The LETL Rat: A Model for IDDM Without Lymphopenia

Takashi Natori and Kazuya Kawano

INTRODUCTION

Diabetes mellitus (DM) is a disease with several different pathogenetic origins. The heterogeneity of the origin of DM is also manifest in experimental animals, in which diabetes is linked in some with sex (NOD mice) and in others with lymphopenia [BBDP (formerly BB) rats]. Although of great interest, these characteristics are restricted to the respective animal models and do not reflect characteristics of human insulin-dependent diabetes mellitus (IDDM). The strain we describe here represents an animal model that more closely reflects the pathology of human IDDM.

ESTABLISHMENT OF LETL STRAIN

The LETL strain originated from a few pairs of outbred Long-Evans rats purchased from Charles River Canada (St. Constant, Quebec) in 1982. During the course of establishing a breeding colony, some rats showed a sudden onset of polyuria and polydipsia and were determined to be diabetic by measuring glucose in the urine and blood glucose levels. Diabetic animals exhibited retarded growth and died within 30 days of the appearance of glycosuria. Unfortunately, diabetic females reproduced poorly, and males could reproduce only at an early stage of diabetes. Consequently, we attempted to establish an inbred strain by selectively breeding brothers in early-stage diabetes with their nondiabetic sisters. Nondiabetic brothers were also used occasionally to avoid increasing the incidence of diabetes to a level that would result in poor overall breeding performance of the colony. Brother x sister matings were continued in this manner for 7 years, maintaining an incidence of diabetes in less than 30 percent of the rats. In 1989, the strain, which was designated LETL (Long-Evans Tokushima Lean), reached the twentieth generation of selective inbreeding (Kawano et al., 1991b) (Figure 1). A nondiabetic line was also established by brother x sister matings of nondiabetic animals from the same parental stock. That line, the

LETO (Long Evans Tokushima Otsuka), was used as the normal control for LETL. We have recently established from the same parental stock another diabetic line, called OLETF, that spontaneously develops long-term hyperglycemia and exhibits diabetic complications resembling those of human type II diabetes (Kawano et al., 1991a).

CLINICAL FEATURES

LETL rats display no characteristic clinical signs until the onset of glycosuria. Rats are observed daily for wetness of the bedding. For those rats with bedding wetter than those of the controls, glycosuria is confirmed by Ket-Diastix (Miles-Sankyo, Tokyo). Once glycosuria is confirmed, the rats' blood glucose, urinary glucose, and ketone levels are monitored every 4 weeks with a Glucose B test kit (Wako, Osaka, Japan) throughout their life spans. Onset of diabetic symptoms in LETL rats occurs abruptly between 8 and 20 weeks of age, with equal frequency and severity in both sexes. The mean age of diabetes onset after birth is 15.9 weeks in males and 14.1 weeks in females. Without insulin therapy, most affected rats die within 30 days after diabetes onset. Body weights decrease to 50 percent of prediabetes weight, urinary volumes increase from 10-20 g/day to 50-100 g/day, and water intake increases from 20-25 g/day to 100-150 g/day. A prominent clinical feature in LETL rats is an elevated plasma glucose level. The level is 6–9 mM before diabetes onset but increases to greater than 30 mM after onset. Plasma insulin decreases abruptly at onset to

Takashi Natori, M.D., is a senior advisor at the Tokushima Research Institute, Otsuka Pharmaceutical Company, Tokushima, Japan, and director of the PALM Institute in Sapporo, Japan. Kazuya Kawana, D.V.M., is a chief scientist at the Tokushima Research Institute, Otsuka Pharmaceutical Company, Tokushima, Japan.
below the lower limit of detection (8.7 pM). The urinary glucose level correlates well with that of plasma glucose. Urinary ketones are detected a few days after the onset of diabetes.

The average incidence of diabetes during the inbreeding process was approximately 21 percent in males and 15 percent in females. The incidence of diabetes depended on whether diabetic parents were used in mating. Of those animals with two diabetic parents, 64.2 percent (18/28) were diabetic, whereas of those with two nondiabetic parents, 13.7 percent (91/664) were diabetic. When diabetic males were mated with nondiabetic females, the incidence was 41.7 percent (71/170), whereas with the reverse mating (i.e., when diabetic females were mated with nondiabetic males), the incidence was 23.5 percent (4/17). In the LETO control line, no diabetic rats were found during the 20 generations of inbreeding.

