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Technical Note

Anti-BSA antibodies are a major cause of non-specific binding in insulin autoantibody radiobinding assays

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ABSTRACT

Insulin autoantibodies (IAA) are usually the first risk-markers detected during the type 1 diabetes prodrome, but precise measurement is difficult as insulin binding is often low. Non-specific binding (NSB) of ^{125}I-labelled insulin necessitates competitive displacement with unlabelled insulin to demonstrate specificity. NSB varies with different batches of label, suggesting that it is caused by impurities in the label. Addition of bovine serum albumin (BSA) can reduce NSB, so we investigated whether BSA antibodies cause lack of specificity in IAA assays.

Samples from patients with newly-diagnosed type 1 diabetes, healthy schoolchildren previously found to have raised ^{125}I-insulin binding (≥0.4 units) and IAA-negative schoolchildren were re-assayed for IAA by radiobinding microassay using commercial ^{125}I-insulin with and without 1 g/dl BSA added to the buffer.

Of 100 patients, 68 were IAA-positive on re-assay with BSA compared to 72 without BSA ($p = 0.125$). Of 154 schoolchildren who previously had raised ^{125}I-insulin binding, only 45 had ^{125}I-insulin binding ≥0.4 units on re-assay with BSA compared to 90 without BSA ($p < 0.001$). Following competitive displacement with unlabelled insulin, 40 were IAA-positive with BSA compared to 48 without BSA ($p = 0.02$). No IAA-negative schoolchildren were IAA-positive on re-assay. Levels of NSB were associated with antibodies binding ^{125}I-BSA and purification of labelled insulin reduced NSB.

Addition of BSA to assay buffer improves the screening efficiency of the IAA assay without reducing disease sensitivity in patients. High titre BSA antibodies interfere with IAA measurement because of ^{125}I-BSA present in some insulin labels. Improved purification of insulin labels should obviate the need for competitive displacement.

1. Introduction

Insulin autoantibodies (IAA) are very useful for identifying children likely to progress to type 1 diabetes (Palmer et al., 1983). Unfortunately, the majority of IAA associated with type 1 diabetes show low levels of insulin binding, which makes measurement difficult. The microassay using ^{125}I-labelled insulin is currently the method of choice for measuring IAA (Williams et al., 1997; Naserke et al., 1998; Yu et al., 2000) and competitive displacement with excess unlabelled insulin is used routinely in order to demonstrate specificity to insulin (Vardi et al., 1987). This displacement step increases the cost and complexity of the assay, but has proved necessary because many sera which bind ^{125}I-labelled insulin do not show specificity to insulin (Hegewald et al., 1992; Williams et al., 1997). Most laboratories assay all samples with competitive displacement, but to economize our laboratory screens all samples for ^{125}I-insulin binding and only competes those samples with raised ^{125}I-insulin binding.

The level of non-insulin specific binding varies with different batches of label, suggesting that it is caused by an

Abbreviations: NSB, non-specific binding; BSA, bovine serum albumin; IAA, insulin autoantibodies;SNSC, screen-negative schoolchildren; SPSC, screen-positive schoolchildren.

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impurity in the label. Further, some IAA microassays add 1 g/dl bovine serum albumin (BSA) to the label to reduce NSB (Yu et al., 2000) and manufacturers may also add BSA directly to the label (Perkin Elmer LAS, Beaconsfield, Bucks UK). We have therefore investigated whether adding BSA to the buffer can reduce this non-insulin specific binding and tried to determine the nature of the label impurity.

2. Materials and methods

2.1. Samples

Sera were available from subjects living in the Oxford region of the UK which had previously been assayed for IAA using ^{125}I-insulin from Amersham Biosciences (Bingley et al., 1997, 1999). These samples (Fig. 1) were collected from (1) 100 children and adolescents with newly-diagnosed type 1 diabetes (median age 10.1 years, range 1.3 to 20.5 years) with a disease duration of less than 2 weeks (median 0 days, range −9 to 10 days) of whom 70 had previously been found IAA-positive following competitive displacement, (2) 154 of 156 healthy schoolchildren (median age 11.5 years, range 9.1 to 13.6 years) who had previously shown increased binding (≥0.4 units) of ^{125}I-labelled insulin prior to competitive displacement (screen-positive) and of whom 71 were subsequently found IAA-positive following competitive displacement, (3) 154 of 2704 schoolchildren (median age 11.4 years, range 9.0 to 13.7 years) who had previously shown low levels of binding (<0.4 units) of ^{125}I-labelled insulin (screen-negative) and were therefore classified IAA-negative. Sera from 5 first-degree relatives of patients with type 1 diabetes and 3 healthy adult controls were also used for anti-BSA antibody displacement analysis. The study was approved by the Local Research Ethics Committees.

