Restriction fragment length polymorphism analysis of major histocompatibility complex genes in the non-obese diabetic mouse strain and its non-diabetic sister strains

Y. Fujishima¹, Y. Koide¹, T. Kaidoh¹, M. Nishimura² and T. O. Yoshida¹

¹ Department of Microbiology and Immunology, and

² Institute for Experimental Animals, Hamamatsu University School of Medicine, Handa, Hamamatsu, Japan

Summary. It has been suggested that one of the recessive genes controlling diabetes in non-obese diabetic mice is linked to the major histocompatibility complex. We, therefore, performed restriction fragment length polymorphism studies of major histocompatibility complex genes (class I, II, and III) in non-obese diabetic mice in comparison with those of their non-diabetic sister strains, non-obese non-diabetic, cataract, and ILI mice which were derived from the same Jcl-ICR mice as the non-obese diabetic mouse was. When class II and III probes and a minimum of four restriction enzymes were used, class II and III genes of non-obese diabetic mice were indistinguishable from those of cataract and ILI mice but totally different from those of non-obese non-diabetic mice. The studies also indicated that A β , E β , and C4-Slp genes of non-obese diabetic, cataract, and ILI mice, and A α , A β , E β and C4-Slp genes of non-obese non-diabetic mice are different from those of BALB/c and C57BL/6 mice, respectively. While non-obese non-diabetic mice expressed the E α gene, non-obese diabetic, cataract, and ILI mice appeared to carry a deletion in the 5' end of the E α gene resulting in failure to

transcribe the E α gene. When class I probe was used, cataract mice showed very different band patterns from those of the other ICR-derived mice. It is suggested that non-obese diabetic, non-obese non-diabetic, and ILI mice contain only a single class I D region gene. Taken together, these results indicate that, although class I loci of non-obese diabetic and ILI mice were serologically typed as K^d and D^b, and those of non-obese non-diabetic mice were typed as K^b and D^b, no H-2^g type recombination between K and D loci of non-obese diabetic, cataract, and ILI mice was evident. Since cataract and ILI mice are suggested to share the same class II and III regions with non-obese diabetic mice, they should be feasible strains for further studies to characterise the major histocompatibility complex-linked diabetogenic gene(s) of the non-obese diabetic mouse strain.

Key words: Major histocompatibility complex, non-obese diabetic mouse, non-obese non-diabetic mouse, cataract mouse, ILI mouse, insulin-dependent diabetes, restriction fragment length polymorphisms.

The non-obese diabetic (NOD) mouse strain spontaneously develops insulin-dependent diabetes mellitus. The disease is presumed to be of autoimmune etiology since it is characterised by lymphocyte infiltration into the pancreatic islets (insulinitis) [1–4], autoantibodies against islet cells [3, 5], and the ability of the disease to be transferred to NOD nu/nu mice by splenic lymphocytes of NOD mice [6]. In breeding studies with NOD and C57BL/6 mice, it has been revealed that two recessive genes on independent chromosomes are responsible for the insulinitis [7]. In crosses of NOD mice with normal C3H mice, Hattori et al. [8] detected a recessive diabetogenic gene linked to the major histocompatibility complex (MHC). In separate reports [9, 10], it has been suggested that at least three recessive genes, including one linked to the MHC, control the develop-

ment of diabetes in NOD mice. From the cDNA sequencing studies, Acha-Orbea and McDevitt [11] suggested that the unique sequence of I-A β allele in NOD mice may make the A β gene the MHC-linked susceptibility gene. Nishimoto et al. [12] reported that the expression of I-E molecules in NOD mice can prevent the development of insulinitis and suggested that the failure of the E α gene expression, in concert with other recessive gene(s) controlling insulinitis, allows NOD mice to develop insulinitis. However, precise characterisation and localisation of the MHC-linked diabetogenic gene(s) in NOD mice have been not yet determined. Therefore, further studies of MHC genes in the NOD mouse and its sister strains provide very useful information to characterise MHC-linked diabetogenic gene(s) in NOD mice.

