EXTENDED REPORT

An epistatic effect of the female specific loci on the development of autoimmune vasculitis and antinuclear autoantibody in murine lupus

M-C Zhang, N Misu, H Furukawa, Y Watanabe, M Terada, H Komori, T Miyazaki, M Nose, M Ono

Ann Rheum Dis 2006;65:495–500. doi: 10.1136/ard.2005.040832

See end of article for authors' affiliations

Correspondence to: Professor Masao Ono, Department of Pathology, Tohoku University Graduate School of Medicine, 2-1 Seiryō, Aoba-ku, Sendai, Miyagi 980-8575, Japan; onomasao@mail.tains.tohoku.ac.jp

Accepted 26 August 2005
Published Online First
8 September 2005

Objective: To identify the genetic loci regulating the incidence and severity of renal autoimmune vasculitis developed in murine lupus.

Methods: Vasculitis of renal arteries was histopathologically evaluated in MRL/Mp-Fas^{lpr} (MRL/lpr), C57BL/6-Fas^{lpr} (B6/lpr), (MRL/lpr×B6/lpr) F₁, and MRL/lpr×(MRL/lpr×B6/lpr) F₁ backcross mice. Using genomic DNA samples of the backcross mice, genome-wide scans, association studies, and linkage analyses were carried out based on genotypes of polymorphic microsatellite markers. Correlations of vasculitis grade and levels of various autoantibodies were also evaluated.

Results: Two recessive susceptibility loci of the MRL allele were identified on chromosomes 4 and 1, which had previously been defined as the autoimmune related loci termed *Arvm1* and *Sle-1/Nba2*, respectively. The former was epistatic to the latter in a female specific manner. The titre of antinuclear autoantibody (ANA) in IgG class, but not ANA in IgM class or anti-dsDNA in either IgG or IgM class, correlated significantly with vasculitis grade.

Conclusions: The present loci have been reported in previous studies using a different set of murine strains, suggesting that they are of importance in the development of autoimmune vasculitis in murine models. The concomitance of autoimmune vasculitis and IgG ANA suggests a shared genetic factor regulating these traits.

Several studies have shown a significant genetic predisposition towards developing common autoimmune diseases, including systemic lupus erythematosus (SLE). Taking advantage of inbred genetic composition and high genetic penetrance to disease onset, murine autoimmune models have provided not only evidence to support a genetic predisposition, but also valuable clues to the genetic interpretation of human diseases—that is, polygenic modes of inheritance, candidate genes, and genetic interactions.

The MRL/lpr mice spontaneously develop a spectrum of autoimmunity resembling human SLE. These mice stably develop glomerulonephritis, systemic vasculitis, arthritis, and sialadenitis in parallel with serum autoimmune traits and remarkable lymphoproliferation.¹ MRL/lpr is the mutant strain defective in expression of the *cd95* gene.² As *cd95* encodes the receptor CD95 which mediates the apoptosis signal in lymphocytes,³ its defective mutation (*lpr*) may explain lymphoproliferation and a defect in activation-induced cell death of T cells.^{2,4,5} The *cd95* sufficient mice MRL/+ are known to develop glomerulonephritis and pancreatitis with serum autoimmune traits very late in life,⁶ but the severity of glomerulonephritis and the levels of autoantibodies are much reduced in comparison with MRL/lpr, indicating that the *lpr* is primarily associated with the accelerated pathogenesis of autoimmunity in MRL/lpr mice. Importantly, the *lpr* congenic mouse B6/lpr barely develops autoimmune diseases.¹ This further indicates that MRL specific genetic factors other than the *lpr* are a prerequisite for the development of autoimmune diseases in MRL/lpr mice.^{7,8} Serum autoimmune traits such as ANA and anti-dsDNA antibody are also known to depend on a genetic background other than the *lpr*.⁸ It is unknown what strain specific genetic factors are critically involved in the development of autoimmune traits.

We previously reported that renal vasculitis and glomerulonephritis were genetically segregated in the MRL/lpr×(MRL/lpr×B6/lpr) F₁ backcross mouse.⁹ The results suggested a differential genetic control for the development of each disease. However, this remains to be substantiated. Our primary aim in the present study was to examine the genetic loci associated with vasculitis. To achieve this, we prepared a large number of MRL/lpr×(MRL/lpr×B6/lpr) F₁ backcross mice (MBN₂). In all, 458 MBN₂ mice were subjected to genetic study to define the susceptibility loci to renal vasculitis. Quantitative trait locus (QTL) analysis finally showed two significant loci explaining the pathogenesis in female mice. The two loci were on chromosomes 4 and 1 in a recessive susceptibility mode of inheritance, and the former locus had an epistatic effect on the latter. As the latter locus overlapped the common interval previously represented as *Sle-1* or *Nba2*—which was known to be crucially associated with ANA production¹⁰—we sought for a correlation between vasculitis and autoantibody emergence in MBN₂ female mice. We present new evidence for epistatic interaction of the known loci and a serum autoimmune trait indicative of autoimmune vasculitis.

