Circulating Immune Complexes in the Serum of Diabetes Mellitus in Childhood by a Modified $^{125}$I-C₁q Binding Test

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Synopsis

The $^{125}$I-C₁q binding test for the detection of soluble immune complexes in native unheated human serum was applied to the study of sera from 52 patients with diabetes mellitus in childhood. This radiolabeled C₁q binding test is more sensitive and reproducible among the various methods proposed for the detection of immune complexes.

The $^{125}$I-C₁q binding activity in 52 sera from diabetes mellitus in childhood was 9.47±0.36% compared to 6.94±0.74% in normal controls. $^{125}$I-C₁q binding values in diabetes mellitus in childhood were significantly higher than normal controls. Slight high values were seen in 3 patients with positive anti-DNA-antibodies in diabetes mellitus in childhood.

$^{125}$I-C₁q binding was not significantly increased in patients with positive antithyroid antibodies and insulin antibodies. There was no significant correlation between the duration of diabetes and $^{125}$I-C₁q binding activity.

Recent investigations suggest that autoimmune processes may be responsible for the pathogenesis in insulin-dependent diabetics (IDD) (Nerup et al., 1976; Irvine et al., 1977).

The clinical association between IDD and autoimmune diseases and a high rate of occurrence of organ-specific antibodies in IDD have already been reported (Irvine et al., 1970; Nerup et al., 1973). Antibodies to pancreatic islet cells in IDD have been detected by using an indirect immunofluorescence technique (Irvine et al., 1977; Lendrum et al., 1975; Bottazzo et al., 1974). The fact that this antibody was a complement fixing and of IgG class was demonstrated by Bottazzo (1974) and Lendrum (1975).

Various methods have been used for the detection of soluble immune complexes in serum. For instance, biochemical methods such as chromatography and ultracentrifugation in sucrose density gradients were used for the detection. The radiolabeled C₁q has been known to react with sera containing immune complexes. This new method could be more sensitive and reproducible than previous methods (Creighton et al., 1973). This C₁q binding test has been applied to the study of sera from patients with SLE or to the carriers of the hepatitis B antigen (Nydegger et al., 1974).

The purpose of the present study was to detect the immune complexes in the native unheated serum of diabetes mellitus in childhood by a modified $^{125}$I-C₁q binding test which was reported by Zubler (1976) and to present evidence in support of the concept of immune complex diseases in some of those patients.
Materials and Methods

Patients studied
The patients were insulin-dependent diabetics who were attending Kinki region-summer camp of diabetic children in 1975 and 1976. They were 29 girls aged 3 to 15 years and 23 boys aged 8 to 14 years. 96 males and 176 females were chosen as normal controls. The age and sex matched control group consisted of 13 healthy children (5 boys and 8 girls) aged 0 to 15 years. These sera have been stored at -70°C.

Measurement of immune complexes
C₁q was purified by a rapid purification method for the preparation of monospecific antisera (Yonemasu et al., 1971).

The purification of C₁q was ascertained by the immunoelectrophoresis and disc electrophoresis, and latex agglutination test. Radioiodination of C₁q was performed by lactoperoxidase method of Heusser (1973) and was stored at -70°C. The radiolabeled C₁q was diluted in 5 to 8 ml of Veronal Buffered Saline containing 1% bovine serum albumin and centrifuged at 18000 g for 40 min at 4°C to discard aggregates of C₁q before use (Tasaka et al., 1978).

The ¹²⁵I-C₁q binding test was performed according to the modified method by Zubler (1976). First, 50 μl of serum sample was mixed with 0.2 M EDTA (pH 7.5) 100 μl and incubated for 30 min at 37°C. The next step, 50 μl of ¹²⁵I-C₁q and 3% polyethylene glycol (PEG) 1000 μl were added. The mixture was stored for 60 min at 0°C and then centrifuged at 1500 g for 20 min at 4°C.

The supernates were discarded and radioactivity in the precipitates was measured by a gamma counter. Results were expressed as percent of ¹²⁵I-C₁q precipitated as compared with the radioactivity precipitated in a trichloroacetic acid “TCA control” tube in which 1 ml 15% TCA was added to 150 μl of normal human serum mixed with 50 μl of ¹²⁵I-C₁q. All tests were done in duplicate.

Autoantibodies
Circulating antibodies to thyroglobulin and to microsome of thyroid epithelial cells were determined by the tanned red cell hemagglutination method, using a commercially prepared reagents (Thyroid test, Microsome test-Fuji Zoki Co. Tokyo). Antithyroglobulin and antimicrosomal antibody titres were regarded as positive if hemagglutination occurred in 1:160 dilution of sera. For titration v-shaped wells of plastic agglutination trays (Cooke Instruments) were used because the settling pattern of the cells in the cup could be observed from the bottom. Anti-DNA-antibodies were tested with Anti-DNA kits from The Radio Chemical Center-Kaken Kagaku Co. Tokyo.

Insulin-antibody was detected by a slight modification of the method of Wright (1966). Insulin-antibody titres were regarded as positive when B corrected/F values were higher than 0.0375.

