Note

Suppression of Overt Diabetes in NOD Mice by Anti-thymocyte Serum or Anti-Thy 1, 2 Antibody

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Effects of anti-thymocyte serum (ATS) and anti-Thy 1, 2 monoclonal antibody on the spontaneously occurring diabetes in NOD mice were examined. Spontaneous diabetes in female mice was markedly suppressed by intravenous injection of rabbit anti-mouse thymocyte serum diluted to 1:4 on three consecutive days during the time period from 70 to 100 days after birth; the cumulative incidence of overt diabetes up to 195 days of age was greatly reduced and the onset of diabetes was delayed. Similar effect was observed with anti-Thy 1, 2 antibody treatment. These findings suggest that T lymphocytes play a role in the production of spontaneous diabetes in this mouse strain.

Non-obese diabetes-prone (NOD) mouse is a novel inbred strain which develops spontaneous insulin-dependent diabetes mellitus. The features of the pathogenesis have been described elsewhere [3, 4]. This strain has been regarded as a good animal model for human Type I insulin-dependent diabetes, and therefore, the pathogenic mechanism is now being investigated from various standpoints. Attention has been particularly paid to the possibility that autoimmunities to pancreatic islet cells of the humoral and/or cellular types might be involved in some stages in the development of overt diabetes. To examine this possibility, we carried out a series of experiments to ascertain whether immunological treatment could affect the pathogenesis. In the present study, on the analogy of the report that spontaneous diabetes in the BB rat is suppressed by multiple injections of anti-lymphocyte serum[2] we studied the suppressive effects of anti-thymocyte serum(ATS)and anti-Thy 1, 2 monoclonal antibody preparation on spontaneous diabetes in NOD mice.

Female NOD mice aged 55-100 days were kept under specific pathogen-free conditions. Female DS/Shi mice (aged 60 days) were employed as the source of thymocytes for the determination of antibody activity of ATS. All the mice were given a commercial diet CA-1 (Japan Clea, Tokyo, JPN) and tap water ad libitum.

Rabbit antiserum to mouse thymocytes was purchased from Cappel Laboratories, Cockranville, PA, USA (Lot 11940). Antibodies to mouse tissue antigens other than those specific to thymocytes were removed by absorption with acetone powder of mouse liver (Cappel Laboratories, Lot 14769). In brief, 8 ml of ATS was mixed with 1 g acetone powder of mouse liver which had previously been washed three times with phosphate-buffered saline solution (pH
After 18-h incubation at 4°C with gentle stirring, the mixture was spun down at 2,000 rpm for 5 minutes and the supernatant was separated. This procedure was repeated twice. ATS was then diluted with Hanks’ balanced salt solution and the antibody activity was assessed by a 4-h $^{51}$Cr-release test [1] using thymocytes of DS/Shi mice as the target cells. The absorbed ATS diluted to 1:320 lysed half of the 4×10^5 cells of $^{51}$Cr-labelled thymocytes in the presence of fresh normal rabbit serum (finally diluted to 1:400) as complement. Anti-Thyl, 2 monoclonal antibody (ascites) was purchased from Olac, Blackthorn Bicester, Oxon, UK (Lot B4L2, the thymocyte-lytic activity being 1:500,000).

The effects of ATS and the anti-Thy 1, 2 antibody preparation were examined against spontaneous diabetes in females. Mice aged 70–100 days were injected intravenously with 0.2 ml of ATS or anti-Thy 1, 2 antibody preparation diluted to 1:4 and 1:32 respectively with phosphate-buffered saline solution (PBS, pH 7.0) once a day for 3 consecutive days. As the controls, untreated mice were used in Experiment 1, and those treated with normal rabbit serum (NRS) diluted to 1:4 were employed in the other experiments (Experiments 2–5). The urinary glucose level of these mice was checked every day using Tes-Tape® (Eli-Lilly, Ill, USA) and the animals showing grade +2 or +4 (glucose>28 mmol/l) were recorded as diabetic. Plasma glucose concentration was measured quantitatively by the glucose oxidase method using a commercial kit, Blood Sugar-GOD-Perid Test® (Boehringer Mannheim–Yamanouchi, Tokyo, JPN) [4] at the time designated in the text.

Student’s t test was performed to evaluate the significance of the difference of plasma glucose levels between Tes-Tape®–positive and negative groups.