Fig. 1. Schematic map of the major histocompatibility complex (MHC) genes. **a** Class II region and hybridisation probes for restriction fragment length polymorphism (RFLP) analysis of class II genes (adapted from Steinmetz et al. [13]). Genes are shown as open boxes (□). **b** Class I region and hybridisation probes for RFLP analysis of class I genes. Genes are shown as open boxes (□). The “7 cross” indicates a sequence that cross-hybridises strongly with probe 7 (adapted from Stephan et al. [14]). **c** Class III region and hybridisation probes for RFLP analysis of class III genes (adapted from Nonaka et al. [16]). Genes are shown as closed boxes (■).

In this study, we have performed restriction fragment length polymorphism (RFLP) studies of MHC genes (class I, II, and III) in NOD mice in comparison with those of non-obese non-diabetic (NON), cataract (CTS), and ILI mice, which are non-diabetic inbred strains but are derived from the same Jcl-ICR mice as NOD mice was.

Materials and methods

Mouse strains

NOD, NON, BALB/c, and C57BL/6 mice were bred in our Institute for Experimental Animals. CTS mice were kindly provided for a preparation of DNA and RNA by Dr. S. Makino (Aburahi Laboratory, Shionogi, Shiga, Japan). ILI mice were kindly provided by Dr.

Fig. 2. Southern blot analysis of ICR-derived mice DNA with class II probe 3 (Eβ) after digestion with Kpn I or Hind III. Ten μg of enzyme-digested DNA per lane was run on 0.7% agarose gels, blotted, and hybridised with the probe. Sizes of DNA marker fragments are shown at the left of the figure

H. Kato (Central Institute for Experimental Animals, Kawasaki, Japan). The NOD and NON strains were established in the course of the establishment of the CTS mouse strain from commercial outbred Jcl-ICR mice [1]. The ILI strain was also established from Jcl-ICR mice in Central Institute for Experimental Animals (Kawasaki, Japan).

DNA probes

The hybridisation probes were derived from the following cosmid clones isolated from BALB/c or AKR DNA libraries [13, 14]. Class II MHC probes (Fig. 1-(a)): Probe 1 (Aβ) 5.2 kb Eco RI fragment from 41.1; probe 2 (Aα), 4.7 kb Eco RI-Bam HI fragment from 24.2; probe 3 (Eβ), 2.0 kb Eco RI fragment from 24.2; probe 4 (5' probe of Eα), 3.4 kb Sal I fragment from 32.1; probe 5 (3' probe of Eα), 1.6 kb Sal I-Sma I fragment from 32.1; probe 6, 0.4 kb Acc I-Sac I fragment (the sequence spanning the promoter region and the signal peptide exon of the Eα^d gene) from 32.1. Class I D region probes (Fig. 1-(b)): probe 7, 6.2 kb Hpa I fragment (the region between D^d and D₂^d genes) from 11.20; probe 9, 0.8 kb Hpa I fragment (5' flanking sequences of the D^k gene) from k 1.3; probe 10, 0.6 kb Dpn I fragment (the segment between D₃^d and D₄^d genes) from the 5.2 kb Nru I-Sma I fragment of 16.1. These class II and class I region cosmid clones were kindly provided by Dr. M. Steinmetz (Basel Institute for Immunology, Basel, Switzerland). Class I MHC probe (Fig. 1-(b)): probe L is a 1.6 kb Xba I fragment (5' half of the coding region of the L^d gene) from genomic clone pL^d-4 [15] which was kindly provided by Dr. T. Shiroishi (National Institute of Genetics, Mishima, Japan). Class III MHC probe (Fig. 1-(c)): probe 8 is “sex-limited protein (Slp)” γ chain cDNA, isolated from cDNA clone pFSIp/5.2 [16] which was kindly provided by Dr. M. Takahashi (Department of Immunobiology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan).

