Breeding of a Non-Obese, Diabetic Strain of Mice

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(Received for publication : April 9, 1979)

A female mouse spontaneously exhibiting polyuria and glucosuria accompanied by rapid weight loss was found in one of two sublines derived from the CTS mice. Eight mating pairs were made using its offspring and selection was performed for both spontaneous diabetes and reproductive ability. After six generations of the selective breeding the diabetic (nod) and the control (non) lines were established.

A marked sex difference was observed in the incidence of diabetic symptoms in the nod mouse. The cumulative incidence of the onset up to 30 weeks of age was 80% in females and less than 20% in males. The onset of diabetes was abrupt in both sexes, and spontaneous remission was not observed. However, daily administration of insulin induced an increase of body weight and a prolongation of life span.

Diabetic symptoms are biochemically characterized by polyuria, polydipsia, hyperglycemia, glucosuria and hypercholesteremia. Pathological examination revealed a high frequency of lymphocyte infiltration around and/or into the Langerhans' islet. It was observed even at the prediabetic stage over five weeks of both sexes. The number and size of the islets were markedly reduced in the overt diabetic mice.

Although the mechanism of the pathogenesis is not clear yet, the nod mouse may be a useful animal model for investigating the human juvenile type diabetes.

Many hereditary diabetic strains of mice are summerized by Renold [25] and Hunt et al. [13]. All of them are characterized by spontaneous hyperglycemia, hyperinsulinemia and obesity. These mice are valuable for the research of maturity-onset type diabetes in man. However no suitable mouse model for the insulin-dependent juvenile type diabetes had been reported, except for the diabetes induced with EMC virus [6] and chemicals such as alloxan.

A female mouse which exhibited polyuria and glucosuria accompanied by weight

loss was found during our routine breeding work. This mouse died within a month following rapid deterioration. These features were so interesting that an attempt was made to establish a non-obese diabetic strain. The present paper reports the process of the selective breeding and the characteristics of the diabetic mice.

Materials and Methods

Animals : Two sublines were separated from the CTS strain [22] at the 6th generation and temporarily named A and B,

respectively. The CTS mouse was derived from the JCL-ICR mice and characterized by cataract eyes with microphthalmia [21]. However, both A and B sublines had normal eyes. In 1974, a female mouse showing spontaneous diabetic symptoms was discovered in the A line at the 20th generation. This female mouse had given birth to two litters before showing the diabetic symptoms. As the initial matings for the selective breeding of a diabetic line, five matings between these two litters and three matings between the mice of subline B were performed. The offspring whose one or both parents showed spontaneous diabetes and high reproductivity were selected for breeding. The total number of mice used in the present study was ca. 1500. The check for diabetes was done using Tes-Tape® (Eli-Lilly) as described below.

The animals were maintained under conventional conditions at constant temperature (22-25°C) until the 4th or 5th generation, and thereafter, the pups were placed under aseptic conditions by Caesarean section and fostered by SPF-ICR mothers. Under the conventional conditions, the mice were fed on commercial diet CA-1 (Clea Japan, Inc.) and tap water was available ad libitum. Under the barrier-sustained conditions, the same diet was given after having been autoclaved at 121°C for 7 min and the drinking water at 121°C for 35 min.

Laboratory tests: Non-fasting blood samples were obtained with a heparinized syringe by heart puncture under light ether anesthesia, and the plasma was separated quickly at 4°C and kept at -20°C until analysis. The plasma glucose level was determined with a Technicon Auto Analyzer, and the plasma cholesterol by the method of Zak-Henly [12,30].

The test for detecting urinary glucose was done directly using Tes-Tape® (Eli-Lilly). This method was used as the criterion for the selective breeding. Animals which showed Tes-Tape values of 1+ or

higher were classified as diabetic. Urinary glucose concentrations were also quantitatively determined by the glucose oxidase method (Glucostat, Worthington) on 24-hour urine samples. These samples were collected by placing the mice in polycarbonate metabolic cages with toluene as a preservative. For determination of ketonuria, Lab-Stix® (Ames) was used.