METHODS

Mice

MRL/lpr and B6/lpr mice were originally purchased from the Jackson Laboratory (Bar Harbor, Maine, USA) and bred under specific-pathogen-free condition in the Integrated Centre for Sciences (INCS) in Ehime University. Using (MRL/lpr×B6/lpr) F₁ (MBF₁) and MRL/lpr×(MRL/lpr×B6/lpr) F₁ backcross mice (MBN₂) were prepared and housed in

Abbreviations: ANA, antinuclear autoantibody; QTL, quantitative trait locus; SLE, systemic lupus erythematosus

Table 1 Incidence of vasculitis in MRL/lpr, B6/lpr, MBF1, and MBN2 mice

Strain	Sex	Vasculitis grade			Incidence‡
		0	1	2	
MRL/lpr	Female	10	26	20	46/50 (82.1%)
	Male	4	9	13	22/26 (84.6%)
	Total	14	35	33	68/82 (82.9%)
B6/lpr	Female	10	0	0	0/10 (0%)
	Male	10	0	0	0/10 (0%)
	Total	10	0	0	0/10 (0%)
MBF1*	Female	25	7	1	8/33 (24.2%)
	Male	18	3	0	3/21 (14.3%)
	Total	43	10	1	11/54 (20.4%)
MBN2†	Female	124	49	37	86/210 (41.0%)
	Male	155	64	29	93/248 (37.5%)
	Total	279	113	66	179/458 (39.1%)

* (MRL/lpr × B6/lpr)F1.
† MRL/lpr × (MRL/lpr × B6/lpr)F1.
‡ Grades 1 and 2 were regarded as positive for vasculitis.

the same centre and in the laboratory animal centre of Kawashima Co, Gifu, Japan. For all animal experiments, we observed the Ehime University guidelines for animal experimentation.

Histological examination and grading criteria of vasculitis lesion

Mice were killed under ether anaesthesia at 19 to 20 weeks of age. Serum samples were collected and stored at -80°C until use. The kidneys were fixed with 10% formalin in 0.01 M phosphate buffer (pH 7.2), and embedded in paraffin. Tissue sections were stained with haematoxylin and eosin (H&E) or elastica-Masson (EM) for histological examination by light microscopy. Histopathological grading for renal vasculitic lesions was done as described elsewhere.¹¹ In brief, the severity of vasculitis was graded as: 0, normal to minimal perivascular lymphocyte infiltration; 1, moderate perivascular cell infiltration associated with destruction of external elastic lamina; 2, intimal thickening with the destruction of internal elastic lamina in addition to the findings of grade 1. We observed more than 20 arteries in six sections prepared from bilateral kidneys, and defined the vasculitis grade of the animal with the maximum grade in those observations. An animal with one or more vasculitic lesions diagnosed as grade 1 or 2 was considered to have vasculitis.

Measurements of autoantibodies

The ANA titre was measured by immunoreactivity to normal murine hepatocytes as described previously.¹² Nuclear samples were prepared by stamping minced liver tissue on a glass slide, drying it completely, and fixing it in ice cold acetone for 10 minutes. Before co-incubation of serum samples and nuclear samples, serum samples were incubated with 5 µg/ml dsDNA for two hours at 4°C to adsorb possible cross reactivity to dsDNA. After masking nuclear samples with 10% normal goat serum, serial dilutions of the serum samples were incubated on nuclear samples for two hours at 4°C. After washing with phosphate buffered saline, they were further incubated with the secondary antibody, either FITC conjugated γ chain specific anti-mouse IgG (Zymed, South San Francisco, California, USA) or μ chain specific anti-mouse IgM (Zymed).

The titre of the ANA index was determined as the maximum dilution point at which the nuclear signal could still be identified: 0, no staining with 1/100 dilution; 1, faintly stained with 1/100 dilution; 2, strongly stained with 1:100 dilution; 3, stained with 1:10³ dilution; 4, stained with 1:10⁴

dilution; 5, stained with 1:3 × 10⁴ dilution; 6, stained with 1:9 × 10⁴ dilution. Anti-dsDNA titres were measured by

Figure 1 Plots of the logarithm of odds (LOD) scores of the quantitative trait locus (QTL) for renal vasculitis on (A) chromosome 4 and (B) chromosome 1. We adopted composite interval mapping of model 6 in the Windows QTL Cartographer (V2.5) software. The control marker number and window size were 5 and 10 cM, respectively. Horizontal lines indicate the threshold levels of statistic significance, which were determined by the permutation test (1000 times permutations, p=0.05). Genetic positions with MIT markers are indicated on the horizontal axis.

enzyme linked immunosorbent assay (ELISA) as described previously.¹³ A unit for anti-dsDNA titre was defined as an equivalent titre in the serum of female MRL/lpr mice.

