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Immunology Letters 49 (1996) 123–126

immunology letters

Islet cell and glutamic acid decarboxylase antibodies and heat-shock protein 65 responses in children with newly diagnosed insulin-dependent diabetes mellitus

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Received 27 May 1995; revised 12 December 1995; accepted 28 December 1995

Abstract

Islet cell antibodies (ICA) were detected in 66% and glutamic acid decarboxylase (GAD) antibodies in 64% of children (n = 47) with newly diagnosed insulin-dependent diabetes mellitus (IDDM). Fifteen percent of the patients had neither GAD nor ICA antibodies. Responses to mycobacterial heat-shock protein 65 (Hsp65) were detected in all patients. There was a significant correlation between anti-GAD antibodies and proliferation of peripheral blood mononuclear cells to Hsp65, and between ICA and antibodies to Hsp65.

Keywords: IDDM; ICA; Anti-GAD; Hsp65 responses

1. Introduction

Antibodies against pancreatic islet-associated antigens are frequently detected in patients with IDDM of recent onset. These include. 'classical' islet cell antibodies (ICA) as well as, e.g., antibodies against glutamic acid decarboxylase (GAD) and autologous insulin [1–6]. Antibodies against other antigens such as heat-shock protein 65 (Hsp65) and bovine serum albumin (BSA) may also be detected [7–9]. Patients' blood T cells and T cell clones derived from blood T cells have been shown to proliferate, e.g., in response to insulin and GAD [10–12]. We tested the relationship between islet cell, GAD, and Hsp65 antibodies as well as proliferation to Hsp65 and BSA in children with newly diagnosed insulin-dependent diabetes and control children. Anti-GAD antibodies were found to correlate with proliferation to Hsp65, and ICA with antibodies to Hsp65.

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SSDI 0165-2478(95)02493-5

2. Materials and methods

2.1. Patients and controls

Samples were taken from 47 children (mean age 8.5 ± 0.6 years, range 1.0–15.8 years) with newly diagnosed IDDM as soon as the diabetes was well stabilized (within 2 weeks of diagnosis). The controls were 22 children (mean age 11.0 ± 0.9 years, range 2.1–16.9 years) undergoing minor elective surgery. Informed consent was obtained from the patients and/or their parents and the study was approved by the ethical committee of the hospital.

2.2. Peripheral blood mononuclear cells and sera

Peripheral blood mononuclear cells (PBMC) were obtained as previously described [13], aliquoted and stored in liquid nitrogen until used. Sera were obtained at the same time as PBMC, aliquoted and stored at −20 until tested.

2.3. Antigens

E. coli clone with the full-length human GAD 65 cDNA was a kind gift from Dr. Allan E. Karlsen and Catherine E. Grubin, University of Washington, Seattle, USA [14]. Heat-shock protein 65 K of *Mycobacterium bovis* (Hsp65, batch MA-10B) was a kind gift from Prof. Marc Feldmann, London, UK. Bovine serum albumin (BSA, Sigma Chemical Company, St. Louis, MO, USA), purified protein derivative of Bacillus Calmette-Guerain (Tuberculin, PPD, Statens Serum Institut, Denmark) and staphylococcal enterotoxin B (SEB, Sigma) were also used.

2.4. Proliferation assays

Proliferative responses by PBMC were tested as described previously [10,13]. The antigen concentrations used were: BSA, 1 and 0.1%/well; PPD, 10 and 1 μg/well; Hsp65, 3 μg/well; and SEB, 1 μg/well. ^{3}H-Thymidine (0.5 μCi/well) was added after 72 or 96 h of culture. Six hours later the cells were harvested. Cellular proliferation was expressed as the stimulation index (SI, mean cpm incorporated in the presence of antigen divided by mean cpm incorporated in cultures with medium alone).

2.5. Antibody assays

GAD65-Ab were determined by a radiobinding assay (RBA) described by Grubin et al. [15] and modified by Falorni et al. [16]. Briefly, ^{35}S-labelled recombinant human GAD65 or rat GAD67, produced by in vitro transcription/translation, was incubated with serum diluted 1:25. Immunocomplexes were precipitated with Protein A-sepharose (Pharmacia, Uppsala, Sweden) and counted for radioactivity in a liquid scintillation analyzer. A strongly positive reference serum was included on each plate along with three negative reference controls. The results were expressed as relative units (RU), i.e., the ratio of (sample cpm − mean cpm of three negative controls)/(reference serum cpm − mean cpm of three negative controls) × 100. Using the cut-off value of ≥ 6 RU/ml the assay had a sensitivity of 79.5% and specificity of 100% when the samples distributed by the Second Glutamic Acid Antibody Workshop (Chantilly, France, 1994) were analyzed. Islet cell (ICA) and Hsp65 antibodies were assayed as previously described [17,18].

