Differences in the Contribution of the CTLA4 Gene to Susceptibility to Fulminant and Type 1A Diabetes in Japanese Patients

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OBJECTIVE — To examine the contribution of the CTLA4 gene in the susceptibility to fulminant type 1 diabetes and compare it with classic type 1A diabetes.

RESEARCH DESIGN AND METHODS — We genotyped the +49G>A and CT60G>A variants of the CTLA4 gene in fulminant type 1 diabetic patients (n = 55), classic type 1A diabetic patients (n = 91), and healthy control subjects (n = 369). We also assessed serum levels of the soluble form of CTLA4 (sCTLA4).

RESULTS — The +49GG and CT60GG genotypes were associated with type 1A diabetes (P < 0.001). In contrast, the CT60AA genotype, but not the +49G>A variation, was associated with fulminant type 1 diabetes (P < 0.05), especially in patients carrying HLA-DR4 (P < 0.01). Serum levels of sCTLA4 were significantly decreased in patients with fulminant type 1 diabetes (P < 0.05).

CONCLUSIONS — These results suggest that CTLA4 CT60 affects the genetic susceptibility to fulminant type 1 diabetes. Furthermore, the contribution of CTLA4 to disease susceptibility is distinct between fulminant type 1 diabetes and classic type 1A diabetes.

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Table 1—CTLA4 polymorphisms in fulminant type 1 diabetic patients, type 1A diabetic patients, and healthy control subjects

| Polymorphism | Fulminant vs. control | Type 1A vs. control | Fulminant vs. type 1A | Fulminant vs. control |
|--------------|-----------------------|--------------------|-----------------------|----------------------|
| +94G vs. GC  |           |                   |                       |                      |
| G (0.07)    | 1.00 (0.0)           | 0.026              | 1.00 (0.0)           | 0.026                |
| A (0.03)    | 0.05 (0.0)           | 0.005              | 0.05 (0.0)           | 0.005                |
| +92A vs. GG  |                       |                   |                       |                      |
| C (0.12)    | 1.00 (0.0)           | 0.026              | 1.00 (0.0)           | 0.026                |
| G (0.03)    | 0.05 (0.0)           | 0.005              | 0.05 (0.0)           | 0.005                |

Data are n (%) The interaction between CTLA4 polymorphisms and HLA-DR4 was assessed by χ² test with a 2 × 2 contingency table (+94G vs. GC or +92A vs. GG) in DR4-positive or -negative patients and the corresponding control subjects.
positive individuals, the frequency of the CT60AA genotype was significantly increased in patients with fulminant type 1 diabetes \( (P = 0.005) \). However, stratification of patients by the presence or absence of HLA-DR4 did not affect the association between the +49GG genotype and type 1A diabetes (Table 1).

It has been reported that the CT60G allele might be associated with lower production of sCTLA4 mRNA (3). We therefore determined serum sCTLA4 levels. The mean sCTLA4 levels in fulminant type 1 diabetic patients \( (0.56 \pm 0.24 \text{ ng/ml [mean \pm SD], } n = 36) \) was significantly lower than those in type 1A diabetic patients and control subjects \( (0.94 \pm 0.87 \text{ ng/ml, } n = 45) \) and control subjects \( (0.89 \pm 0.76 \text{ ng/ml, } n = 23) \) \( (P = 0.043) \). A mixed-model ANOVA using phenotypic group (fulminant type 1 diabetes, type 1A diabetes, and control) and CT60 genotype (GG and GA+AA) as factorial fixed effects revealed no differences in sCTLA4 levels between CT60 genotypes \( (P = 0.76) \) or phenotype/genotype interactions \( (P = 0.40) \).

**CONCLUSIONS** — CTLA4, which delivers inhibitory signals to T-cell activation, is expressed on the surface of activated T-cells and regulatory T-cells, and the lack of CTLA4 results in uncontrolled T-cell–mediated lymphoproliferative disease (6). Furthermore, CTLA4 also has a significant biological role in attenuating T-cell responses in the context of an inflammatory environment, such as infection with a pathogen (7). We showed that CTLA4 is associated with an increased risk of fulminant type 1 diabetes and that its contribution is distinct from classic type 1A diabetes. As reported previously (5,8), a significant association between classic type 1A diabetes and +49GG and CT60GG genotype was also found in the present study. However, the CT60AA genotype contributes to the susceptibility to fulminant type 1 diabetes. Moreover, it is implicated that HLA-DR4 influences the association of fulminant type 1 diabetes with the CT60AA genotype.

In this study, we also revealed that serum sCTLA4 level in fulminant type 1 diabetic patients were significantly lower than those in type 1A diabetic patients and control subjects. Although it remains unknown how sCTLA4 regulates T-cell activation, recombinant sCTLA4 inhibits T-cell proliferation in vitro. Furthermore, sCTLA4 is constituently expressed in nonstimulated T-cells, and its expression is downregulated after T-cell activation (9). Therefore, the decreased levels of sCTLA4 might indicate a lower potential of T-cell inhibition in fulminant type 1 diabetes, which might be caused by functional defects leading to reduced production of sCTLA4 or ongoing activation of the immune system eventually leading to decreased levels of sCTLA4.

In conclusion, the present study implicates that CTLA4 confers susceptibility to fulminant type 1 diabetes. Furthermore, the different contributions of CTLA4 to susceptibility to fulminant and classic type 1A diabetes indicate that the underlying immune process–prized \( \beta \)-cell injury might be distinct between these subtypes of type 1 diabetes.

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