Mapping of vasculitis susceptibility loci

Genotypes of the MBN₂ mice were determined by microsatellite analyses. We first carried out selective mapping using 48 samples consisting of 24 non-affected mice (grade 0) and 24 affected mice (grade 2). In all, 126 microsatellite markers were used, which provided full coverage of the mouse autosomes, with an average of 12.9 cM and a maximum distance of 23 cM. The candidate chromosomes were determined using a statistical index ($p < 0.05$) given by the χ^2 test. Second, QTL analysis was undertaken on candidate chromosomes using whole MBN₂ samples. We determined the polymorphism of the *cd72* gene (forward 5'-CAGCGGCTTAGAGTTGC-3' and reverse 5'-GCAGGGCTTATGGAAGTAAT-3') and the *fcgr2b* gene, for which primers was kindly provided by Dr S Hirose (Juntendo University School of Medicine, Tokyo, Japan). The genetic positions of microsatellite markers and genes were based on information from the Mouse Genome Informatics (MGI), Jackson Laboratory.

Statistical analysis

In the association study, $p < 0.0034$ ($\chi^2 > 8.58$, 1df) and $p < 0.0001$ ($\chi^2 > 15.13$, 1df) were accepted as suggestive and significant linkages, respectively.¹⁴ The QTL analysis was undertaken using Windows QTL Cartographer (V2.5)

software developed by Zeng *et al* with histopathological grades of vasculitis as the indicator for the phenotype.¹⁵ In detail, composite interval mapping of model 6 was adopted, and the control marker number and window size was 5 and 10 cM, respectively. The walk speed was 2 cM, and the forward regression method was selected. The threshold level of statistical significance for QTL was determined by the permutation test (1000 times permutation at $p = 0.05$) developed by Churchill and Doerge.¹⁶ The Kruskal–Wallis test was used to determine the association between the autoantibody titre and vasculitis. We regarded $p < 0.05$ as significant in the Kruskal–Wallis test and the χ^2 test, and $p < 0.01$ in the paired comparison in three groups.

RESULTS

Incidence of renal vasculitis

In MBF₁, MBN₂, and MRL/lpr, renal vasculitis was characterised by a granulomatous arterial lesion associated with mononuclear cell infiltration in the perivascular region and destruction of the external elastic lamina. In addition, some mice showed intimal thickening accompanied by destruction of the internal elastic lamina. In an affected mouse with vasculitis, we were able to identify one or at most only a few lesions in 20 arteries studied, suggesting a sporadic incidence of vasculitis in the kidney. Irrespective of the severity, vasculitis generally affected a larger artery such as an interlobar or arcuate artery in the kidney. The pathological characteristics were very similar to those observed in MRL/lpr mice and intercrosses of MRL/lpr and C3H/HeJ-*Fas*^{lpr} (C3H/

Table 2 Association of microsatellite genotype and incidence of renal vasculitis in MBN2 mice

Chromosome/sex (marker in <i>italics</i>)	Position (cM)	Vasculitis				χ^2 , 1 df	p Value‡
		Negative*		Positive*			
		MM†	MB	MM	MB		
A							
Chr 4/Female							
<i>D4Mit319</i>	12.1	47	77	54	32	12.6	0.00039
<i>D4Mit271</i>	20.8	47	77	58	28	17.7	<0.0001¶
<i>cd72</i>	22.5	46	78	56	30	16.0	<0.0001¶
<i>D4Mit17</i>	31.4	52	72	58	28	13.2	0.00027
Chr 4/Male							
<i>D4Mit319</i>	12.1	70	85	48	45	0.97	0.32
<i>D4Mit271</i>	20.8	74	81	49	44	0.57	0.45
<i>cd72</i>	22.5	71	84	49	44	1.10	0.29
<i>D4Mit17</i>	31.4	65	90	44	49	0.68	0.41
Chr 4/Total							
<i>D4Mit319</i>	12.1	116	163	102	77	10.4	0.0013
<i>D4Mit271</i>	20.8	120	159	107	72	12.3	0.0005§
<i>cd72</i>	22.5	116	163	105	74	12.7	0.0004§
<i>D4Mit17</i>	31.4	116	163	102	77	10.4	0.0013
B							
Chr 1/Female							
<i>D1Mit268</i>	83.4	56	68	47	39	1.83	0.18
<i>fcgr2b</i>	92.3	54	70	50	36	4.32	0.04
<i>D1Mit356</i>	95.8	56	68	51	35	4.06	0.04
<i>D1Mit291</i>	101.5	60	64	44	42	0.16	0.69
Chr 1/Male							
<i>D1Mit268</i>	83.4	68	87	48	45	1.40	0.24
<i>fcgr2b</i>	92.3	69	86	48	45	1.17	0.28
<i>D1Mit356</i>	95.8	69	86	49	44	1.56	0.21
<i>D1Mit291</i>	101.5	69	86	49	44	1.56	0.21
Chr 1/Total							
<i>D1Mit268</i>	83.4	124	155	95	84	3.25	0.07
<i>fcgr2b</i>	92.3	123	156	98	81	4.96	0.03
<i>D1Mit356</i>	95.8	125	154	100	79	5.34	0.02
<i>D1Mit291</i>	101.5	129	150	93	86	1.43	0.23

