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Complement activation pathways associated with islet cell surface antibody (ICSA) derived from child patients with insulin-dependent diabetes mellitus (IDDM).

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Complement activation pathways associated with islet cell surface antibody (ICSA) derived from child patients with insulin-dependent diabetes mellitus (IDDM).*

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Abstract

We studied the pathways of complement activation associated with the islet cell surface antibody (ICSA) obtained from sera of 7 patients (age less than 15 years) with insulin dependent diabetes mellitus (IDDM). The target cells were 51CR labelled rat islet cells and the complement source was human AB serum. Complement-dependent antibody mediated cytotoxicity (CAMC activity) was obtained using the percentage of cytotoxicity. CAMC activity of untreated sera was significantly inhibited by treating with EGTA or EDTA (p less than 0.001). The CAMC activity of EDTA-treated sera was significantly lower than that of EGTA-treated sera (p less than 0.001). In the inactivated human AB serum, it was lower than that of EGTA-treated sera (p less than 0.05), but not different from that of EDTA-treated sera. These results show that the complement activation associated with ICSA in patients occurred not only via the classical pathway but also via the alternative pathway.

KEYWORDS: complement activation, islet surface antibody, insulin-dependent diabetes mellitus, complement-dependent antibody mediated cytotoxicity, classical pathway, alternative pathway

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Complement Activation Pathways Associated with Islet Cell Surface Antibody (ICSA) Derived from Child Patients with Insulin-Dependent Diabetes Mellitus (IDDM)

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We studied the pathways of complement activation associated with the islet cell surface antibody (ICSA) obtained from sera of 7 patients (age < 15 years) with insulin dependent diabetes mellitus (IDDM). The target cells were ⁵¹CR labelled rat islet cells and the complement source was human AB serum. Complement-dependent antibody mediated cytotoxicity (CAMC activity) was obtained using the percentage of cytotoxicity. CAMC activity of untreated sera was significantly inhibited by treating with EGTA or EDTA ($p < 0.001$). The CAMC activity of EDTA-treated sera was significantly lower than that of EGTA-treated sera ($p < 0.001$). In the inactivated human AB serum, it was lower than that of EGTA-treated sera ($p < 0.05$), but not different from that of EDTA-treated sera. These results show that the complement activation associated with ICSA in patients occurred not only via the classical pathway but also via the alternative pathway.

Key words : complement activation, islet surface antibody, insulin-dependent diabetes mellitus, complement-dependent antibody mediated cytotoxicity, classical pathway, alternative pathway

There has been indirect evidence of complement activation in IDDM (1). In our previous report, we showed the evidence of complement activation associated with an islet cell surface antibody derived from child patients with IDDM (2). In this report, we have clarified the pathways of the complement activation *in vitro*.

Materials and Methods

The fasting blood samples of 7 patients were col-

lected from the peripheral vein. Four of the patients were female and 3 were male. All of them were below 15 years of age (mean age, 10 ± 2.2). Sera containing ICSA were separated from blood samples by centrifugation. It was then stored at -20°C . All sera were assayed within 2 months of collection and were heat activated at 56°C for 30 min prior to assay.

ICSA assay. ICSA was assayed by indirect immunofluorescence using rat pancreatic islet cells according to the method of Lernmark (3). CAMC assay and *in vitro* rat pancreatic islet cell culture were done according to our previous method (2).

Statistical analysis. All results were expressed as mean \pm SD. CAMC activity was tested for statistically significant differences using Student's paired *t*-test.

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Results

ICSA was positive in all the 7 subjects. CAMC activity of untreated sera ($19.0 \pm 4.0\%$, $n=7$), was inhibited by treating with EGTA ($7.1 \pm 4.9\%$) and with EDTA ($2.5 \pm 0.9\%$), respectively ($p < 0.001$). The CAMC activity of EDTA-treated sera was significantly lower than that of EGTA-treated sera ($n=7$, $p < 0.05$) (Table 1). CAMC activity in the untreated sera of inactivated human AB type ($2.9 \pm 0.6\%$) was lower than that of those treated with EGTA ($7.1 \pm 4.9\%$) ($p < 0.05$), but not different from that of those treated with EDTA ($2.5 \pm 0.9\%$) (Table 1).

Table 1 Comparison of complement-dependent antibody mediated cytotoxicity (CAMC) activity between treated and untreated sera

Sera	CAMC activity (%)	Comparison (vs)
Untreated sera (U)	19.0 ± 4.0	G** D**
Treated sera with EGTA (G)	7.1 ± 4.9	U** D*
Treated sera with EDTA (D)	2.5 ± 0.9	G* U**
Untreated sera of inactivated human AB type (UI)	2.9 ± 0.6	G* D ^{ns}

* $p < 0.05$ ** $p < 0.001$ ns, not significant

Discussion

Indirect evidence is present for complement activation in IDDM (1). In our previous report, we showed the evidence of complement activation in IDDM (2). In the present paper, we have tried to clarify the pathway of the complement activation by immune complex in association with ICSA derived from patients with IDDM *in vitro*. The fact that CAMC activity in untreated sera was significantly inhibited by treating with EGTA implies that the complement activation occurs via classical pathway. Because, the complement

activation via classical pathway is completely inhibited by EGTA, but not the alternative pathway. In addition, CAMC activity in EDTA-treated sera was significantly lower than that of EGTA-treated sera, but not different from that of untreated sera of inactivated human AB serum. Because the complement activation via both classical and alternative pathways completely inhibited by EDTA, and the sera of inactivated human AB type did not activate any of the complement pathways. This inhibition by EDTA implies that the complements are also activated via alternative pathway. In this study, however, it was not determined whether the classical or alternative pathway predominantly contribute to the complement activation.

From these results, it can be suggested that the complement activation in IDDM occurs via two pathways and that complement activation by immune complex associated with ICSA occurs not only via classical pathway but also via alternative pathway.

References

1. Dobersen MJ, Scharff JE, Ginsberg-Fellmer F and Notkins AL: Cytotoxic autoantibodies to beta cells in the serum of patients with insulin-dependent diabetes mellitus. *N Engl J Med* (1980) **303**, 1493-1498.
2. Okada S, Sato K, Miyai Y, Masaki Y, Higuchi T and Ota Z: Serum C3 and C4 levels and complement-dependent antibody-mediated cytotoxic activity of islet cell surface antibody in type 1 (insulin-dependent) diabetic children. *Diabetologia* (1987) **30**, 869-873.
3. Lernmark A, Sehlin J, Taljedal IB, Kromann H and Nerup J: Possible toxic effects of normal and diabetic patient serum of pancreatic B cells. *Diabetologia* (1978) **14**, 25-31.
4. Lacy PE, and Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* (1967) **16**, 35-39.
5. Meda P, Hooghe-Peters EL and Trci L: Monolayer cultures of adult pancreatic islet cells on osmotically disrupted fibroblasts. *Diabetes* (1980) **29**, 497-500.

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