Effects of three consecutive daily injections of ATS and anti-Thy 1, 2 monoclonal antibody preparation against the spontaneous diabetes in females are shown in Table 1. In Experiment 1, the Tes-Tape® test revealed that such treatments at the age of 70–100 days completely suppressed the onset of diabetes. This was confirmed by quantitative determination of

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Treatment</th>
<th>Age at first treatment (days)</th>
<th>Total No. of mice tested</th>
<th>Before treatment</th>
<th>130 days</th>
<th>165 days</th>
<th>195 days</th>
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<tr>
<td>1</td>
<td>ATS*</td>
<td>70</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Not tested</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Anti-Thy 1, 2</td>
<td>70</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>5</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>10</td>
<td>0</td>
<td>4(40%)#</td>
<td>6(60%)</td>
<td>7(70%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ATS</td>
<td>82</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1(14%)</td>
<td>1(14%)</td>
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<tr>
<td></td>
<td>NRS†</td>
<td>9</td>
<td>0</td>
<td>3(33%)</td>
<td>5(56%)</td>
<td>6(67%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ATS</td>
<td>92</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>2(18%)</td>
<td>3(27%)</td>
</tr>
<tr>
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<td>0</td>
<td>5(50%)</td>
<td>7(70%)</td>
<td>8(80%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ATS</td>
<td>92</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>2(18%)</td>
<td>3(27%)</td>
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<tr>
<td></td>
<td>NRS</td>
<td>10</td>
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<td>5(50%)</td>
<td>8(80%)</td>
<td>8(80%)</td>
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<tr>
<td>5</td>
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<td>1(11%)</td>
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<td>1(11%)</td>
</tr>
<tr>
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<td>NRS</td>
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<td>5(62%)</td>
<td>6(75%)</td>
<td>7(88%)</td>
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</tbody>
</table>

* ATS: 1:4 dilution
† NRS: 1:4 dilution
# Numerals in parentheses indicate incidence of overt diabetes.
plasma glucose. The plasma glucose concentrations of non-diabetic (Tes-Tape*-negative) ATS-treated and anti-Thy 1, 2 antibody-treated groups were 7.4±1.4 mmol/l (mean±SD, n=7) and 9.2±2.4 mmol/l (n=7), respectively, at the termination of the observation (i.e., at ages 165 and 195 days), both being much lower than those of diabetic (Tes-Tape*-positive) mice in the untreated control group (40.6±9.3 mmol/l, n=7, determined 14 days after the onset of diabetes). The difference between the Tes-Tape*-positive and -negative animals was statistically significant (p<0.001). In Experiments 2–5, suppression by ATS-treatment was not complete but still striking, the incidence of diabetes of ATS-treated groups being only 1/8 to 1/3 of those of the control (NRS-treated) groups.

The present study demonstrates that ATS suppresses spontaneous diabetes in NOD mice. This observation agrees with the finding of Like et al.[2] that anti-lymphocyte serum of rabbit origin prevents diabetes in BB rats. Because the ATS preparation used in this study was pre-treated with large quantities of acetone powder of mouse liver to eliminate antibodies to the common antigens of mouse tissues, selective depletion of T-lymphocytes appears to have suppressed diabetes pathogenesis. More definite support for this hypothesis comes from the suppressive effect of anti-Thy 1, 2 monoclonal antibody. In the present study, only three consecutive injections of ATS or anti-Thy 1, 2 antibody at 70–100 days caused long-lasting suppression of spontaneously occurring diabetes in females up to the age of 195 days. A possible mechanism for such a long suppression would be that the autoimmunity to pancreatic islets proceeds so slowly that only a few doses of these antibody preparations are sufficient to keep the autoimmunity at a reduced level for a long time.

Although hyperglycemia and elevated plasma glucose concentration have been reported to return to normal levels after treatment with anti-lymphocyte serum (ALS) in approximately 38% of diabetic BB rats [2], ATS treatment after the onset of diabetes failed to exert such a curative effect in five out of six NOD mice. The discrepancy between the findings in the BB rat and the NOD mouse might be due to the difference in the frequency of the treatment (ALS was injected to the rats three times weekly for 30 days).

Acknowledgment

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References

NOD マウスにおける抗胸腺細胞血清ならびに抗 Thy 1, 2 抗体
投与による顕性糖尿発症の抑制

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NOD 雌マウスにおける顕性糖尿の自然発症に及ぼす
ウサギ抗マウス胸腺細胞血清 (ATS) ならびに抗 Thy
1, 2 モノクロナル抗体の影響をしらべた。70〜100 日齢
の期間にて ATS (4 倍希釈液 0.2ml) を連続 3 日間静
注し、165〜195日齢までの発症を観察したところ、無処
置またはウサギ正常血清投与の対照群に比べ、累積発症
率は低く、且つ、発症が遅延されることが見出された。
抗 Thy 1, 2 抗体を投与した場合も抑制は顕著であった。
これらの結果から、NOD マウスにおける糖尿の自然発
症には T リンバ球が関与していることが示唆される。