*Grades 1 and 2 were regarded as positive for vasculitis.

†Genotypes of MM and MB indicate MRL/MRL homozygote and MRL/B6 heterozygote, respectively

‡ χ^2 test (df, degrees of freedom).

§Significant linkage.

¶Suggestive linkage.

Table 3 Association of microsatellite genotype and incidence of renal vasculitis in the *CD72MM* group* of MBN2 mice

Chromosome/sex (marker in <i>italics</i>)	Position (cM)	Vasculitis				χ^2 , 1 df	p Value
		Negative†		Positive†			
		MM‡	MB	MM	MB		
Chr 1/Female							
<i>D1Mit268</i>	83.4	17	28	32	24	3.75	0.05
<i>fcgr2b</i>	92.3	13	32	32	24	8.06	0.005
<i>D1Mit356</i>	95.8	13	32	32	24	8.06	0.005
<i>D1Mit291</i>	101.5	15	30	28	28	2.83	0.09
Chr 1/Male							
<i>D1Mit268</i>	83.4	25	46	24	25	2.27	0.13
<i>fcgr2b</i>	92.3	24	47	24	25	2.78	0.10
<i>D1Mit356</i>	95.8	24	47	25	24	3.56	0.06
<i>D1Mit291</i>	101.5	24	47	24	25	2.78	0.10
Chr 1/Total							
<i>D1Mit268</i>	83.4	42	74	56	49	6.55	0.01
<i>fcgr2b</i>	92.3	37	79	56	49	10.4	0.0013§
<i>D1Mit356</i>	95.8	37	79	57	48	11.3	0.0008§
<i>D1Mit291</i>	101.5	39	77	52	53	5.75	0.02

**CD72MM* group includes MRL/MRL homozygotes at the *cd72* locus.

†Grades 1 and 2 were regarded as positive individuals for vasculitis.

‡Genotypes of MM and MB indicate MRL/MRL homozygote and MRL/B6 heterozygote, respectively.

§Suggestive linkage (χ^2 test).

lpr) strains.¹¹ The histological evaluation of the severity of vasculitis in B6/*lpr*, MBF₁ and MBN₂ groups of mice is summarised in table 1. No vasculitis was observed in the B6/*lpr* group. On the other hand, the MBF₁ and MBN₂ groups showed a 20.4% and a 39.1% incidence of vasculitis, respectively. These rates were much lower than that of the parental MRL/*lpr* group (82.9%; $p < 0.01$ for MBF₁ and MBN₂). Vasculitis seemed inherited from MRL/*lpr* in an incompletely recessive manner. A sex difference in vasculitis incidence was not evident in all affected groups.

Mapping of vasculitis susceptibility loci

We first determined four candidate chromosomes in a genome-wide search using 126 informative microsatellite markers. Then, in an association study with all samples, we defined two markers, *D4Mit271* (20.8 cM) and *cd72* (22.5 cM), on chromosome 4 with statistically suggestive linkage ($p < 0.0034$) (table 2A). Of particular importance, significant linkage to these markers was shown only in the female group ($p < 0.0001$). These results were supported by

QTL analysis, in which the highest LOD (logarithm of odds) score (2.083) was given at *D4Mit271* only by the female group (fig 1A).

Epistasis of the two loci associated with the incidence and severity of vasculitis

The distal region of mouse chromosome 1 has often been reported as a susceptibility locus for autoimmune traits, including glomerulonephritis and autoantibody emergence.^{10 17–19} The present results showed a trace linkage of this region to vasculitis, though this was not supported by the statistics (table 2B). We then sorted a large panel of mice into two groups by *cd72* genotype, thereafter termed the MM group (MRL homozygote) and the MB group (MRL/B6 heterozygote), and each group was analysed in the way described above. We were able to identify a suggestive linkage of *fcgr2b* (92.3 cM) and *D1Mit356* (95.8 cM) in the MM group ($p < 0.0034$), but no linkage in the MB group (table 3). In QTL analysis, the MM group but not the MB group again showed a significant linkage (LOD = 3.408) at *D1Mit356* (fig 1B).