Southern blot analysis

The procedure used was adapted from the protocol of Nasmyth [17]. Genomic DNA (10 μg) from mouse spleens or testes was digested with restriction enzymes, and was electrophoresed in a 0.7% agarose gel, and transferred to Hybond N nylon filters (Amersham, Tokyo,

Japan). The membrane was hybridised to the probe labelled by nick-translation or by random hexanucleotide priming (a specific activity of about $1\sim3\times10^8$ c.p.m./ μg). Pre-hybridisation treatment of the membrane was carried out for 1 h at room temperature in a mixture containing 54% Nasmyth solution (0.55 mol/l NaCl, 0.166 mol/l Na_2HPO_4 , 5.5 mmol/l EDTA pH 6.2, 1.85% sodium lauryl sarcosinate, 18.5% dextran sulfate) and 0.46% of 10 mg/ml salmon sperm DNA. Hybridisation was performed in the same buffer at 65°C for 48 h. The filters were washed with $3\times\text{SSC}$ (0.3 mol/l NaCl, 0.03 mol/l sodium citrate), 0.1% sodium dodecyl sulfate (SDS) for 5 min at room temperature and then washed with $2\times\text{SSC}$, 0.1% SDS for 5 min at room temperature, and with $1\times\text{SSC}$, 0.1% SDS for 15 min at 68°C, and with $0.2\times\text{SSC}$, 0.1% SDS for 15 min at 68°C. The filters were dried and exposed to X-ray films for 3–4 days at -70°C with intensifying screen.

Northern blot analysis

Twenty μg of total RNA prepared from mouse spleens by the guanidinium-isothiocyanate method [18] was electrophoresed on a 1.0% agarose formaldehyde gel, and transferred to Hybond N. The membrane was hybridised to a ^{32}P -labelled nick-translated probe in a mixture containing 54% Nasmyth solution [17], 46% formamide and 0.46% of 10 mg/ml Salmon sperm DNA for 24 h at 37°C. After hybridisation, the filters were washed down to $0.2\times\text{SSC}$ at 68°C, dried, and exposed to X-ray films.

Statistical analysis

RFLP analysis using class I probe L was evaluated by the percentage of coincidence of RFLP to NOD DNA. The formula for this calculations as follows: percent coincidence of RFLP = $(1 - \text{discordant band number to NOD of test DNA} / \text{total fragment number detected in mice studied with probe L}) \times 100$.

Results

Restriction fragment length polymorphism analysis using class II major histocompatibility complex probes

Mouse strains established from Jcl-ICR mice, NOD (K^d , D^b), NON (K^b , D^b) (K. Moriwaki, personal communication), CTS (?), and ILI (K^d , D^b) and the two mouse strains BALB/c (H-2^d) and C57BL/6 (H-2^b) were analysed for RFLP of class II genes. BALB/c and C57BL/6 mice were chosen as control strains since the class I loci of the NOD mouse have been typed serologically as K^d and D^b . The genomic loci of each probes are shown in Figure 1-(a). We chose these probes because RFLP patterns in various MHC haplotypes of mouse strains have been reported using them and restriction enzymes, Eco RI, Bam HI, Hind III, and Kpn I [13, 19]. The results are presented in Table 1. Southern blot analysis using probe 3 (E β) with Kpn I and Hind III is illustrated in Figure 2. With probe 1 (A β), RFLP patterns of NOD, CTS, and ILI DNA were indistinguishable, but NON mice showed quite different band patterns from the other three ICR-derived

Table 1. Results of restriction fragment length polymorphism analysis using class II major histocompatibility complex probes

