

## Superiority of Radiobinding Assay Over ELISA for Detection of IAAs in Newly Diagnosed Type I Diabetic Children

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**Objective:** Liquid- or solid-phase assays have been used for insulin autoantibody (IAA) determination, and the method of IAA measurement has not been standardized. **Research Design and Methods:** IAAs were determined by radiobinding assay (RBA) and enzyme-linked immunosorbent assay (ELISA) in two large age-matched groups of nondiabetic and newly diagnosed insulin-dependent (type I) diabetic children. **Results:** Positivity for IAA by RBA ( $\geq$  nondiabetic mean + 3SD) was 2 of 178 (1.1%) and 55 of 173 (32%) in nondiabetic and diabetic children, respectively. Prevalence of IAA by RBA was significantly higher in the youngest age-group (63% between 0–4 yr). Positivity for IAA by ELISA was 1 of 178 (0.6%) and 8 of 169 (4.7%) in nondiabetic and diabetic children, respectively. Concordance rates between both assays were 0 of 3 (0%) in control subjects and 5 of 58 (8.6%) in diabetic children. **Conclusions:** We conclude that RBA is more appropriate than ELISA for IAA detection at the onset of the disease. In addition, because available data suggest that IAAs detected by RBA only are high-affinity antibodies, it is tempting to speculate that IAAs reflect a mature immune reaction against endogenous insulin. *Diabetes Care* 14:61–63, 1991

international workshops did not reach a consensus or provide an explanation for the variations observed in results by both methods (3). The purpose of this study was to compare the results obtained for IAA by RBA and ELISA in two large groups of nondiabetic and newly diagnosed type I diabetic children. Consequently, the aim was also to select the more appropriate assay for IAA detection at the onset of the disease.

Newly diagnosed type I diabetic children <20 yr of age were recruited from the incidence study organized in four regions of France (4). Three hundred nine new type I diabetic cases were identified between 1 January 1988 and 30 November 1989. A serum sample was obtained from 283 children; 173 of 283 (56%) sera, drawn before or within 48 h after initiation of insulin therapy, were used for the 2 IAA assays.

Sera from nondiabetic children were obtained from the Regional Institute of Health in Tours, France. In 1989, 178 sera were randomly and anonymously collected from age-matched children attending a free health checkup.

### RESEARCH DESIGN AND METHODS

Serum extraction was performed as described by Srikantha et al. (5). Insulin binding was measured by incubating a constant amount ( $\sim 20,000$  counts  $\cdot$  min $^{-1}$   $\cdot$  tube $^{-1}$ ) of human A14-Tyr- $^{125}$ I-labeled insulin (sp act 350  $\mu$ Ci/ $\mu$ g) with 80  $\mu$ l of each serum extract for 48 h

**I**nulin autoantibodies (IAAs) in the sera of newly diagnosed insulin-dependent (type I) diabetic patients can be detected either by liquid-phase assay (radiobinding assay; RBA; 1) or solid-phase assay (enzyme-linked immunosorbent assay; ELISA; 2). Two

at 4°C. The bound insulin fraction was then precipitated and separated according to the method of Palmer et al. (1). Nonspecific binding was simultaneously determined on each sample by addition of an excess of human cold insulin ( $7.1 \times 10^{-5}$  M). Results are expressed as percentage of insulin binding. Interassay precision was 16 and 10% for an insulin binding at 2.3 and 9.9%, respectively. Intra-assay precision was 6 and 2% for levels at 23 and 39%, respectively.

ELISA of insulin-IgG was performed as previously described (6) with human insulin-coated microplates and a mouse monoclonal anti-human IgG. A rabbit anti-mouse Ig serum, conjugated to peroxidase, was used to bind preformed immune complexes. To measure nonspecific binding, 1:5 diluted serum samples were preincubated overnight at 4°C with human insulin ( $7 \times 10^{-4}$  M) and were thereafter similarly processed. Interassay precision was 34 and 14.5% at the level of 0.2 and 1.0 optical density (OD), respectively. Intra-assay precision was 31 and 13.4% at the level of 0.2 and 1.0 OD units, respectively. Results are means  $\pm$  1SD. For both methods, threshold of positivity was defined as the mean of control + 3SD in the direct assay, together with a percentage of inhibition in the competitive assay  $\geq$  mean percentage of control + 3SD.

## RESULTS

In nondiabetic children ( $n = 178$ ), the mean percentage of binding was  $2.08 \pm 0.27\%$  in the direct assay, and the percentage of inhibition was  $0.53 \pm 0.20\%$  in the competitive assay. There was no variation related to age. Frequencies of IAA<sup>+</sup> sera in both nondiabetic and newly diabetic children are shown in Table 1. Two of

**TABLE 1**  
Results for insulin autoantibody (IAA) measured by radiobinding assay (RBA) and enzyme-linked immunosorbent assay (ELISA) in children

	Age (yr)				
	0-4	5-9	10-14	15-19	0-19
Nondiabetic					
<i>n</i>	26	51	54	47	178
IAA <sup>+</sup>					
RBA	1	0	1	0	2 (1.1)
ELISA	0	0	1	0	1 (0.6)
Diabetic					
<i>n</i>	19	45	67	42	173
IAA <sup>+</sup>					
RBA	12 (63)	17 (38)	16 (24)	10 (24)	55 (32)
<i>n</i>	18	45	66	40	169
ELISA	1 (5.5)	2 (4.5)	1 (1.5)	4 (10)	8 (4.7)

Numbers in parentheses are percentages of children testing IAA<sup>+</sup> by test indicated. Frequency of IAA<sup>+</sup> sera were significantly related to age by RBA ( $P < 0.01$ ) but not by ELISA in newly diagnosed insulin-dependent diabetic children.