Next, we evaluated a combined effect of the two susceptibility loci, *D1Mit356* and *cd72*, on the incidence or the severity of vasculitis (table 4). The two susceptibility loci showed an additive effect on the incidence and the severity of vasculitis in a female specific manner. An additive effect was not proven statistically in the male group. Importantly, a susceptible contribution of the *D1Mit356* locus to the incidence and the severity of vasculitis was highly influenced by the *cd72* locus. These findings indicate epistasis of the *cd72* locus to the *D1Mit356* locus.

Autoantibody correlated with vasculitis

It has often been reported that autoantibody emergence is linked to a genetic interval in the distal region of chromosome 1 in murine lupus models.^{10 17–19} Because the present results proved a genetic linkage of this region to vasculitis in the female group, we looked for a quantitative correlation between vasculitis and autoantibody in the female group. ANA titres and anti-dsDNA were measured in serum samples collected from the 181 MBN₂ female mice. The results showed a significant correlation between ANA titre in the IgG class and vasculitis in the MM group of the *cd72* locus ($p = 0.00064$), but no correlation in the MB group ($p = 0.31$).

Table 4 Epistatic interaction of the *Arvm1* locus (*cd72*) and *D1Mit356*

Sex	Genotype		Incidence* (%)	Mean grade
	<i>cd72</i>	<i>D1Mit356</i>		
Female	MM †	MM	71.1‡	1.04§
	MM	MB	42.9‡	0.57§
	MB	MM	30.6	0.47
	MB	MB	23.4	0.32
Male	MM	MM	51.0	0.65
	MM	MB	33.8	0.42
	MB	MM	34.8	0.48
	MB	MB	33.9	0.46
Total	MM	MM	60.6‡	0.84§
	MM	MB	37.8‡	0.49§
	MB	MM	32.8	0.47
	MB	MB	29.2	0.40

*Percentage positive for vasculitis (grades 1 and 2).

†Genotypes of MM and MB indicate MRL/MRL homozygote and MRL/B6 heterozygote, respectively.

‡ $p < 0.01$ (χ^2 test).§ $p < 0.01$ (Mann-Whitney test).

Table 5 Correlation of autoantibody titre and vasculitis grade in MBN2 female mice

Genotype of <i>cd72</i> and autoantibody	Vasculitis grade†			p Value
	0	1	2	
CD72-MM*	n=37	n=31	n=19	
ANA-IgM	1.81 (0.97)	1.77 (1.15)	2.11 (0.88)	0.46
ANA-IgG	3.27 (0.84)	4.06 (1.09)	4.31 (1.11)	0.00064‡
dsDNA-IgM	0.47 (0.53)	0.44 (0.38)	0.51 (0.48)	0.41
dsDNA-IgG	0.19 (0.26)	0.23 (0.24)	0.14 (0.09)	0.59
CD72-MB*	n=70	n=13	n=11	
ANA-IgM	1.91 (1.11)	2.08 (1.19)	2.00 (0.77)	0.74
ANA-IgG	3.78 (1.23)	4.08 (1.32)	4.45 (1.37)	0.31
dsDNA-IgM	0.56 (0.54)	0.55 (0.49)	0.67 (0.48)	0.66
dsDNA-IgG	0.37 (0.56)	0.18 (0.19)	0.68 (1.15)	0.29

*Genotypes of CD72-MM and CD72-MB indicate MRL/MRL homozygous and MRL/B6 heterozygous at the *cd72* locus, respectively.

†Mean titre (SD) of autoantibody.

‡Significant difference in autoantibody titres by the three vasculitis grades (Kruskal–Wallis test).

Neither ANA titre in the IgM class nor anti-dsDNA in either class was significantly associated with vasculitis (table 5).

DISCUSSION

The two loci identified in this study are in agreement with previous studies that revealed similar autoimmune loci. The chromosome 4 locus in our study was found to overlap the *Arvm1* locus identified in the previous study.¹¹ The *Arvm1* locus was shown to be a significant susceptibility locus for renal vasculitis when a group of differently backcrossed mice—MRL/lpr×(MRL/lpr×C3H/lpr) F₁ (MCN₂)—was analysed. That study further suggested that an allelic variant of the *cd72* gene was a candidate genetic factor for vasculitis in the *Arvm1* locus. Indeed, the primary structure of CD72 is highly polymorphic between MRL/lpr and C3H/lpr.¹¹ CD72 is an inhibitory receptor influencing B cell antigen receptor signalling.^{20–22} It is possible that this allelic difference in CD72 affects B cell antigen receptor signalling, and somehow causes the development of autoimmunity. As the primary structure of CD72 in B6/lpr is exactly same as that of C3H/lpr, the *cd72* gene is highly polymorphic between MRL/lpr and B6/lpr.^{11 23} Again, our study supports the candidacy of the *Arvm1* locus. We would like to emphasise the significance of the *Arvm1* locus on an MRL/lpr genetic background for the development of autoimmune vasculitis.

