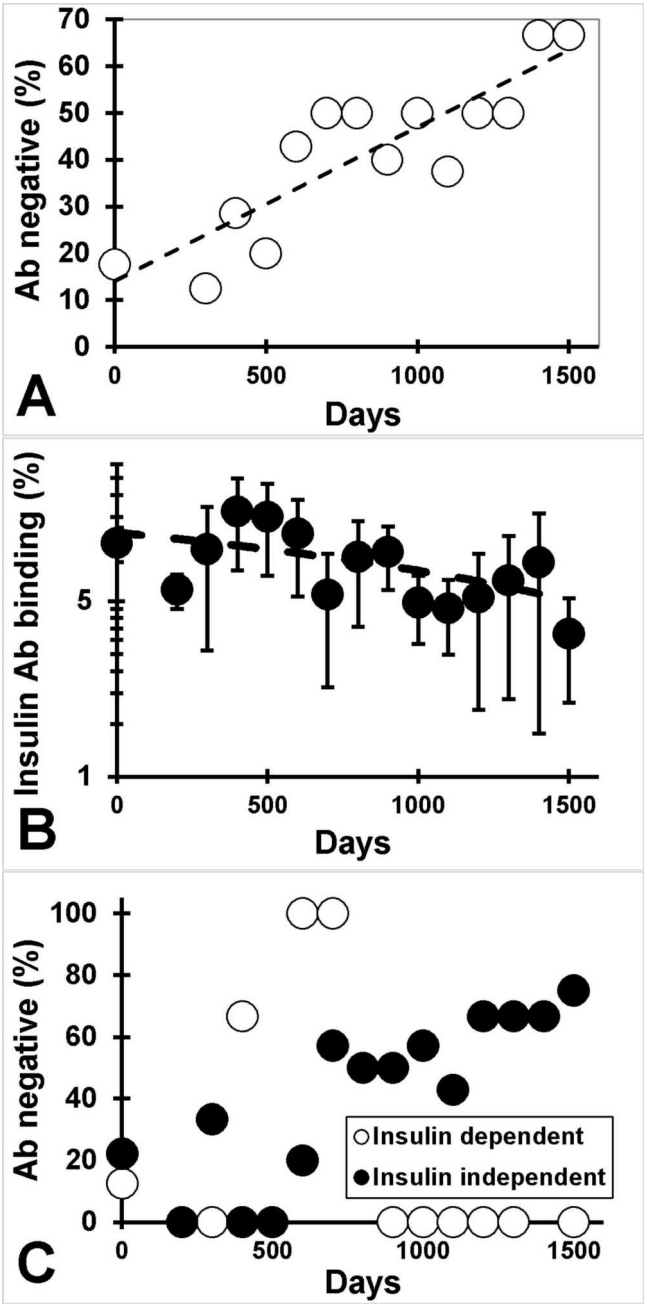
In summary, we report a higher rate of insulin antibody positivity in people with T1D than in non-T1D subjects and higher antibody positivity in T1D adults referred for ICT. ICT recipients who gained insulin independence were more likely to become antibody negative than those requiring exogenous insulin. Our study is strengthened by all measurements performed in the same laboratory with large numbers of non-T1D and non-ICT T1D subjects being assessed alongside adults with T1D enrolled in our tertiary referral centre-based ICT program. Study limitations include potential for selection bias and low subject numbers, although our study

size is similar to or larger to other reports. Our results support a role of insulin antibodies in the marked glucose lability of adults with T1D who may require ICT and that post-ICT insulin antibodies may decline. Larger studies or a meta-analysis of the usually small ICT studies regarding the significance of insulin antibodies in ICT or pancreas transplant patients are merited, ideally with comparator groups as reported herein.

References.

1. O'Connell PJ, Holmes-Walker DJ, Goodman D, Hawthorne WJ, Loudovaris T, Gunton JE, et al. Multicenter Australian trial of islet transplantation: improving accessibility and outcomes. Am J Transplant. 2013;13(7):1850-8.
2. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science. 1983;222(4630):1337-9.
3. Holmes-Walker DJ, Gunton JE, Hawthorne W, Payk M, Anderson P, Donath S, et al. Islet Transplantation Provides Superior Glycemic Control With Less Hypoglycemia Compared With Continuous Subcutaneous Insulin Infusion or Multiple Daily Insulin Injections. Transplantation. 2017;101(6):1268-75.
4. Shapiro AM, Pokrywczynska M, Ricordi C. Clinical pancreatic islet transplantation. Nat Rev Endocrinol. 2017;13(5):268-77.
5. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ. Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab. 1965;25(10):1375-84.
6. Albano JD, Ekins RP, Maritz G, Turner RC. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol (Copenh). 1972;70(3):487-509.
7. Van Haeften TW. Clinical significance of insulin antibodies in insulin-treated diabetic patients. Diabetes Care. 1989;12(9):641-8.
8. Van den Driessche A, Eenkhoorn V, Van Gaal L, De Block C. Type 1 diabetes and autoimmune polyglandular syndrome: a clinical review. Neth J Med. 2009;67(11):376-87.
9. Goldman J, Baldwin D, Rubenstein AH, Klink DD, Blackard WG, Fisher LK, et al. Characterization of circulating insulin and proinsulin-binding antibodies in autoimmune hypoglycemia. J Clin Invest. 1979;63(5):1050-9.
10. Shen Y, Song X, Ren Y. Insulin autoimmune syndrome induced by exogenous insulin injection: a four-case series. BMC Endocr Disord. 2019;19(1):148.
11. Lablanche S, Borot S, Thaunat O, Bayle F, Badet L, Morelon E, et al. Impact of anti-insulin antibodies on islet transplantation outcome: data from the GRAGIL Network. Transplantation. 2014;98(4):475-82.

Figure 1
Antibody over time in ICT recipients: A – antibody status, B – mean antibody level, C – antibody status in ICT recipients according to insulin independence (assessed post-ICT).



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