At autopsy, the pancreas was removed and fixed in Bouin's solution. The specimens were dehydrated, embedded in Tissue Prep®, then stained with hematoxylin eosin or Gomori's aldehyde fuchsin. Histological observation of the liver, kidney, heart, hypophysis, adrenal glands, testes and ovaries was performed by hematoxylin eosin staining after fixing in a 10% formalin solution.

Results

1. Process of selective breeding

The course of selective breeding is outlined in Fig. 1. Among the initial eight pairs, five females (Nos. 3, 4, 25, 26, and 27) and two males (Nos. 3 and 31) exhibited diabetic symptoms. All of them belonged to the A line. Eighteen litters consisting of 66 females and 72 males were obtained from the initial matings. Of these F1 mice, 12 females and 3 males were diabetics. The offspring from the three females (Nos. 28, 29, and 117) which showed good reproductive performance were selected for next brother-sister matings and the process was repeated. The diabetic and non-diabetic lines were clearly separated at the second generation. In the subsequent generations, the three diabetic lines kept showing the same diabetic symptoms.

Caesarean operation was performed in each line, and the pups were carried into the barrier-sustained room to be maintained under SPF conditions. The onset and the syndrome of diabetes under the SPF conditions were almost the same as those observed under the conventional conditions.

Fig. 1. The pedigree of the diabetic and non-diabetic lines.

The upper and lower numbers in the brackets show the individual numbers of female and male, respectively. Male and female mice were numbered separately.

● : Diabetic female. ○ : Normal female. ■ : Diabetic male.
□ : Normal male. * : Discontinued. ** : Caesarean operation.
※ : Sterile.

Table 1. Changes in diabetic condition accompanied with progress of generation

Item	Sex	Generation						
		0	1	2	3	4	5	6
Incidence of diabetes (%)	♀	62.5 (8)	84.6 (13)	83.3 (24)	51.7 (29)	73.7 (19)	67.4 (43)	61.8 (34)
	♂	33.3 (6)	33.3 (9)	6.7 (15)	7.1 (14)	10.5 (19)	9.5 (21)	0 (25)
Age at onset (days)	♀	194 (2)	172 (10)	179 (19)	179 (14)	149 (12)	147 (25)	139 (21)
	♂	246 (2)	312 (3)	276 (1)	248 (1)	168 (1)	177 (2)	—
Time from onset to death (days)	♀	52 (2)	36 (9)	45 (17)	41 (12)	44 (10)	27 (5)	24 (5)
	♂	61 (2)	—	—	135 (1)	—	43 (1)	—

Each value shows the mean calculated from the breeding record of the mice that survived beyond 150 days of age.

() : No. of mice examined.

— : No data were available.

The incidence, age at onset and duration of diabetes in each generation in the course of selective breeding are shown in Table 1. These data were taken from the

breeding record of the mice that survived beyond 150 days of age. The incidence in females was constant up to the 6th generation, being about 60 to 80%. In contrast,

the incidence in males decreased with the progress of generations. Namely, the incidence was 33.3% in the first two generations, but became less than 10% after the second generation.

The onset of overt diabetes in female mice was around the age of 200 days at the early stage of selection, but tended to occur about 50 days earlier after the fourth generation. The onset was late in males compared with the females, and took more than 200 days during the first four generations. After the fourth generation, the age of onset shortened as the females. The survival time after the onset was very short in both sexes. In females, the survival time was more than 40 days in early generations, but later shortened to about 25 days.

2. Clinical symptoms

Fig. 2. Cumulative incidences of the diabetic mice.

● — ● : Female (n=79).
○ — ○ : Male (n=45).

Table 2. Clinical characteristics and biochemical values in the diabetic mice before onset of diabetes