Our findings showed a female specific efficacy for the *Arvm1* locus in MBN₂ mice. This is not found in previous studies with MCN₂ mice. Three possible reasons come to mind to explain this discrepancy. First, there could be a suppressive effect of minor loci in B6/lpr on the susceptibility of the *Arvm1* locus in the male. We might have overlooked such minor loci in our study. Second, there could be an epistatic effect of the B6/lpr Y chromosome on the *Arvm1* locus. The Y chromosome of MCN₂ male mouse originated from C3H/lpr, while that of MBN₂ male mouse originated from B6/lpr. To prove this point, experiments are necessary with reciprocal breeding to generate BMN₂ mice. Third, there might be a difference in the genes involved in the genesis of vasculitis in MCN₂ and MBN₂ cases. In this case, the function of the susceptibility gene could be controlled in a sex sensitive manner. Further studies are needed to explore these possible reasons.

The distal region of chromosome 1 defined in this study overlaps the loci previously defined as autoimmune susceptibility ones. Several lines of studies with different combinations of mouse strains and the congenic strains harbouring this region have emphasized the significance of this region in the development of autoimmunity, including glomerulonephritis and emergence of ANA.^{10 24 25} It is a new finding that

this region is a potent susceptibility locus to renal vasculitis in a sensitive mode to the *Arvm1* locus. This region is known to consist of many genes of immunological importance—for example, the inhibitory Fc receptor gene (*fcgr2b*),^{26–28} the signalling lymphocyte activation molecule (SLAM) family genes,^{29–34} and interferon inducible protein genes (*Ifi*).^{35 36} There is recent evidence for genetic polymorphism of this region in relation to mouse autoimmunity. According to the latest evidence, B6/lpr belongs to the group III of *fcgr2b* gene and the haplotype 1 of SLAM family genes, typical of non-autoimmune prone haplotypes. On the other hand, MRL/lpr and C3H/lpr can be defined as the group I or *autoimmune fcgr2b promoter haplotype* (AIH) of *fcgr2b* gene, and the haplotype 2 of SLAM family genes, typical of autoimmune prone haplotypes.^{37–39} It is possible that this haplotypic difference between B6/lpr and MRL/lpr is also involved in the development of vasculitis in association with ANA production. It is of particular interest to identify the genes in this region that are commonly involved in a broad spectrum of autoimmunity. In the previous study using MCN₂ mice, this region was not shown to be susceptible to vasculitis. This may reflect an overall correspondence of this region between C3H/lpr and MRL/lpr.

Our current data show an epistatic effect of the *Arvm1* locus on the susceptibility of the chromosome 1 locus. Further studies should be carried out to determine the mechanism of the epistatic interaction of the two loci. Interestingly, a recent study on human SLE has shown that *cd72* polymorphisms associated with alternative splicing modify the susceptibility to human SLE through epistatic interaction with *fcgr2b*.⁴⁰ The human loci, including the *cd72* and *fcgr2b* genes, are syntenic of the *Arvm1* locus and the distal region of chromosome 1, respectively. Although it is unclear whether or not the epistatic effect in human SLE is related to vasculitis, epistatic modes of complex loci may be important in understanding the genetic basis of the pathogenesis of SLE.

SLE usually develops a variety of disorders, including glomerulonephritis, vasculitis, dermatitis, interstitial pneumonia, and so on. There is increasing evidence from genetic studies on mouse SLE model to show that the development of each type of SLE disorder is controlled by a different set of genetic factors.⁹ This provides a possible interpretation for the pathogenic mechanism whereby human SLE has such a varied spectrum. Increased serum levels of autoantibodies are a universal feature of SLE in both human and murine models. Many kinds of autoantibody are detectable, but the genetic basis for the link between autoantibodies and SLE disease manifestations is unclear. Although the present study

was not designed to address this issue fully, our findings indicate a correlation between ANA of IgG type and vasculitis. Moreover, the difference in the contribution of each class of autoantibody to vasculitis suggests differential genetic regulation of the development of these antibodies, as suggested previously.⁸⁻⁴¹ ANA and anti-dsDNA are known to be correlated with the onset of glomerulonephritis; thus a single specificity of ANA for vasculitis is unlikely. Combined evaluation of two or more autoantibodies may be useful for the specific diagnosis of SLE variants.