2.6. Statistical analysis

The results are given as mean ± SEM. Mann-Whitney *U*-test was used to test the significance of difference between the groups. For correlations Spearman's rank test was used.

3. Results

3.1. Proliferation

The SIs induced by Hsp65 in diabetics and controls were 4.0 ± 1.7 and 4.7 ± 1.2 ($P < 0.001$), those by BSA 9.1 ± 1.8 and 8.8 ± 1.7 (P = ns), PPD 7.6 ± 2.0 and 12.2 ± 3.2 ($P < 0.05$) and SEB 105.5 ± 16.1 and 80.4 ± 12.3 (P = ns). SIs to Hsp65 in patients and controls are shown in Fig. 1.

3.2. Antibodies

ICA in titres ≥ 2.5 JDFU/ml were detected in 66% (31/47) and anti-GAD antibodies at levels ≥ 6 RU/ml in 64% (30/47) of the patients. The controls did not have ICA or anti-GAD antibodies. Ten of the 17 patients (59%) without GAD antibodies had ICA, and nine of the 16 patients (56%) without ICA had GAD antibodies. Seven patients (15%) had neither anti-GAD nor ICA. Hsp65 antibodies were detected in all patients and controls with mean levels of 71.7 ± 13.0 and 65.3 ± 11.6 U/ml (P = ns).

3.3. Correlation between antibodies and proliferation

There was a significant correlation between anti-GAD and islet cell antibodies ($P < 0.01$, Fig. 2), anti-GAD antibodies and proliferation to Hsp65 ($P < 0.01$, Fig. 3) as well as ICA (JDFU/ml) and Hsp65 antibodies ($P < 0.05$; Fig. 4). In diabetics but not in controls there was a correlation between proliferation in response to Hsp65 and PPD ($P < 0.001$) and

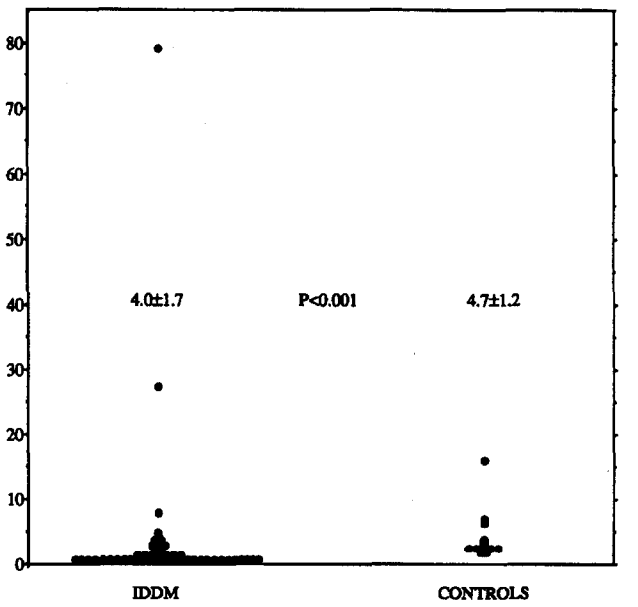

Fig. 1. Stimulation indices (SI) induced by Hsp65 in diabetics and controls.

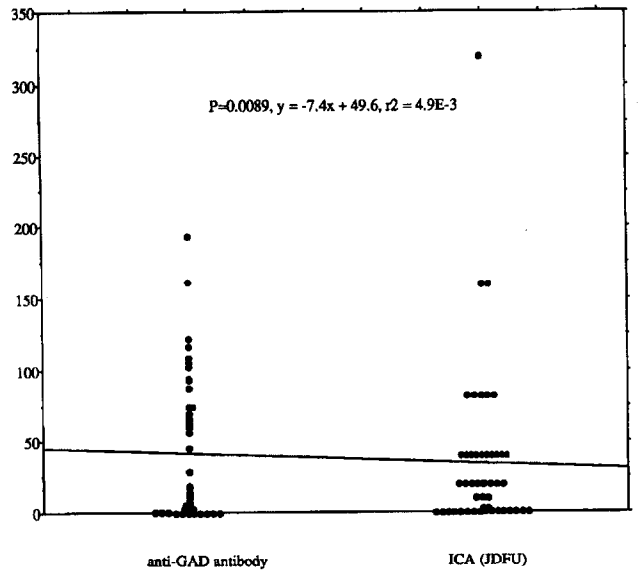

Fig. 2. Correlation between anti-GAD and islet cell antibodies (ICA) in patients with insulin-dependent diabetes mellitus.

proliferation to Hsp65 and BSA ($P < 0.001$). There was no correlation between Hsp65 proliferation and Hsp65 antibodies.