Age in weeks	Sex	Body weight (g)	Water intake (ml/day)	Urine volume (ml/day)	Food consumption (g/day)
5	♀	20.1±1.3 (75)	5.0±1.0 (75)	0.8±0.4 (75)	3.3±0.6 (71)
	♂	24.8±1.8 (54)	5.9±1.2 (54)	1.0±0.5 (54)	3.9±0.5 (50)
9	♀	21.9±1.9 (11)	5.1±1.2 (11)	1.1±0.8 (11)	3.2±0.4 (7)
	♂	28.8±1.5 (10)	4.7±0.7 (10)	0.8±0.3 (10)	3.5±0.6 (6)
17	♀	25.2±1.6 (15)	5.4±1.4 (15)	1.2±1.4 (15)	3.3±0.5 (6)
	♂	32.7±1.6 (16)	5.7±1.4 (16)	0.9±0.4 (16)	4.0±1.5 (5)
Age in weeks	Sex	Plasma glucose (mg/dl)	Urinary glucose (mg/dl)	Plasma cholesterol (mg/dl)	Ketonuria* (positive %)
5	♀	190.8± 33.4 (31)	22.4± 28.3 (75)	76.1± 9.3 (10)	0 (4)
	♂	187.1± 41.2 (33)	12.9± 20.3 (53)	103.0± 10.0 (9)	0 (4)
9	♀	163.7± 34.6 (55)	182.5±147.7 (4)	126.7± 31.6 (25)	0 (4)
	♂	171.5± 41.5 (49)	72.5± 5.0 (4)	140.6± 28.5 (13)	0 (4)
17	♀	225.7± 63.8 (9)	115.0±85.1 (4)	78.6± 0.2 (3)	0 (4)
	♂	163.0± 22.6 (9)	53.4±102.1 (9)	106.6± 0.2 (8)	0 (4)

Each value represents the mean±standard deviation.

() : No. of mice examined.

* : Ketonuria was determined using Lab-Stix (Ames).

In order to investigate the time course of the onset and progress of diabetes, the diabetic mice were inspected with Tes-

Tape once a week from 13 to 30 weeks of age. As shown in Fig. 2, overt diabetic symptoms in the females were first ob-

Table 3. Clinical characteristics and biochemical values in the diabetic mice after onset of diabetes

After onset (weeks)	Sex	Body weight (g)	Water intake (ml/day)	Urine volume (ml/day)	Food consumption (g/day)
0 - 2	♀	28.1 ± 4.4 (18)	22.6 ± 6.9 (14)	17.0 ± 5.9 (14)	4.8 ± 1.2 (14)
	♂	29.9 ± 2.5 (3)	21.6 ± 9.3 (2)	14.8 ± 7.1 (2)	4.8 ± 1.5 (3)
2 - 4	♀	25.8 ± 4.6 (28)	26.4 ± 4.8 (22)	19.8 ± 4.3 (19)	6.1 ± 1.6 (19)
	♂	17.7 (1)	21.4 (1)	19.3 (1)	4.7 (1)
> 4	♀	24.6 ± 3.7 (21)	26.3 ± 6.1 (9)	18.4 ± 4.7 (5)	5.0 ± 1.4 (5)
	♂	26.6 ± 3.9 (12)	23.2 ± 6.5 (8)	17.9 ± 6.8 (8)	5.1 ± 1.2 (9)

After onset (weeks)	Sex	Plasma glucose (mg/dl)	Urinary glucose (mg/dl)	Plasma cholesterol (mg/dl)	Ketonuria* (positive%)
0 - 2	♀	644.9 ± 140.5 (6)	13.8 ± 4.9 (12)	162.2 ± 63.7 (6)	16.7 (12)
	♂	239.4 (1)	12.7 ± 4.0 (3)	—	0 (3)
2 - 4	♀	730.7 ± 166.7 (8)	12.3 ± 5.3 (18)	601.1 ± 304.2 (12)	26.7 (15)
	♂	666.6 (1)	9.6 (1)	—	0 (1)
> 4	♀	714.2 ± 260.2 (10)	10.1 ± 6.3 (9)	836.4 ± 185.4 (4)	66.7 (6)
	♂	597.5 ± 186.4 (6)	10.5 ± 5.7 (8)	1902 (1)	0 (10)

Each value represents the mean ± standard deviation.

() : No. of mice examined.

-- : No data were available.

* : Ketonuria was determined using Lab-Stix (Ames).

served at the age of 90 days, and the occurrence of diabetes showed a steady rise with age. The incidences of diabetes up to 130 days and 210 days were about 50% and 80%, respectively. No significant difference was observed in the incidence of diabetes between the multiparous and virgin females.

On the other hand, the onset occurred in males 60 days later than in females. The cumulative incidence of the diabetes up to 210 days was very low, not exceeding 20%. Thus, significant sex differences were confirmed with respect to the frequency and the time of the onset.