Conclusions

We have identified a female specific vasculitis locus on chromosome 4 using MRL/lpr × (MRL/lpr × B6/lpr) F₁ back-cross mice. Epistasis of this locus to the major autoimmune related locus on chromosome 1 was demonstrated. Taking advantage of the murine autoimmune model, we should make further efforts to uncover the genetic interactions of the complex loci associated with polygenic diseases. Improvements in our understanding of this field will provide new insights into the pathogenic mechanisms of human SLE.

ACKNOWLEDGEMENTS

We thank Dr H Umeda, Dr T Tsubaki, and Dr M R Itoh for technical help; and Drs K Hata and Y Nozaki in the Laboratory Animal Centre of Kawashima Co Ltd and the members of the Integrated Centre for Sciences (INCS), Ehime University, for animal breeding. The study was supported by grants-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan to MO and MN.

Authors' affiliations

M-C Zhang, N Misu, H Furukawa, M Ono, Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan
Y Watanabe, M Terada, H Komori, T Miyazaki, M Nose, Department of Pathology, Ehime University School of Medicine, Toon, Japan

REFERENCES

- Theofilopoulos AN, Dixon FJ. Murine models of systemic lupus erythematosus. *Adv Immunol* 1985;**37**:269-390.
- Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 1992;**356**:314-17.
- Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 1991;**66**:233-43.
- Watson ML, Rao JK, Gilkeson GS, Ruiz P, Eichler EM, Pisetsky DS, et al. Genetic analysis of MRL-lpr mice: relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. *J Exp Med* 1992;**176**:1645-56.
- Alderson MR, Tough TW, Davis-Smith T, Braddy S, Falk B, Schooley KA, et al. Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* 1995;**181**:71-7.
- Kanno H, Nose M, Itoh J, Taniguchi Y, Kyogoku M. Spontaneous development of pancreatitis in the MRL/Mp strain of mice in autoimmune mechanism. *Clin Exp Immunol* 1992;**89**:68-73.
- Wang Y, Nose M, Kamoto T, Nishimura M, Hiai H. Host modifier genes affect mouse autoimmunity induced by the lpr gene. *Am J Pathol* 1997;**151**:1791-8.
- Vidal S, Kono DH, Theofilopoulos AN. Loci predisposing to autoimmunity in MRL-Fas lpr and C57BL/6-Fas lpr mice. *J Clin Invest* 1998;**101**:696-702.
- Nose M, Nishimura M, Kyogoku M. Analysis of granulomatous arteritis in MRL/Mp autoimmune disease mice bearing lymphoproliferative genes. The use of mouse genetics to dissociate the development of arteritis and glomerulonephritis. *Am J Pathol* 1989;**135**:271-80.
- Mohan C, Alas E, Morel L, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis. *Sle1* on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J Clin Invest* 1998;**101**:1362-72.
- Qu WM, Miyazaki T, Terada M, Lu LM, Nishihara M, Yamada A, et al. Genetic dissection of vasculitis in MRL/lpr lupus mice: a novel susceptibility locus involving the *CD72* allele. *Eur J Immunol* 2000;**30**:2027-37.
- Tosca A, Hatzis J, Haidas A, Varelzidis A. Evaluation of various antigenic substrates for the detection of antinuclear antibodies. *Dermatologica* 1981;**163**:401-7.
- Takahashi S, Nose M, Sasaki J, Yamamoto T, Kyogoku M. IgG3 production in MRL/lpr mice is responsible for development of lupus nephritis. *J Immunol* 1991;**147**:515-19.
- Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995;**11**:241-7.
- Zeng ZB. Precision mapping of quantitative trait loci. *Genetics* 1994;**136**:1457-68.
- Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics* 1994;**138**:963-71.
- Morel L, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1994;**1**:219-29.
- Vyse TJ, Rozzo SJ, Drake CG, Izui S, Kotzin BL. Control of multiple autoantibodies linked with a lupus nephritis susceptibility locus in New Zealand black mice. *J Immunol* 1997;**158**:5566-74.
- Hogarth MB, Slingsby JH, Allen PJ, Thompson EM, Chandler P, Davies KA, et al. Multiple lupus susceptibility loci map to chromosome 1 in BXSB mice. *J Immunol* 1998;**161**:2753-61.
- Adachi T, Wakabayashi C, Nakayama T, Yakura H, Tsubata T. *CD72* negatively regulates signaling through the antigen receptor of B cells. *J Immunol* 2000;**164**:1223-9.