![Fig. 1. ¹²⁵I-C₁q binding test in normal controls.](image1)

![Fig. 2. ¹²⁵I-C₁q binding test in normal controls and diabetes mellitus in childhood.](image2)
Results

This modified $^{125}$I-C1q binding test was applied to the study of normal controls consisting of 96 males and 176 females in each 10-year age group over 1 to 100 years. In females, $^{125}$I-C1q binding activity ($^{125}$I-C1q BA) showed a progressive increase to the age of 40 years and a decrease over the age of 40 years. In males, maximum values of $^{125}$I-C1q BA were found in the latter half of 20 years and 60 years. The $^{125}$I-C1q BA in 96 males was 9.21 ± 0.54 compared to 10.15 ± 0.48 in 176 females. (Fig. 1)

52 insulin-dependent diabetes mellitus in childhood showed significantly higher values when compared to the values obtained from 13 normal controls (0-15 years old). The mean value ($\pm$ SE) for the diabetics group was $9.47 \pm 0.36\%$ $^{125}$I-C1q BA. The mean $\pm$ SE of the normal controls was $6.94 \pm 0.74\%$. (Fig. 2)

3 patients with positive anti-DNA-antibodies in diabetes mellitus in childhood showed 8.56, 10.8, 11.6% $^{125}$I-C1q BA. (Fig. 3)

No significantly increased $^{125}$I-C1q binding activity was seen in patients with positive antithyroid antibodies and insulin antibodies. There was no significant difference of $^{125}$I-C1q precipitation between diabetics with antithyroid antibodies and those without antithyroid antibodies. (Fig. 4) $^{125}$I-C1q BA in diabetics with antiinsulin antibodies and those without antiinsulin antibodies was much the same. (Fig. 5)

Table 1 illustrates $^{125}$I-C1q BA in diabetics according to the duration of diabetes. There was no significant correlation between the duration of diabetes and $^{125}$I-C1q BA.
Discussion

Although the pathogenesis and etiology of diabetes mellitus remain undetermined, evidence accumulated in recent years suggest that an autoimmune mechanism is responsible for insulin-dependent diabetics (Nerup et al., 1976; Irvine et al., 1977). Increased clinical association of diabetes mellitus with Hashimoto's thyroiditis, Addison's disease and pernicious anemia, and increased frequencies of organ-specific antibodies such as thyroid, gastric parietal cells support that autoimmune mechanism is in pathogenesis of insulin-dependent diabetics.

Antibodies to pancreatic islet cells were found to be of complementary fixing and of IgG class using an indirect immunofluorescence method (Bottazzo et al., 1974; Lendrum et al., 1975).

The detection of circulating immune complexes has been performed by a great variety of methods such as chromatographic separation, ultracentrifugation based on biochemical methods, platelet aggregation test, Raji cell method, ADCC inhibition test and $^{125}$I-C$_{1q}$ binding test. The advantage of this $^{125}$I-C$_{1q}$ binding test is that it is possible to treat about three hundred specimen in duplicate in half a day by using a small amount of C$_{1q}$. On the contrary, the disadvantage is that it is necessary to use a considerable accomodation of treating radioisotope and the activity of C$_{1q}$ has a tendency to diminish. As heparinized blood showed extremely high values of $^{125}$C-C$_{1q}$ BA, we excluded heparinized specimen. The condition of preservation of serum samples is very important. Only fresh and stored samples at $-70^\circ$C must be used in this test. And heat-inactivated serum has been found to reduce the C$_{1q}$ binding activity of immune complexes mixed with native serum. So we mixed 50 $\mu$l of serum sample with 0.2 M EDTA (pH 7.5) 100 $\mu$l in order to prevent the
integration of $^{125}$I-$C_{1q}$ into the intrinsic $C_{1q}$ complex by Zubler's modified method. PEG was used at a lower concentration (final concentration 2.5%) in order to precipitate only the soluble antigen-antibody complexes. Using an aggregated human-globulins (agg HGG), we have already reported that agg HGG between the concentration of 0.5 $\gamma$/ml and that of 3 mg/ml were detectable for positive controls and the $C_{1q}$ BA of the agg HGG increased nearly linearly (Tasaka et al., 1978). And in 44 patients with SLE, $^{125}$I-$C_{1q}$ BA correlated with the titres of antinuclear factors, positive LE test and in nephrotic syndrome (membranous type), $C_{1q}$ BA showed high values. $C_{1q}$ BA in normal females between 30 and 49 years was significantly different from that in normal age matched males ($p<0.01$). This difference is very important, compared with the fact that various autoimmune diseases are found frequently in females aged 30 to 50 years. Using a Raji cell assay, Irvine et al. (1977) revealed significant prevalence of soluble immune complexes in newly diagnosed IDD with positive islet cell antibody. And they have already clarified that islet cell antibody was most common in newly diagnosed, IDD of recent onset and the prevalence of humoral islet cell antibody was strongly dependent on the duration of the diabetics. In our experiments, significantly higher values of $^{125}$I-$C_{1q}$ BA were also found compared to normal aged matched control group, and, no significant correlation between the duration of diabetes and $^{125}$I-$C_{1q}$ BA was found. The reason for this discrepancy is not clear, but various factors including procedures for detecting immune complexes might be responsible.

References