Probe 1		H-2	Eco RI	Bam HI	Hind III	Kpn I
mouse strain						
NOD	g	6.6 ^a	12.5	2.7	6.4 5.5	1.9 13
NON	b	7.0	9.6	2.7 2.3	8.2	>30
CTS	?	6.6	12.5	2.7	6.4 5.5	1.9 13
ILI	g	6.6	12.5	2.7	6.4 5.5	1.9 13
BALB/c	d	6.6	12.5	2.7	6.4 2.8	1.9 >30
C57BL/6	b	7.0	9.6	2.3	8.2 4.7	>30
Probe 2		H-2	Eco RI	Bam HI	Hind III	Kpn I
mouse strain						
NOD	g	11.5		6.0	11.0	11.0
NON	b	11.5		6.0	6.1 2.7	15.0
CTS	?	11.5		6.0	11.0	11.0
ILI	g	11.5		6.0	11.0	11.0
BALB/c	d	11.5		6.0	11.0	11.0
C57BL/6	b	11.5		6.0	8.2 1.3	23.5
Probe 3		H-2	Eco RI	Bam HI	Hind III	Kpn I
mouse strain						
NOD	g	2.1		13.0	8.0	23.5
NON	b	2.1		4.8	3.1	15.0
CTS	?	2.1		13.0	8.0	23.5
ILI	g	2.1		13.0	8.0	23.5
BALB/c	d	1.9		5.5	8.0	15.0
C57BL/6	b	2.1		13.0	8.0	10.5
Probe 4		H-2	Eco RI	Bam HI	Hind III	Kpn I
mouse strain						
NOD	g	8.4	3.0	7.4 7.0	7.2	8.6 3.0
NON	b	9.6	3.0	7.4	8.1	8.6 3.7
CTS	?	8.4	3.0	7.4 7.0	7.2	8.6 3.0
ILI	g	8.4	3.0	7.4 7.0	7.2	8.6 3.0
BALB/c	d	9.6	3.0	7.4	8.1	8.6 3.7
C57BL/6	b	8.4	3.0	7.4 7.0	7.2	8.6 3.0
Probe 5		H-2	Eco RI	Bam HI	Hind III	Kpn I
mouse strain						
NOD	g	7.6		5.6	8.6	3.2
NON	b	8.4		6.2	9.0	4.0
CTS	?	7.6		5.6	8.6	3.2
ILI	g	7.6		5.6	8.6	3.2
BALB/c	d	8.4		6.2	9.0	4.0
C57BL/6	b	7.6		5.6	8.6	3.2

^a Fragment sizes are in kb

mice with all four restriction enzymes. NOD, CTS, and ILI mice were different from BALB/c mice with Hind III and Kpn I and also different from C57BL/6 mice with every four enzymes. NON mice were distinct from BALB/c mice when Eco RI, Bam HI, and Hind III were used and also distinct from C57BL/6 mice with Bam HI and Hind III. With probe 2 (A α), Hind III and Kpn I generated polymorphism. NOD, CTS, ILI and BALB/c mice showed the same RFLP patterns. NON mice, however, showed distinct patterns from the other ICR-derived mice, BALB/c, and C57BL/6 mice. With probe 3 (E β), NOD, CTS, and ILI mice showed identical band patterns. However, NON,

Fig. 3. Northern blot analysis of A α , A β , E α , and E β transcripts in NOD and NON mice. Twenty μ g of total RNA per lane was electrophoresed on 1% agarose formaldehyde gels, blotted, and hybridised with class II probes. A α (probe 2), A β (probe 1), E α (probe 4), E β (probe 3) indicate probes used

Fig. 4. Southern blot analysis of ICR-derived mice with probe 6 after digestion with Kpn I. Probe 6 (0.4 kb Acc I-Sac I fragment from cosmid clone 32.1) corresponds to the fragment spanning the promoter region and the signal peptide exon of the E α gene. Sizes of DNA marker fragments are shown at the left of the figure

BALB/c, or C57BL/6 mice exhibited distinct RFLP patterns, respectively, and were different from NOD, CTS, and ILI mice. With probe 4 (5' probe of E α) and 5 (3' probe of E α), NOD, CTS, and ILI mice shared the RFLP patterns with C57BL/6 mice. NON and BALB/c mice shared another pattern.