- Pan C, Baumgarth N, Parnes JR. *CD72*-deficient mice reveal nonredundant roles of *CD72* in B cell development and activation. *Immunity* 1999;**11**:495-506.
- Kumanogoh A, Watanabe C, Lee I, Wang X, Shi W, Araki H, et al. Identification of *CD72* as a lymphocyte receptor for the class IV semaphorin *CD100*: a novel mechanism for regulating B cell signaling. *Immunity* 2003;**16**:321-31.
- Robinson WH, Ying H, Miceli MC, Parnes JR. Extensive polymorphism in the extracellular domain of the mouse B cell differentiation antigen *Lyb-2/CD72*. *J Immunol* 1992;**149**:880-6.
- Morel L, Blenman KR, Croker BP, Wakeland EK. The major murine systemic lupus erythematosus susceptibility locus, *Sle1*, is a cluster of functionally related genes. *Proc Natl Acad Sci USA* 2001;**98**:1787-92.
- Haywood ME, Rogers NJ, Rose SJ, Boyle J, McDermott A, Rankin JM, et al. Dissection of BXSB lupus phenotype using mice congenic for chromosome 1 demonstrates that separate intervals direct different aspects of disease. *J Immunol* 2004;**173**:4277-85.
- Bolland S, Ravetch JV. Spontaneous autoimmune disease in *FcγRIIB*-deficient mice results from strain-specific epistasis. *Immunity* 2000;**13**:277-85.
- Bolland S, Yim YS, Tus K, Wakeland EK, Ravetch JV. Genetic modifiers of systemic lupus erythematosus in *FcγRIIB*^{-/-} mice. *J Exp Med* 2002;**195**:1167-74.
- McGaha TL, Sorrentino B, Ravetch JV. Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science* 2005;**307**:590-3.
- Lee JK, Boles KS, Mathew PA. Molecular and functional characterization of a CS1 (CRACC) splice variant expressed in human NK cells that does not contain immunoreceptor tyrosine-based switch motifs. *Eur J Immunol* 2004;**34**:2791-9.
- Howie D, Simarro M, Sayos J, Guirado M, Sancho J, Terhorst C. Molecular dissection of the signaling and costimulatory functions of *CD150* (SLAMF1): *CD150*/SAP binding and *CD150*-mediated costimulation. *Blood* 2002;**99**:957-65.
- Tangye SG, Nichols KE, Hare NJ, van de Weert BC. Functional requirements for interactions between *CD84* and *Src* homology 2 domain-containing proteins and their contribution to human T cell activation. *J Immunol* 2003;**171**:2485-95.
- Tovar V, del Valle J, Zapater N, Martin M, Romero X, Pizcueta P, et al. Mouse novel *Ly9*: a new member of the expanding *CD150* (SLAMF1) family of leukocyte cell-surface receptors. *Immunogenetics* 2002;**54**:394-402.
- Lee KM, Bhawan S, Majima T, Wei H, Nishimura MI, Yagita H, et al. Cutting edge: the NK cell receptor 2B4 augments antigen-specific T cell cytotoxicity through *CD48* ligation on neighboring T cells. *J Immunol* 2003;**170**:4881-5.
- Peck SR, Ruley HE. *Ly108*: a new member of the mouse *CD2* family of cell surface proteins. *Immunogenetics* 2000;**52**:63-72.
- Hueber W, Zeng D, Strober S, Utz PJ. Interferon- α -inducible proteins are novel autoantigens in murine lupus. *Arthritis Rheum* 2004;**50**:3239-49.
- Rozzo SJ, Allard JD, Choubey D, Vyse TJ, Izui S, Peltz G, et al. Evidence for an interferon-inducible gene, *Ifi202*, in the susceptibility to systemic lupus. *Immunity* 2001;**15**:435-43.
- Jiang Y, Hirose S, Abe M, Sanokawa-Akakra R, Ohtsuiji M, Mi X, et al. Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. *Immunogenetics* 2000;**51**:429-35.
- Pritchard NR, Cutler AJ, Uribe S, Chadban SJ, Morley BJ, Smith KG. Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor *FcγRII*. *Curr Biol* 2000;**10**:227-30.
- Wandstrat AE, Nguyen C, Limaye N, Chan AY, Subramanian S, Tian XH, et al. Association of extensive polymorphisms in the *SLAMF1/CD2* gene cluster with murine lupus. *Immunity* 2004;**21**:769-80.
- Hitomi Y, Tsuchiya N, Kawasaki A, Ohashi J, Suzuki T, Kyogoku C, et al. *CD72* polymorphisms associated with alternative splicing modify susceptibility to human systemic lupus erythematosus through epistatic interaction with *FCGR2B*. *Hum Mol Genet* 2004;**13**:2907-17.
- Haywood ME, Hogarth MB, Slingsby JH, Rose SJ, Allen PJ, Thompson EM, et al. Identification of intervals on chromosomes 1, 3, and 13 linked to the development of lupus in BXSB mice. *Arthritis Rheum* 2000;**43**:349-5.