HISTOPATHOLOGY

The histological appearance of the pancreas of an LETL rat is shown in Figure 2. A characteristic feature is lymphocyte infiltration into the peri-islet area and the islets. Lymphocyte infiltration appeared approximately 4–5 days before the onset of clinical diabetes in roughly half of the islets examined, then expanded to almost all islets during the clinical onset period. However, the insulitis gradually regressed after the onset of clinical diabetes. Lymphocytes were rarely found in atrophic islets more than a week after diabetes onset, and insulin-containing cells were not immunohistochemically detected in affected animals (Figure 3A). On the other hand, insulitis was still prominent and insulin-positive cells were detected in diabetic rats examined 2 days after onset of clinical diabetes (Figure 3B). The distribution of glucagon and somatostatin were normal. Insulitis was not observed in nondiabetic animals, including LETO control rats.

FIGURE 2 Histological appearance of the pancreas of an LETL rat. (Reprinted with permission from Kawano et al., 1991b, p. 1377. Copyright © 1991 by American Diabetes Association, Inc.)

FIGURE 3 Immunohistochemical staining of insulin-containing cells (immunoperoxidase-hematoxylin staining), (a) Pancreatic islet cells of an affected rat (13 weeks old); (b) Pancreatic islets obtained 2 days after onset of clinical diabetes. (Reprinted with permission from Kawano et al., 1991b, p. 1378. Copyright © 1991 by American Diabetes Association, Inc.)

Lymphoid cell infiltration was observed in other organs, such as the salivary gland (21.4 percent) and the lacrimal glands (30 percent)—but not in the thyroid glands, reproductive organs, or thymus—in 559 25-week-old rats, of which 286 were female and 273 were male. At later stages of the disease, glycogen deposition was observed in the kidney tubules, but no glomerular lesions were seen. Lymphocyte subsets in the peripheral blood and the spleen were analyzed in a fluorescence-activated cell sorter (FACS; Spectrum III, Ortho Westwood, Massachusetts) with monoclonal antibodies OX6, OX8, OX33, and OX19 (Table 1). The proportion of pan T lymphocytes was 37 percent in diabetic LETL rats, 45.7 percent in nondiabetic LETL rats, and 42 percent in control LETO rats. The proportion of helper to cytotoxic/suppressor T lymphocytes followed a similar pattern—proportions were lower in diabetic LETL rats than in nondiabetic or control LETO rats—although the differences were not statistically significant. No significant differences in the numbers of splenic lymphocytes were found in the three groups.

GENETICS

The general genetic profile of the LETL strain was reported before, and was compared to the LETO strain (Kawano et al., 1989). The two strains appear to share the same haplotype at the 22 loci that were tested. Both strains carry RT1". The frequencies of insulitis in F1, F2, and backcross progenies are shown in Table 2. No insulitis was found in any F1 hybrids [(F344 × LETL)F1 and (WKAH × LETL)F1]. In [(F344 × LETL)F1 × (F344 × LETL)F1]F2 animals, insulitis was found in 5.4 percent of the offspring, whereas in [(WKAH × LETL)F1 × (WKAH × LETL)F1]F2 animals, the frequency was 3.3 percent. All F2 rats with insulitis (7/130) appeared to carry ho-
TABLE 1 Lymphocyte subsets in LETO, nondiabetic LETL, and diabetic LETL rats

<table>
<thead>
<tr>
<th>Source/strain</th>
<th>Peripheral blood lymphocytes</th>
<th>Splenic lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia(OX6&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>B lymphocytes (OX33&lt;sup&gt;b&lt;/sup&gt;)</td>
</tr>
<tr>
<td>LETO (control)</td>
<td>22.6±3.5</td>
<td>23.5±2.7</td>
</tr>
<tr>
<td>LETL (nondiabetic)</td>
<td>22.5±3.9</td>
<td>21.7±7.5</td>
</tr>
<tr>
<td>LETL (diabetic)</td>
<td>32.7±6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.5±7.6</td>
</tr>
</tbody>
</table>

All values are means ± SD, LETL, Long-Evans Tokushima Lean; LETO, Long-Evans Tokushima Otsuka; OX6, OX33, and OX19 were the monoclonal antibodies used. (Reprinted with permission from Kawano et al., 1991b, p. 1379. Copyright © 1991 by American Diabetes Association, Inc.)