The E β ₂ probe, 3.2 kb Eco RI fragment from cosmid clone 32.1 [13], with the four restriction enzymes, did not generate any polymorphism (data not shown). To analyse RFLP of A β ₂ and A β ₃, we used probe 1 and a human DP β cDNA probe. Probe 1 cross-hybridised with A β ₂ [20], and the human DP β probe cross-hybridised with A β ₃ [21]. Probe 1 with Pvu II and Pst I, and

Fig. 5. Southern blot analysis of ICR-derived mice with class I probe L after digestion with Bam HI or Eco RI. Ten μ g of DNA was electrophoresed on 0.7% agarose gels, blotted, and hybridised with the probe. Sizes of DNA marker fragments are shown at the left of the figure

DP β probe with Eco RI showed no polymorphism in A β ₂ and A β ₃, respectively, of the mice studied (data not shown).

Taken together, these results indicate that the NOD class II MHC genes are indistinguishable from those of CTS, and ILI mice as long as the four restriction enzymes, Eco RI, Bam HI, Hind III, and Kpn I, were used. NON class II genes were distinct from those of NOD, CTS, and ILI mice. A β and E β genes of NOD, CTS, and ILI mice were different from those of BALB/c and C57BL/6 mice. A α , A β , and E β genes of NON mice were also different from those of BALB/c and C57BL/6 mice.

Class II major histocompatibility complex gene expression

NOD and NON mice were examined for class II gene expression. Northern blot analysis indicates that both NOD and NON mice synthesise A α , A β , and E β RNA, and that, while NON mice expressed E α RNA, NOD mice did not (Fig. 3). We have pursued the molecular mechanisms by which this defect in I-E expression is generated. Mice of haplotypes b and s bear a deletion spanning 627 bp which includes the promoter region and the signal peptide exon of the E α gene [22]. With probe 4, the 5' region of the E α gene, and the four re-

Fig. 6. Schematic representation of the results of RFLP analysis of ICR-derived mice with class I probe L. DNA was digested with each of the restriction enzymes, Eco RI, Bam HI, Hind III, and Kpn I. Black vertical bars (■) indicate hybridised bands

striction enzymes, DNA from NOD mice showed the same RFLP patterns as did those from C57BL/6 mice (Table 1). It is conceivable that the failure of the *Eα* gene expression in NOD mice is due to the same deletion of the *Eα* gene as observed in C57BL/6 mice.

To confirm our interpretation, we prepared probe 6 which is deleted in mice of haplotype b [23]. Probe 6 hybridised with 4.0 kb Kpn I fragments in NON and BALB/c mice; however, no hybridised band was evident in NOD, CTS, ILI, and C57BL/6 mice (Fig. 4). It is suggested that NOD, CTS, and ILI mice bear the same deletion spanning the promoter region and the signal peptide exon of the *Eα* gene, as do mice of haplotypes b and s [22–24]. This mutation must prevent transcription of the *Eα* gene and, hence, functional expression of the *Eα*–*Eβ* dimer on the cell surface in NOD, CTS, and ILI mice.

Restriction fragment length polymorphism analysis using class I major histocompatibility complex probe

To analyse RFLP of class I MHC, we prepared probe L (Fig. 1-(b)) which covers leader peptide, C₁, C₂ domains of L^d gene [15]. Figure 5 shows RFLP patterns using probe L with Bam HI and Eco RI. Since class I probes derived from coding regions cross-hybridise with not only the K and D genes but also the Qa genes [25], probe L detected many hybridised bands with every four enzymes. The results are schematically presented

Fig. 7. Southern blot analysis of ICR-derived mice with D region probes after digestion with Bam HI. Ten µg of enzyme-digested DNA per lane was run on 0.7% agarose gels, blotted, and hybridised with the probes. Probe 9; located in the 5' flanking sequences of D^k gene, probe 7; located in the region between D^d and D₂^d genes, probe 10; located in the segment between D₃^d and D₄^d genes. The approximate sizes of the fragments are shown at the right of the figure

in Figure 6. We analysed total hybridised bands as percentage of coincidence of RFLP to NOD class I DNA (see Statistical analysis). The percent coincidence of RFLP to NOD class I DNA of NON, CTS, ILI, BALB/c, and C57BL/6 DNA were approximately 88%, 39%, 90%, 57%, and 70%, respectively.