178 children, aged 1 and 13 yr, respectively, were found to be IAA<sup>+</sup>.

In nondiabetic children, mean OD was  $0.090 \pm 0.109$  in the direct assay, and the magnitude of inhibition was  $0.006 \pm 0.105$  in the competitive assay. Frequencies of IAA<sup>+</sup> sera in both groups of children are reported in Table 1. One 12-yr-old control child was positive, and IAAs of that child were human insulin specific. Among the three diabetic children positive by ELISA only, two had non-species-specific IAA, and one had human insulin-specific IAA.

Five of 169 (2.9%) sera of diabetic children were positive by both assays, and via ELISA, IAAs of these children proved to be non-species specific. None of the control sera were positive by both assays. The overall concordance rates of the two assays were 5 of 58 (8.6%) in type I diabetic children and 0 of 3 in the control population.

## CONCLUSIONS

This study demonstrates that positivity for IAA in newly diagnosed type I diabetic children varies with the assay method used. The frequency of RBA<sup>+</sup> sera was in agreement with previous reports (1,7), although the lack of definite standardization of IAA measurement makes the comparison difficult. Positivity for IAA by ELISA has been poorly documented in children at onset of diabetes. Wilkin et al. (2) identified 38% of such patients above the normal range of 100 healthy adults. In our study, the specificity of the two techniques was ensured by a competitive assay. Moreover, the criteria for positivity were precisely defined, because a large group of age-matched randomly selected children was taken as nondiabetic control subjects. The higher prevalence of IAA by RBA in children  $< 5$  yr of age has already been reported (7). We observed opposite, although not significant, findings for IAA positivity by ELISA. Concordance rates of the results between RBA and ELISA were very low in both control and diabetic groups, suggesting that the two assays are quite different. It has been postulated that the amount of antibody measured in a given serum can vary markedly depending on the epitopes made available to each detection method (8). Another difference may stem from the fact that insulin iodination may alter its affinity for antibody binding in the liquid-phase assay (9). Therefore, RBA and ELISA probably detect antibodies under fundamentally different thermodynamic conditions. In RBA, the antigen-antibody interaction follows the law of mass action, and the concentration of radioligand is extremely low. Thus, RBA depends strongly on the affinity of the antibody. By contrast, in ELISA, an excess of coated insulin promotes antibody binding, thereby minimizing the role of antibody affinity. Recent studies with monoclonal antibodies against insulin and proinsulin support the contention that ELISA detects antibodies on a large window of affinities (capacity assay), whereas RBA is highly sensitive

to affinity and preferentially detects high-affinity antibodies.

In our diabetic population, most IAA<sup>+</sup> sera were identified by RBA, suggesting that these IAAs are of high affinity. As such, they might be the witness of a mature process directed against endogenous insulin. In each of the two groups of children, there was an ELISA<sup>+</sup> serum containing low-affinity human insulin-specific IAAs, similar to the clonally-restricted antibodies found in the nondiabetic population at the frequency of 1% (10).

In conclusion, RBA and ELISA yield discrepant results for IAA detection in newly diagnosed type I diabetic children. In terms of IAA prevalence and age-related positivity, RBA definitely appears to be more appropriate than ELISA for IAA detection.

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Received for publication 16 April 1990 and accepted in revised form 27 July 1990.

#### ACKNOWLEDGMENTS

This work was supported by grants from the Fonds de la Recherche Scientifique Médicale (Brussels), Caisse Nationale d'Assurance Maladie-Institut National de la Santé et de la Recherche Médicale (998-197, Paris), and Novo Nordisk (Copenhagen).

We are grateful to Drs. C. de Beaufort, J. Doutreix, V. Froment, and J. Voirin for dedicated and skillful assistance.

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## Serum Levels of Tumor Necrosis Factor and IL-1 $\alpha$ and IL-1 $\beta$ in Diabetic Patients

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**Objective:** To determine whether chronic hyperglycemia causes increased levels of serum tumor necrosis factor (TNF) and interleukin 1 $\alpha$  (IL-1 $\alpha$ ) and IL-1 $\beta$ . **Research Design and Methods:** Sera were obtained from 59 diabetic patients, 44 chronically ill nondiabetic patients, and 34 age-matched healthy control subjects. Mononuclear cells were isolated from a subgroup of diabetic patients and healthy control subjects. **Results:** Except for a modest increase in the prevalence of detectable serum TNF levels in diabetic patients, the serum cytokines measured in this study did not appear to be altered in diabetes. **In vitro** TNF production by mononuclear cells was not altered in diabetic patients. **However, in vitro** IL-1 $\beta$  secretion, in response to lipopolysaccharides, was reduced. **Conclusions:** Diabetes mellitus is not associated with significant

changes in serum levels of TNF, IL-1 $\alpha$ , or IL-1 $\beta$ . **In vitro** secretion of IL-1 $\beta$  in response to lipopolysaccharides may be reduced in diabetes. *Diabetes Care* 14:63-65, 1991

Various biological sequelae have been attributed to the advanced glycosylation end products (AGE) of various proteins (1,2). Of particular interest are the recent *in vitro* studies that have shown that AGE attached to proteins can specifically stimulate monocyte production of tumor necrosis factor (TNF) and interleukin 1 $\beta$  (IL-1 $\beta$ ; 3). This observation