<sup>a</sup>P<0.01  
<sup>b</sup>P<0.001, vs. LETO

mozygous RT1<sup>a</sup>. In the backcross progenies [(F344 × LETL)F1 × LETL], 26.3 percent of the animals developed insulitis, whereas in the [(WKAH × LETL)F1 × LETL] animals, the frequency was 6.6 percent. [(F344 × LETL)F1 × F344] animals appeared to have no insulitis. These results suggest that at least two recessive genes are involved in induction of insulitis, one of which is closely linked with RT1<sup>a</sup>. The clinical and pathological features of LETL rats are similar to those of BBDP rats, except that LETL rats do not develop T lymphopenia. The proportions of lymphocyte subsets in peripheral and spleen lymphocytes remained within the normal ranges (Table 1). In addition, the lymphocytes appeared to show normal functional reactivity against allogeneic stimulator cells (Table 3). These features contrast with those of BBDP rat lymphocytes, which are abnormal in quantity (Jackson et al., 1981; Elder and Maclaren, 1983; Woda et al., 1986) and function (Elder and Maclaren, 1983; Bellgrau et al., 1982; Greiner et al., 1986). Like et al. (1986) reported on a strain related to BBDP, called BBDR, that have no lymphopenia, and Guberski et al. (1991) have recently reported that the outbreak of diabetes is associated with Kilham’s rat virus infection. It is likely that the lymphopenia itself in BBDR rats is not functionally associated with diabetes, although several reports suggest that it is in BBDP (Guttmann et al., 1983; Jackson et al., 1984; Georgiou et al., 1988). Thus, we have concluded that the LETL strain represents another model of IDDM without lymphopenia and shows no direct association between lymphopenia and diabetes.

Encephalomyocarditis virus (or enterovirus) and Coxsackie B4 virus cause insulitis and subsequently, IDDM in mice (Craighead and McLane, 1968; Burch et al., 1971); however, it is unlikely that infectious agents, such as viruses, are responsible for the IDDM of LETL rats. First, transfer of serum and supernatants of pancreas or spleen homogenates from diabetic LETL rats to naive young adult LETO or LEA/Otk rats does not cause diabetes (unpublished data). Second, the LETO control line has never developed diabetes, even though the animals are kept in the same room or, sometimes, in the same cage as LETL rats. However, we cannot exclude the possibility of involvement of Kilham’s rat virus, as reported by Guberski et al. (1991).

TABLE 2 Mode of Inheritance of insulitis in LETL rats

<table>
<thead>
<tr>
<th>Cross</th>
<th>n</th>
<th>Insulitis (n)</th>
<th>Insulitis frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;(WKAH/Hkm × LETL)</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;(F344/Ducrj × LETL)</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt;(WKAH/Hkm × LETL) × (WKAH/Hkm × LETL)</td>
<td>59</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt;(F344/Ducrj × LETL) × (WKAH/Hkm × LETL)</td>
<td>130</td>
<td>7</td>
<td>5.4</td>
</tr>
<tr>
<td>BC&lt;sub&gt;1&lt;/sub&gt;(WKAH/Hkm × LETL) × LETL</td>
<td>15</td>
<td>1</td>
<td>6.6</td>
</tr>
<tr>
<td>BC&lt;sub&gt;1&lt;/sub&gt;(F344/Ducrj × LETL) × LETL</td>
<td>19</td>
<td>5</td>
<td>26.3</td>
</tr>
<tr>
<td>BC&lt;sub&gt;2&lt;/sub&gt;(F344/Ducrj × LETL) × F344/Ducrj</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