D region gene organisation

Five D region class I genes, D^d, D₂^d, D₃^d, D₄^d, and L^d have been identified in the BALB/c mouse, while only one has been found in mouse strains AKR and C57BL/10 [14]. To compare the D region gene organisation in the ICR-derived strains, probes 9, 7, 10 and restriction enzymes Bam HI were employed (Fig. 7). Probe 9, isolated from the 5' flanking sequences of the D^k gene (Fig. 1-(b)), detected the same restriction fragment in NOD, NON, CTS, ILI, and C57BL/6 DNA as in BALB/c DNA. On the other hand, Probe 7, isolated from the region between D^d and D₂^d genes (Fig. 1-(b)),

Fig. 8. Southern blot analysis of ICR-derived mice with class III probe 8 after digestion with each of the restriction enzymes Eco RI, Bam HI, Hind III, and Pst I. Ten μ g of enzyme-digested DNA per lane was electrophoresed on 0.7% agarose gels, blotted, and hybridised with the probe. The approximate sizes of the fragments are shown at the right of the figure

picked up a complementary sequence (6.5 kb fragment) only in BALB/c DNA. Probe 7 also picked up the 4.8 kb fragments in all mice tested since this probe cross-hybridises with a sequence, located in the 3' flanking sequence of the L gene [14]. Probe 10, isolated from the segment between D_3^d and D_4^d gene (Fig. 1-(b)), hybridised with the 6.0 kb fragment only in BALB/c DNA. These results suggest that the sequences located between the D and L genes are absent from NOD,

NON, CTS, ILI and C57BL/6 DNA. It is, therefore, possible that NOD, NON, CTS, and ILI mice contain only a single class I D region gene as do C57BL/10 mice.

Restriction fragment length polymorphism analysis using class III probe

Figure 8 shows RFLP studies using probe 8 (Fig. 1-(c)), which hybridises with C4 gene as well as Slp gene(s) [16]. Among ICR-derived strains, NOD, CTS, and ILI mice showed the same RFLP patterns. (Eco RI 23 kb <, 12.5 kb; Bam HI 8.8 kb, 7.4 kb, 5.0 kb; Hind III 23 kb, 4.0 kb; Pst I 2.3 kb, 2.0 kb, 1.0 kb, 0.9 kb), but NON mice showed different patterns when Bam HI was used (14 kb, 8.8 kb, 5.0 kb). NOD, CTS, and ILI mice were different from BALB/c mice with Bam HI and Pst I, and also different from C57BL/6 mice with Eco RI, Bam HI, Hind III and Pst I. NON mice were distinct from BALB/c mice when Pst I was used. NON mice also distinct from C57BL/6 mice when Eco RI, Hind III, and Pst I were used. Kpn I and Pvu II did not generate any polymorphism among all mice tested (data not shown). Taken together, these results indicate that the class III MHC genes of these mice were divided into four groups: (1) NOD, CTS, and ILI; (2) NON; (3) BALB/c; and (4) C57BL/6.

Discussion

In this study, we have performed RFLP studies of MHC genes in NOD mice in comparison with those of NON, CTS, and ILI mice, which are non-diabetic inbred strains but are derived from the same Jcl-ICR mice as NOD mice.

A summary of RFLP analysis using class I, II and III MHC probes and a minimum of four restriction enzymes, and class I D region gene organisation, are schematically presented in Figure 9.

By using class II probes with the four restriction enzymes, Eco RI, Bam HI, Hind III, and Kpn I, NOD mice have been revealed to share RFLP patterns with CTS and ILI mice. $A\beta$ and $E\beta$ RFLP patterns of these mice were different from those obtained not only in BALB/c ($H-2^d$) and C57BL/6 ($H-2^b$) mice but also in AKR ($H-2^k$) and A.SW ($H-2^s$) mice [19]. In contrast to CTS and ILI mice, NON mice showed totally different RFLP patterns from those of NOD mice, and $A\alpha$, $A\beta$, and $E\beta$ genes of NON mice are different from those of b and d haplotypes. RFLP studies using the class III probe divided these mice into four groups, 1) NOD, CTS, and ILI, 2) NON, 3) BALB/c, and 4) C57BL/6.

