Serum Anti-p53 Autoantibodies in Patients with Type 1 Diabetes

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Abstract. The presence of antibodies reacting with the p53 tumor suppressor protein has been described in patients with some autoimmune disorders. In this study we looked for serum anti-p53 antibodies in 64 patients with autoimmune type 1 diabetes mellitus within 4 mo of diagnosis. The presence of anti-p53 antibodies was observed in 6/64 (9.4%) subjects with type 1 diabetes, and in 1/44 (2.3%) subjects with other organ-specific autoimmune diseases (18 primary biliary cirrhosis, 10 autoimmune hepatitis, 16 thyroid diseases), but in none of 45 control subjects. No relationship was found between antibodies directed against islet- and non-islet-specific antigens and anti-p53 antibodies. These findings support a possible role for p53 in some autoimmune disorders. (received 20 March 2001; accepted 15 May 2001)

Keywords: anti-p53 antibodies, type 1 diabetes, autoimmune diseases

Introduction

Type 1 diabetes mellitus is the end result of progressive autoimmune aggression against the insulin-secreting β-cells [1]. Production of antibodies reacting with pancreatic islet cell antigens (ie, insulin, GAD, IA-2, etc) characterizes this process [2]. In addition, various other autoantibodies directed against organ- and non-organ-specific autoantigens have been identified in sera from patients with type 1 diabetes [3,4].

Recent evidence indicates that alterations in cell survival contribute to the pathogenesis of a variety of diseases such as cancer, neurodegenerative disorders, viral infections, and autoimmune diseases, including type 1 diabetes [5]. The p53 tumor suppressor gene plays an important role in the control of cell proliferation and death. The p53 tumor suppressor protein arrests the cell cycle at the G1 phase or induces apoptosis in response to different degrees of cellular DNA damage [6]. Antibodies directed against the p53 protein (anti-p53 antibodies) are detectable in sera from patients with cancer and are reputed to be a reliable expression of the p53 status in tumors [7].

Activation of the p53 gene has recently been associated with the pathogenesis of autoimmune disorders, such as rheumatoid arthritis and Sjogren’s syndrome [8,9]. In addition, anti-p53 antibodies have been found in sera from patients with thyroid autoimmune disease, systemic lupus erythematosus, and other rheumatic diseases [10-12].

In this study we looked for serum anti-p53 antibodies in patients with type 1 diabetes. The presence of other antibodies directed against islet- and non-islet-antigens was also investigated.

Materials and Methods

Serum samples. Serum samples were obtained from 64 patients with type 1 diabetes (30 males, 34 females, mean age 17.7 yr, range 1-39) collected within 4 mo of diagnosis (mean disease duration, 0.3 mo). A subsequent blood sample was available in 10 diabetics during follow-up periods ranging from 2 to 38 mo. Blood samples from 45 healthy subjects served as controls. Sera obtained from 18 patients with primary biliary cirrhosis (PBC), 10 patients with autoimmune hepatitis (AIH), and 16 patients with autoimmune thyroid diseases (ATD) were also analysed. The sera were stored at -30°C until they were used.
Only caucasian people living in the southern part of Italy (Sicily) were included in this study. The criteria for diagnosis of type 1 diabetes included diabetic ketosis and ketoacidosis, polyuria, polydipsia, weight-loss, and assessment of diabetes-related autoantibodies. None of the diabetic patients were obese and none had acanthosis nigricans. Informed consent was obtained from all subjects.

**Anti-p53 antibodies.** Serum anti-p53 antibodies were detected by an enzyme-linked immunosorbent assay (ELISA) kit (Immunotech, Marseille, France), which has been validated for anti-p53 measurement in autoimmune disorders [10]. According to the manufacturer's instructions, results were expressed as an index that represents the ratio between the optical density (OD) of each unknown sample and the OD of the low positive control sample that is supplied with the kit. Sera with an index $\geq 1.1$ were considered positive.

**Diabetes-related antibodies.** Islet cell antibodies (ICA) were detected by indirect immunofluorescence assay on cryostat sections of unfixed human pancreas. Quantification of ICA was performed by dilution of sera until ICA could not be detected, and results were converted to Juvenile Diabetes Foundation (JDF) units by a standard curve based on the international JDF reference serum sample [13]. Values $\geq 5$ JDF units were defined as positive. Antibodies against glutamic acid decarboxylase 65 kDa (GADA) and protein tyrosine phosphatase-2 (IA-2A) were measured by two distinct radioimmunoassay kits (CIS Diagnostici, Italy). Values greater than 1 and 0.75 U/ml, respectively, were considered positive.