4. Discussion

In this study islet cell and/or GAD antibodies were detected in 85% of the children with newly diagnosed insulin-dependent diabetes. One third of the patients, however, tested negative for either islet cell or GAD antibodies. Half of the children without ICA had anti-GAD antibodies, and half of the children without anti-GAD had ICA. Other reports also suggest that ICA and GAD antibodies may not always overlap [4,15,19].

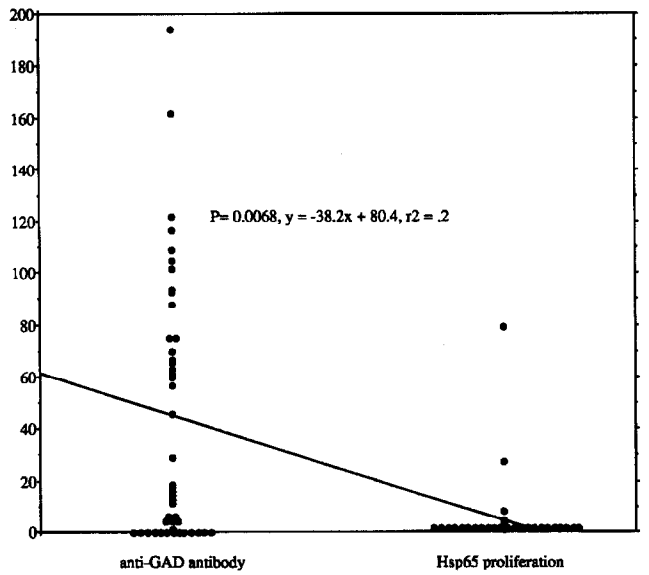

Fig. 3. Correlation between anti-GAD antibodies and proliferative responses to Hsp65 in patients with insulin-dependent mellitus.

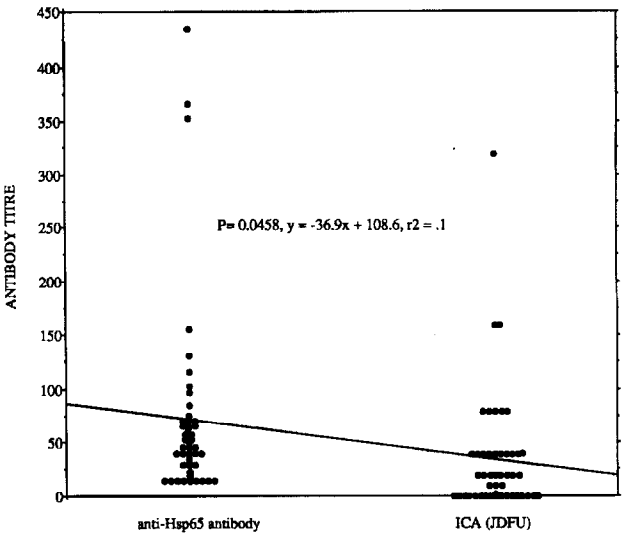

Fig. 4. Correlation between anti-Hsp65 and islet cell antibodies (ICA) in patients with insulin-dependent mellitus.

Similar levels of antibodies against mycobacterial Hsp65 were detected in diabetic and control children. This may be due to the vaccination of all newborns in Finland against tuberculosis using attenuated Bacillus Calmette-Guerain (BCG) vaccine thought to contain Hsp65 [20]. However, proliferative responses against mycobacterial Hsp65 as well as PPD, also contained in the BCG vaccine, were significantly lower in diabetics. As responses against BSA, SEB and PHA were similar in the two groups, responses to Hsp65 and PPD seemed to be specifically affected in the diabetics tested. The reason(s) for this is unknown.

In diabetics proliferation to Hsp65 correlated with anti-GAD antibodies and antibodies to Hsp65 with ICA. Whether the epitopes recognized by ICA and Hsp65 antibodies or anti-GAD antibodies and mononuclear cells responding to Hsp65 are among those shared by mycobacterial Hsp65, human Hsp60 and GAD65 [20], and whether the specificity of responses to Hsp65 is similar in diabetics and normal children remains to be established.

Antibodies against BSA may be found in children with diabetes [9,10]. We found no difference in the proliferation to BSA in diabetic and control children. This does not exclude the possibility that the epitopes recognized by the two groups were different.

Thus GAD and islet cell antibodies were, as expected, frequently detected in children with newly diagnosed IDDM. The presence of the former correlated to proliferation in response to Hsp65, the latter to anti-Hsp65 antibodies. The relationship between Hsp65 and islet cell antigens such as GAD remains to be established.

Acknowledgements

We thank Drs A.E. Karlsen and C.E. Grubin for the GAD65 cDNA and Prof. M. Feldmann for Hsp65, Pirkko Rautio and Riitta Väisänen for technical assistance and Leena Saraste for help with the manuscript. The financial support by the Sigrid Juselius Foundation and Finska Läkaresällskapet is gratefully acknowledged.

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