The clinical characteristics and biochemical parameters of the diabetic mice before and after onset of diabetes are shown in Tables 2 and 3. Daily water consumption and urine excretion prior to onset were within the normal range, regardless of sex or age. However, marked

polydipsia and polyuria developed after onset. Water intake increased about four times and urine excretion 15-20 times. The overt diabetic mice after onset also showed a tendency toward polyphagia.

Plasma glucose concentrations ranged from 163.7 to 225.7 mg/dl prior to onset of diabetes. With onset of glucosuria, plasma glucose levels rose about three-fold and urinary glucose concentrations about a hundred-fold. Plasma cholesterol levels also increased significantly and ketonuria was observed frequently in the overt diabetic females.

Conspicuous weight loss was associated with the abrupt onset of diabetes in both sexes as illustrated in Fig. 3. The decline of body weight was more remarkable in females than in males. The mice at the onset of diabetes weighed more than 30 g, but less than 20 g before the death. All of the diabetic females continuously ex-

Fig. 3. Changes in body weight after the onset of diabetes.

— : Female. ---- : Male. * : Dead. ** : Autopsy.

creted large amounts of glucose in the urine. In males, however, negative glucosuria was occasionally observed after the onset. No spontaneous remission from diabetes was observed.

The response of the diabetic mice to exogenous insulin was examined. Insulin was given subcutaneously at a dose of 40 units per kg of body weight per day in the evening; the dose being comparable to that necessary for survival in alloxan-induced diabetic mice. Daily injection of insulin induced an increase of body weight and a marked prolongation of life span in diabetic mice (Fig. 4). One female mouse with severe symptoms (No. 59) showed a remarkable restoration of body weight with a temporal recovery to 30 g. When the insulin injection was stopped, the body weight decreased again. All the diabetic mice survived during the insulin treatment. These results suggested that the diabetic mice might be suffering from an insulin deficiency.

The reproductive capacities between diabetic and non-diabetic lines are compared in Table 4. The parturition rate of the diabetic line was 39.0%, considerably

Fig. 4. Effect of the daily insulin treatment (40U/kg, sc) on the body weight of the diabetic mice.

The broken lines show the change in body weight when the treatment was stopped.

lower than that of the non-diabetic line. Though many of these diabetic mice were infertile during this experiment, they often became pregnant in subsequent matings. This evidence suggests that long-term mating is necessary for reproduction in the

Table 4. Reproductive performance of the diabetic and non-diabetic lines

Line	Parturition rate* (%)	Litter size	Weaning rate (%)
Non-diabetic	82.4 (17)	9.4 ± 1.0 (14)	100.0 (115)
Diabetic	39.0 (41)	10.1 ± 1.7 (15)	100.0 (129)

The period of mating was 2 weeks.

() : No. of mice examined.

* : No. of delivery females/No. of females mated.

diabetic line. Most of the mothers before the onset of diabetes could nurse their pups well (Table 4). However, most of mothers showing overt diabetes could not complete weaning their pups due to their short life.

Other abnormalities such as abdominal distention and spinal curvature were observed following the onset of diabetes. Some mice became soiled with urine, and their fur became damp and yellowish. At the terminal stage, the animals showed wasting syndromes and finally entered a comatose state and died. The autopsy findings of the diabetic mice revealed atrophy of the thymus and spleen and dilatation of the intestines, gall bladder, and cecum. Elongation of the intestines was also observed. Cataract eyes were noted only in one diabetic mouse.

3. Histological findings

Light-microscopic observations were carried out on the pre-diabetics and the diabetics. Several morphologic alterations of the Langerhans' islets had occurred not only in the diabetics but also in the pre-diabetics over five weeks of age. In the pancreases of the pre-diabetics, the most prominent findings were infiltration of peri- and intra-insular mononuclear cells, mainly lymphocytes. The cellular infiltration was also observed around the peri-ductal and peri-vascular structures (Photo 2). At the more advanced state, most of the original cells constituting the islets decreased in number and were entirely replaced by the lymphocytes (Photo 3). The inflammatory reaction generally showed a tendency to