**Other antibodies.** IgA antibodies to endomysium (EMA) were detected by indirect immunofluorescence assay on monkey esophageal tissue (The Binding Site, Birmingham, UK). Sera showing titres $\geq 1:5$ were considered positive. IgA antibodies to transglutaminase (TGA) were assessed by ELISA using transglutaminase from guinea pig liver as antigen [14]. Levels above 10 AU were defined as positive. Antibodies to parietal cells (PCA), nuclei (ANA), mitochondria (AMA), and smooth muscle (SMA) were detected by indirect immunofluorescence assay on different rat tissues [15]. Titres $\geq 1:40$ were regarded as positive.

**Statistics.** Statistical analysis was performed by Fisher's exact test. Differences were considered statistically significant when the p value was $<0.05$.

**Results**

Sera positive for anti-p53 antibodies were detected in 6/64 (9.4%) patients with type 1 diabetes within 4 mo of diagnosis, but in none of 45 controls ($p < 0.05$) (Fig. 1). Follow-up of 10 diabetics with ($n = 5$) or without ($n = 5$) positivity for anti-p53 was performed. In 3 of 5 patients with anti-p53 antibodies at initial diagnosis, these antibodies disappeared during the disease, while in the other 2 patients, positivity was maintained after 12 and 38 mo respectively (Fig. 2). The 5 diabetic subjects without anti-p53 antibodies all remained negative during the follow-up.
Fig. 2. Follow-up of anti-p53 antibody levels in 10 type 1 diabetic patients with (n = 5) or without (n = 5) positivity for anti-p53 antibody at diagnosis. Values are expressed as an index (sample OD/anti-p53 low positive control OD). Sera with index ≥1.1 (horizontal dashed line) were considered as positive. Characteristics of the patients identified with letters A-E are reported in Table 2.

Table 1. Characteristics of patients with type 1 diabetes, according to the presence or absence of serum anti-p53 antibodies. Values are means and range; ns (not significant) = p >0.05 by Fisher’s exact test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients positive for anti-p53 antibodies (n = 6)</th>
<th>Patients negative for anti-p53 antibodies (n = 58)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>17.5 (3-36)</td>
<td>16.7 (1-39)</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of diabetes (mo)</td>
<td>0.1 (0-2)</td>
<td>0.3 (0-4)</td>
<td>ns</td>
</tr>
<tr>
<td>Males</td>
<td>n (%)</td>
<td>n (%)</td>
<td>ns</td>
</tr>
<tr>
<td>ICA+ (islet cell antibodies)</td>
<td>5 (83)</td>
<td>26 (45)</td>
<td>ns</td>
</tr>
<tr>
<td>GADA+ and/or IA-2A+ (glutamic acid decarboxylase antibodies and/or tyrosine phosphatase-2 antibodies)</td>
<td>5 (83)</td>
<td>39 (67)</td>
<td>ns</td>
</tr>
<tr>
<td>TGA+ (IgA antibodies to transglutaminase)</td>
<td>1 (16)</td>
<td>3 (5)</td>
<td>ns</td>
</tr>
<tr>
<td>EMA+ (IgA antibodies to endomysium)</td>
<td>0 (–)</td>
<td>1 (1.7)</td>
<td>ns</td>
</tr>
<tr>
<td>PCA+ (parietal cell antibodies)</td>
<td>0 (–)</td>
<td>1 (1.7)</td>
<td>ns</td>
</tr>
<tr>
<td>ANA+ (anti-nuclear antibodies)</td>
<td>0 (–)</td>
<td>2 (3)</td>
<td>ns</td>
</tr>
<tr>
<td>SMA+ (anti-smooth muscle antibodies)</td>
<td>1 (16)</td>
<td>8 (13)</td>
<td>ns</td>
</tr>
<tr>
<td>AMA+ (anti-mitochondrial antibodies)</td>
<td>0 (–)</td>
<td>0 (–)</td>
<td>ns</td>
</tr>
</tbody>
</table>
When clinical and laboratory characteristics of the patients with type 1 diabetes were evaluated in relation to the presence of anti-p53, no differences were observed between positive and negative subjects (Table 1). No correlation was found between persistence or disappearance of anti-p53 antibodies and diabetes-related antibodies during follow up (Table 2).

Positivity for anti-p53 antibodies was found in 1 of 44 (2.3%) subjects with other organ-specific autoimmune diseases. The patient, an 18-yr-old man with autoimmune hepatitis, showed persistence of anti-p53 antibodies in 3 serum samples collected during a period of 5 yr.

**Discussion**

In this study, antibodies against the p53 protein were found in 9.4% of patients with type 1 diabetes within 4 mo of diagnosis. Among subjects with other organ-specific autoimmune diseases, only 1 (2.3%) showed anti-p53 antibodies.