According to Stephan et al. [14], the D region of BALB/c mice contains five class I genes, D, D_2 , D_3 , D_4 , and L; while only one has been found in AKR and C57BL/10 mice. From our results with a low-copy

	Class II				Class III	Class I	% coincidence of RFLP	D region gene organization
	A β	A α	E β	E α	Slp-C4			
NOD							100	D
NON							88	D
CTS							39	D
ILI							90	D
BALB/c							57	D, D2, D3, D4, L
C57BL/6							70	D

Fig. 9. Summary of restriction fragment length polymorphism (RFLP) analysis of MHC genes of class I, II and III, and class I D region gene organisation of NOD, NON, CTS, and ILI mice. Open boxes () RFLP patterns unique for NOD mice; oblique lined boxes () RFLP patterns unique for NON mice; dotted boxes () RFLP patterns unique for BALB/c mice; vertical lined boxes () RFLP patterns unique for C57BL/6 mice

probe and two single copy probes isolated from the D region, we suggest that NOD, NON, CTS, and ILI mice bear only a single class I D region gene. RFLP studies using class I probe L suggest that NOD, NON, and ILI class I DNA are similar, but that CTS mice have distinct class I DNA from those of NOD mice.

Taken together, these results indicate that, although the class I loci of the NOD and ILI mouse were serologically typed as K^d and D^b and those of the NON mouse were typed as K^b and D^b, the inter-class I region, covering the class II and class III genes of NOD, CTS, and ILI mice and those of NON mice derived from the same Jcl-ICR mice, are completely different from each other; and that no H-2^s type recombination between K and D loci in NOD, CTS, and ILI mice is evident. Further studies of the MHC genes of the other ICR-derived strains should define the precise events which generated these unique H-2 haplotypes of NOD, CTS, ILI, and NON mice.

Furthermore, these results suggest that the class II and III genes of ILI and CTS mice are identical to those of NOD mice. Although precise characterisation and localisation of the MHC-linked diabetogenic gene(s) in NOD mice have been not yet determined, the possibility exists that ILI and CTS mice, but not NON mice, possess the gene(s). In this case, since ILI and CTS mice are not known to develop insulinitis or diabetes, they are presumed not to possess the other genes necessary for development of insulinitis and diabetes. This possibility could be confirmed by breeding studies.

It has been suggested that NOD mice lack E α mRNA [8]. We found that NOD mice, as well as CTS and ILI mice, bear a deletion spanning the promoter region and the signal peptide exon of the E α gene, resulting in failure of the E α gene expression. In contrast to NOD, CTS, and ILI mice, NON mice express the E α gene. Nishimoto et al. [12] reported that the expression of I-E molecules in NOD mice can prevent the development of auto-immune insulinitis. However, it has been suggested that the other MHC-linked genes including I-A are involved in the development of insulinitis or diabetes [11]. In this context, ILI and CTS mice might be useful for further analysis of the MHC-linked diabetogenic gene(s) of NOD mice.

To investigate the MHC-linked diabetogenic gene, we are constructing congenic or recombinant congenic mice in which the H-2 complex of the NOD mouse is superimposed on the back-ground gene of the NON mouse. It has been suggested that the NON mouse bears one of the two insulinitis genes but lacks the MHC-linked gene [7]. We are also currently engaged in trying to confirm, by breeding studies, our hypothesis that ILI mice bear the MHC-linked diabetogenic gene(s).

Note added in proof

During preparation of this paper, Ikegami et al. reported that, using monoclonal antibodies, the CTS mouse has similar class II antigens to the NOD mouse but has different class I antigens (Diabetologia 31, 254-258, 1988).

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Dr. Y. Fujishima

Department of Microbiology and Immunology

Hamamatsu University School of Medicine

3600 Handa, Hamamatsu

Japan