disappear at the terminal stage of the pre-diabetes (Photo 4). A morphologic picture of a overt diabetic mouse is shown in Photo 5. The pancreatic islet was exceedingly shrunken and small in size as compared with a normal islet (Photo 1). The decrease in the number of islets was also significant. In some cases, lymphocyte infiltration disappeared (Photo 5). These islets were characterized by the absence of aldehyde fuchsin positive cells (Photo 8). In contrast, many beta cells with well preserved aldehyde fuchsin positive granules were seen in the pancreatic islets of the pre-diabetic mouse (Photo 7). The incidence of cellular infiltration at the age of five weeks was 82.0% in females and 57.5% in males, respectively (data not shown). Sex difference was not remarkable for the spontaneous occurrence of cellular infiltration. In some cases, lymph node formation was observed around the pancreatic tissue or adjacent to the pancreatic lobules; however, the exocrine glands of the pancreases showed no remarkable changes in both the diabetic and pre-diabetic stages.

Apart from the pancreas, peri-vascular aggregations of the lymphocytes in the kidney and liver were observed occasionally, but similar changes were also noted in some of the non-diabetic control. Most of the other organs, including the heart, brain, spleen, adrenal glands, hypophysis, testes, and ovaries, appeared to be normal.

Discussion

The present report has shown the

breeding of non-obese type diabetic strain of mice. The incidence of diabetes was constant throughout six generations of selective breeding, being approximately 60 to 80% in females and about 10% in males around the age of 150 days or older. The control line was simultaneously bred from a sister strain. We called these lines "non-obese diabetic : nod" mouse and "non-obese normal : non" mouse.

The characteristic features of this diabetic mouse are : (1) abrupt onset of symptoms with polyuria, polydipsia, glucosuria and hyperglycemia accompanied by rapid weight loss, (2) infiltration of lymphocytes into the islets and conspicuous reduction in the number of beta cells and the size of islets, and (3) insulin deficiency [28]. Recently, Nakhood et al. [19, 20] reported on spontaneous diabetic rats not associated with obesity. Although they did not establish a strain of diabetic rat, the clinical symptoms and pathological findings of the pancreas were almost the same as our results. These characteristics are similar to those of human diabetes of the juvenile-onset type.

Pancreatic insulinitis has been reported by Gepts [10] in juvenile diabetes of patients autopsied shortly after the onset of clinical symptoms. Such a histological change has also been demonstrated recently in streptozotocin-induced diabetic mice [17] and previously in Wistar rats with spontaneous diabetes [19,20]. Two hypotheses can be proposed concerning the pathogenesis of insulinitis, i. e., viral infection and immunological impairment. There is much evidence suggesting a relationship between viral infection and the onset of diabetes, particularly of insulin-dependent juvenile-onset type diabetes [5, 9, 18, 24, 26]. Coxsackie B virus [8, 9, 29], mumps virus [27] and Reovirus [23] have been assumed to be responsible for the etiology of diabetes, though the participation of viruses is still inconclusive except for EMC virus [2,3,4] in mice. EMC virus can definitely produce diabetes characterized by hyperglycemia,

hypoinsulinemia, glucosuria, polydipsia, beta cell destruction, and insulinitis in the islets of pancreas [2,4,11]. The symptoms observed in this report resemble those of diabetes induced with EMC virus in many respects except the following : (1) low incidence of onset in males [2, 7], (2) progressive weight loss [2], (3) 100% mortality [2], and (4) the absence of extensive myocardial necrosis [3].

Recently, the possibility of the presence of an infectious agent in the development of spontaneous diabetes in guinea pigs was suggested by Lang and Munger [15,16]. Although an infectious agent has not yet been isolated, they regard this disease as contagious because healthy guinea pigs introduced into the colony of the diabetic animals develop similar syndromes. During the course of the present study, however, no diabetic symptoms were observed in the DBA/2, C57BL, C3H mice or the other few inbred strains which were kept in the vicinity of the diabetic mice.