2.2. Insulin autoantibodies

Insulin autoantibodies were measured on 5 μl serum using a radiobinding microassay with ^{125}I A-14 monoiodinated human insulin (Perkin Elmer LAS) as previously described (Williams et al., 1997). Immunocomplexes were precipitated with protein A sepharose (GE Healthcare, Little Chalfont, Bucks UK). Results were calculated using a standard curve constructed from dilutions of an insulin antibody positive sample in normal human serum. The assay involved a screening step in which samples were assayed for ^{125}I-insulin binding and all those with levels ≥0.4 units were re-assayed with competitive displacement using 4×10^{-5} mol/l unlabelled human insulin (Actrapid, Novo Nordisk, Bagsvegaerd, Denmark). Samples with IAA ≥0.2 units after competition (representing the 97.5th percentile of the 2860 schoolchildren) were classified as positive (Williams et al., 1997). The inter-assay CV was 21% at 0.67 units. Our laboratory achieved a laboratory defined sensitivity of 42% at 98.9% specificity in the 2009 Diabetes Autoantibody Standardization Program Workshop (Schlosser et al., in press).

2.3. Strategy

Samples from all patients, screen-positive schoolchildren (SPSC) and screen-negative (SNSC) schoolchildren were re-screened for ^{125}I-insulin binding and those with levels ≥0.4 units were re-assayed with competitive displacement (Fig. 1). Samples had previously been assayed without BSA added to the buffer (Bingley et al., 1997), but re-assay was performed with and without 1 g/dl BSA (Sigma, Poole, Dorset, UK). To determine whether the reduced number of schoolchildren screening positive following addition of 1 g/dl BSA was specific to BSA we compared the effect on ^{125}I-insulin binding of adding 1 g/dl BSA or 1 g/dl ovalbumin (Sigma) to

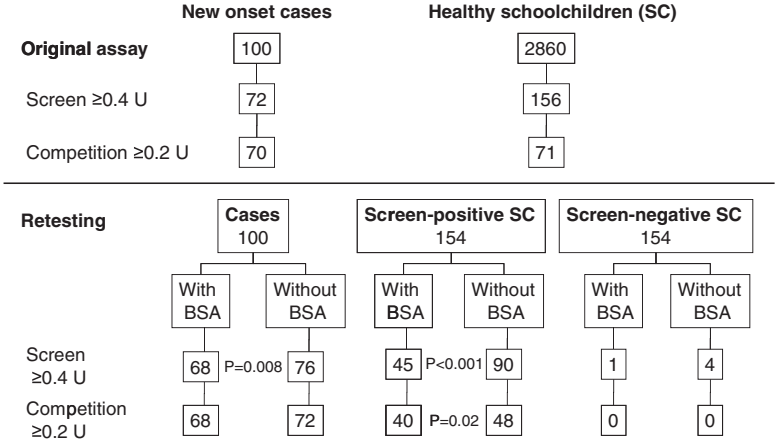

Fig. 1. Diagram showing the strategy adopted and the result of adding 1 g/dl BSA to IAA assay buffer on non-specific binding of ^{125}I-insulin in the IAA microassay. Samples from 100 patients with new-onset type 1 diabetes, 154 of 156 screen-positive schoolchildren (SPSC) and 154 of 2704 screen-negative (SNSC) schoolchildren previously assayed for IAA were re-tested with and without 0.1 g/dl BSA in the assay buffer. The assay included a screening step; only those samples with ^{125}I-insulin binding ≥0.4 units were assayed with competitive displacement. Samples with IAA ≥0.2 units after competition were considered positive. A similar number of patients and schoolchildren were found IAA-positive (≥0.2 units) with and without 1 g/dl BSA, but half as many SPSC re-screened positive in the presence of BSA ($p<0.001$).