BC=Backcross  
(Reprinted with permission from Kawano et al., 1991b, p. 1380. Copyright © 1991 by American Diabetes Association, Inc.)
It is important to note that LETL rats carry the RT1<sup>u</sup> haplotype, as do BBDP rats. Results of mixed lymphocyte reaction (MLR) and restriction-fragment length polymorphism (RFLP) (Kawano et al., 1991b) analyses clearly show that LETL rats carry the u haplotype for the class II region of RT1. This finding is consistent with the results of previous serological typing (unpublished data). In addition, we have reported that the RT1.A haplotype of LETL rats is u (Kawano et al., 1989). Therefore, the results taken together indicate that LETL rats carry RT1.A<sup>H<sub>B</sub>D<sub>B</sub></sup>. Results obtained so far indicate no substantial difference in the u haplotype of LETL (with or without diabetes) and LETO (control line) rats. However, we cannot exclude the possibility that a few substitutions of base pairs exist in sequences of the class II genes that do not affect the RFLP patterns or serological or MLR reactivity, as demonstrated in NOD mice.

**SUMMARY**

Spontaneously diabetic rats with polyuria, polyphagia, and polydipsia were discovered in 1982 in an outbred colony of Long-Evans rats purchased from Charles River Canada in 1982. The diabetic LETL strain was bred from these rats. The characteristic features of the disease in LETL rats are:

- sudden onset of polyuria, polyphagia, hyperglycemia, and weight loss;
- no sex differences in the incidence or severity;
- lymphocyte infiltration into the islets, followed by destruction of beta cells and disappearance of lymphocytes at the onset of diabetes;
- no significant T lymphocytopenia;
- lymphocyte infiltration into the salivary glands and lacrimal glands; and
- involvement of at least two recessive genes in the pathogenesis of insulinis, one of which is closely linked with RT1<sup>u</sup>.

These characteristics closely resemble those of human IDDM.

**REFERENCES**


**TABLE 3** Mixed lymphocyte culture responses of LETO, nondiabetic LETL, and diabetic LETL rats

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>LETO (non DM)</th>
<th>LETL (non DM)</th>
<th>LETL (DM)</th>
<th>F344 (1)</th>
<th>ACI/N (a)</th>
<th>WM (u)</th>
<th>BN/Sea (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td>LETO (non DM)</td>
<td>LETL (non DM)</td>
<td>LETL (DM)</td>
<td>F344 (1)</td>
<td>ACI/N (a)</td>
<td>WM (u)</td>
<td>BN/Sea (n)</td>
</tr>
<tr>
<td>LETO (control)</td>
<td>430±21192</td>
<td>3597±1101</td>
<td>6441±1041</td>
<td>88111±20884</td>
<td>10668±2431</td>
<td>10668±24619</td>
<td>951±1881</td>
</tr>
<tr>
<td>(1.0)</td>
<td>(0.8)</td>
<td>(1.5)</td>
<td>(20.5)</td>
<td>(24.8)</td>
<td>(24.8)</td>
<td>(0.7)</td>
<td>(34.5)</td>
</tr>
<tr>
<td>LETL (non DM)</td>
<td>6790±1479</td>
<td>6965±1358</td>
<td>5089±1891</td>
<td>5677±5984</td>
<td>4742±8584</td>
<td>6666±1154</td>
<td>32247±10098</td>
</tr>
<tr>
<td>(1.0)</td>
<td>(1.0)</td>
<td>(0.7)</td>
<td>(8.2)</td>
<td>(6.8)</td>
<td>(6.8)</td>
<td>(1.0)</td>
<td>(7.5)</td>
</tr>
<tr>
<td>LETL (DM)</td>
<td>552±5639</td>
<td>5714±672</td>
<td>5686±1984</td>
<td>6068±8865</td>
<td>7315±1899</td>
<td>4916±803</td>
<td>105017±8883</td>
</tr>
<tr>
<td>(1.0)</td>
<td>(1.0)</td>
<td>(1.0)</td>
<td>(10.7)</td>
<td>(12.9)</td>
<td>(12.9)</td>
<td>(0.9)</td>
<td>(18.5)</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=4) for cpm of [3H]thymidine incorporation in triplicate cultures with stimulation index in parentheses. DM=Diabetic.

(Reprinted with permission from Kawano et al., 1991b, p. 1379. Copyright © 1991 by American Diabetes Association, Inc.)