Another possible mechanism for the onset of diabetes is pathogenesis through some immunological events. The following four types of experimental immune insulinitis are known (Kawanishi et al. [14], and Basterie [1]) : (1) insulinitis by active immunity to insulin like that which develops in cattle immunized with homologous or heterologous insulin mixed with Freund's adjuvant, (2) insulinitis by passive immunity as seen in mice and rats injected with anti-bovine or anti-porcine insulin serum of guinea pigs, (3) insulinitis due to immunity to pancreas-specific tissue and antigens as produced in mice and rats immunized with pancreas extract, and (4) insulinitis due to deposition of immune complexes in the islets which are formed by the combination of some viruses with high affinity to pancreas and antibodies. The last mechanism is most likely responsible for spontaneous diabetes in nod mice.

A conspicuous sex difference was observed in the spontaneous onset of diabetes.

Such a difference, however, was not seen for the incidence of pancreatic insulinitis. Consequently, we can speculate that the androgenic hormone represses the spontaneous onset of the disease in males. Whether the sex hormones play a role or not is presently under investigation and analysis of the pathogenesis of the nod mouse from genetical, immunological and ultrastructural standpoints is also in progress.

Acknowledgments

We wish to thank Prof. K. Kondo of Nagoya University for his constant interest and guidance in this investigation. We are also grateful to Dr. N. Wakasugi of the Nagoya University for careful review of this manuscript and to Dr. M. Harada of the Shionogi Research Laboratory for his valuable suggestions.

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ヤセ型糖尿マウスの育種

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多尿, 尿糖強陽性を呈し, 急激な衰弱を伴い死亡した 1 匹の雌マウスが CTS 系マウスの亜系で偶然発見された。子孫をもとに 8 対の交配系を作成し, 親の糖尿発症および繁殖力を指標とし, 糖尿マウスの選択を試みた。6 世代にわたる選択により, 糖尿発症系 (nod) とその対照系 (non) の 2 系統が育成された。

nod マウスの顕性糖尿出現には著明な雌雄差が認められ, 30 週令までの累積発症率は雌で 80%, 雄で 20% 程度であった。発症は急激に出現し, 発症後は体重減少, 衰弱が急速であった。自然回復する動物はみられず, 放置すると約 1~2 ケ月で死亡した。しかし, インスリンを連続投与することにより, 体重増加がみられ, ある程度寿命を延長させることが可能であった。

病態の生化学的特徴としては, 多尿, 多飲, 高血糖, 尿糖強陽性, 高コレステロール血症がみられた。また, 病理学的特徴としては膵ラ氏島に種々の程度のリンパ球浸潤が認められた。リンパ球浸潤の出現は, 5 週令以上の糖尿発症前期の動物にも高率に認められ, その出現には糖尿発症でみられたような著明な雌雄差はみられなかった。顕性糖尿マウスの膵ラ氏島はいずれも強い萎縮像を呈し, 島の数ばかりでなく, 島を構成する細胞数にも著減がみられた。

病因については不明であるが, 育種された nod マウスはヒトの若年型インスリン絶対不足糖尿病のモデル動物としての使用が期待出来るものと考えられる。

Explanation of Microphotographs

Photo 1. Pancreatic islet of a normal mouse. H-E. 200x.

Photos 2 to 4. Pancreatic islets of the diabetic mice in the pre-diabetic stage. Lymphocyte infiltration around the islet and the neighboring dilated ductal and vascular structures is visible (Photo 2). When these changes accelerated, most of the cells constituting the islet were entirely replaced by the lymphocytes (Photo 3). Lymphocyte infiltration tended to increase with age, but at the terminal stage of pre-diabetes only a small number of residual lymphocytes was found adjacent to the ductal structures and the islets (Photo 4). H-E. 200x.

Photo 5. Pancreatic islet of a diabetic mouse with hyperglycemia. Compared with a

normal islet (Photo 1), the islet (arrow) is exceedingly small in size. Note the disappearance of lymphocyte infiltration around the atrophied islet. H-E. 200x.

Photo 6. Pancreatic islet of a normal mouse. The islet shows wellgranulated beta cells (dark stain). Aldehyde fuchsin. 200x.

Photo 7. Pancreatic islet of a diabetic mouse in the pre-diabetic stage. Most of the residual cells of the islet are aldehyde fuchsin positive (dark stain). Aldehyde fuchsin. 200x.

Photo 8. Another islet (arrow) found in the same pancreas shown in Photo 5. Note the complete absence of aldehyde fuchsin positive cells. Aldehyde fuchsin. 200x.