Anti-p53 antibodies are usually found in individuals with cancer [16], the prevalence ranging from 4% up to 30% of patients with different malignancies. Anti-p53 antibodies have recently been detected in sera from subjects with some autoimmune disorders. Their presence was noted in 32% of patients with systemic lupus erythematosus [10], in 4.2% of subjects with autoimmune thyroid diseases [12], and in 1.9% of patients with rheumatoid arthritis [11].

The p53 gene plays an important role in the control of cell proliferation and death, encoding a protein that is believed to act as a tumor suppressor. Mutations of the p53 gene have been detected in many human cancers, leading to a biologically altered p53 protein [17]. Accumulation of mutated p53 protein in tumor cells is considered to be a factor in the development of the immune response in cancer patients [18]. However, overexpression of p53 is neither sufficient nor necessary for the generation of anti-p53 antibodies, suggesting that other mechanisms may be involved [19]. Furthermore, the presence of these antibodies has been proposed as an indicator of survival in some types of cancer [7].

The factors that elicit the development of anti-p53 antibodies in subjects with autoimmune disorders are unknown. Events that occur during autoimmune inflammation may be involved in this immune response. Inflammatory loci are characterized by high rates of cell death and compensatory cell proliferation, and recent evidence indicates a potential role of p53 in inflammation. Increased p53 expression has been found in a number of inflammatory diseases, including autoimmune disorders [20-24].

DNA damage caused by oxidizing and nitrating agents, produced in inflamed tissues, may possibly

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**Table 2. Diabetes-related antibodies during the follow-up of 5 diabetic patients who were positive for anti-p53 antibodies.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Time after diagnosis (mo)</th>
<th>anti-p53</th>
<th>ICA</th>
<th>GADA</th>
<th>IA-2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>M</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>27</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>31</td>
<td>M</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>F</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>38</td>
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<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>12</td>
<td>M</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
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<td></td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>E</td>
<td>13</td>
<td>M</td>
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<td></td>
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<td></td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>–</td>
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</tbody>
</table>
account for p53 overexpression [25]. In addition, proinflammatory cytokines may affect p53 activity, and upregulation of p53 can be induced by tumor necrosis factor-alpha (TNF-α) [26]. Nitric oxide and TNF-α are involved in the autoimmune inflammation which characterizes the β-cell destruction in type 1 diabetes [27,28]. Taken together, these findings are compatible with an increased expression of p53 in pancreatic islets during this process; therefore an involvement of islet autoimmune events in the appearance of the anti-p53 antibodies in patients with type 1 diabetes is likely to occur. However, only a minority of our diabetic patients developed anti-p53 antibodies. A low prevalence (15%) of antibodies against the heat-shock protein 60 (hsp60), which is expressed on the surface of β-cells during islet inflammation [29,30], has been reported in type 1 diabetics. Interestingly, in breast cancer, the formation of a complex between heat-shock proteins (hsp) and p53 is necessary to elicit an immune response against p53, suggesting that hsp may play a role in antigenic presentation of p53 [31].

An involvement of non islet-related factors in the development of anti-p53 antibodies cannot be excluded. In fact, antinuclear antibodies (ANA) specific for the p53 protein have been transiently induced in mice immunized with complexes of wild-type p53 and SV40 large T antigen, suggesting that some viral infections might trigger an immune response to p53 [32]. A role of viruses in the etiopathogenesis of autoimmune diabetes has been proposed [33] and anti-nuclear antibodies (ANA) have been described in sera from patients with type 1 diabetes [3]. However, this mechanism seems not to be implicated in the appearance of the anti-p53 antibodies observed in our diabetic group, since none of the patients showed ANA. Localization of p53 protein to mitochondria has been described [34], but the relevance of this observation to the development of anti-p53 antibodies is unknown. No anti-mitochondrial antibodies (AMA) were found in anti-p53 positive diabetics.

In our study, 3 of 5 diabetics positive for anti-p53 antibodies at diagnosis became negative during the disease. As far as we know, no data on follow-up of anti-p53 antibodies have been reported in patients with autoimmune disorders. Various autoantibodies have been identified in sera from patients with type 1 diabetes. Immunological abnormalities are usually observed at disease onset and gradually disappear after diagnosis in most patients [3]. The behaviour of anti-p53 antibodies observed in our diabetic patients is consistent with this tendency.

In conclusion, the results of this study show that anti-p53 antibodies can be detected in sera from patients with type 1 diabetes, which provides support for a role for p53 in certain autoimmune disorders.

References