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Genes for Difference in Eosinophilic Phenotype between MES and BN.MES-*Cyba*^{mes} Rats Are on Chromosomes 9, 5, and 1

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Abstract: The Matsumoto Eosinophilia Shinshu (MES) rat strain develops hereditary blood eosinophilia due to the mutant *Cyba*^{mes} gene. In contrast, BN.MES-*Cyba*^{mes} congenic rats, in which the mutant *Cyba*^{mes} gene introduced into the background of the BN strain, have a normal blood eosinophil level despite showing robust proliferation of eosinophils in the bone marrow. However, the congenic rats manifest focal necrosis with eosinophilic infiltration in the liver, a phenotype rarely observed in the original MES rat strain. To elucidate the genetic basis for the strain differences, (MES × BN.MES-*Cyba*^{mes})F_2 rats were bred, and genetic analyses of phenotypes for eosinophilia were performed. Blood and bone marrow eosinophil levels in the F_2 rats showed broad distributions, suggesting that the traits were under the influence of multiple genes. Genetic association studies revealed that BN-derived marker loci on chromosomes 9 and 5 were responsible for the increase in eosinophil level in the bone marrow, decrease in blood eosinophil level, and the induction of focal necrosis with eosinophilic infiltration in the liver. The BN-derived allele of the marker gene on chromosome 1 was responsible for the decrease of both bone marrow and blood eosinophil levels. These data suggest the existence of genes characterizing/distinguishing the eosinophilic phenotypes of MES and BN.MES-*Cyba*^{mes} on these chromosomes, and form the basis for positional cloning studies of the genes. These studies will advance the understanding of the mechanisms involved in eosinophil mobilization from the bone marrow and recruitment to the organs.
Key words: *Cyba*, eosinophilia, genetic polymorphism, NADPH oxidase, rat strain

Introduction

The eosinophil is a type of white blood cell that plays an important role in the innate immune system [9]. Eosinophils develop and mature in the bone marrow and are subsequently mobilized into the peripheral circulation [1, 9]. The emigration of eosinophils from the bone marrow is a multistep process. Even though several molecules including IL-5, eotaxin, CCR3, adhesion molecules, and phosphatidylinositol 3-kinase are known to

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play critical roles in the release of eosinophils from the bone marrow into the peripheral circulation [6, 10, 25, 27], molecular mechanisms involved in these steps are not well understood. Eosinophils infiltrate into inflammatory sites in disease processes including asthma, and gastrointestinal disorders, and cause progressive damage by releasing eosinophilic factors. Recruitment of eosinophils to inflammatory tissues appears to be regulated by unique cytokine combinations that activate endothelial cells and induce tissue-resident cells to produce eosinophil-active chemokines and other chemoattractants such as eotaxins, which facilitate migration [31]. The precise mechanisms by which eosinophils are recruited to inflammatory sites remain poorly understood. Advances in understanding these issues could lead to new therapeutic options for diseases associated with eosinophils.

The Matsumoto Eosinophilia Shinshu (MES) rat strain has a genetic predisposition to develop eosinophilia [18, 20–24, 34]. In these rats, a marked increase in peripheral blood eosinophils (>500/μl) occurs at about nine weeks of age, with eosinophilia progressing with age until the number of eosinophils eventually exceeds the level that is characteristic of human idiopathic hypereosinophilia (>1,500/μl). Robust eosinophilic proliferation takes place in the bone marrow. Eosinophils infiltrate the mesenteric lymph nodes, where they degenerate, and release eosinophilic factors which cause granulomatous inflammation. Eosinophil-related gastroenteritis or aortitis are also observed. The primary cause of eosinophilia in MES rats is a loss-of-function mutation in the gene for cytochrome b(–245), alpha polypeptide (*Cyba*; also known as $p22^{phox}$), which is an essential component of the superoxide-generating NADPH oxidase complex [14, 19, 20].

In a previous study, we bred and characterized a congenic rat strain in which the mutant $Cyba^{mes}$ gene was introduced into the background of a BN strain (BN.MES-$Cyba^{mes}$) [37]. The congenic rats showed robust proliferation of eosinophils in the bone marrow. Nonetheless, the rats' blood eosinophil levels remained within the normal range. In addition, the rats manifested focal necrosis with eosinophilic infiltration in the liver, a phenotype rarely observed in the original MES rat strain. These results imply genetic polymorphisms exist between MES and BN strains that modulate the mobilization of eosinophils in the bone marrow and recruitment to the organs. We therefore hypothesized that gene(s) in the BN strain potently suppress the blood eosinophil level, but induce (or permit) infiltration of eosinophils in the liver. In order to elucidate the genetic basis for the strain differences, we bred an F_2 population derived from the two rat strains. All rats in this population should be homozygous for the mutant $Cyba^{mes}$ gene, while segregating other genes that might be responsible for the strain differences. Therefore, these rats were expected to segregate the phenotypes associated with eosinophilia, such as blood eosinophil level and focal necrosis with eosinophilic infiltration in the liver, enabling us to perform genetic association studies of these traits.

Materials and Methods

Rats and F_2 production

MES and BN.MES-$Cyba^{mes}$ rats were maintained at the Institute of Experimental Animals, Shinshu University. (MES × BN.MES-$Cyba^{mes}$)F_2 rat progenies were bred at the institute. F_2 rats were weaned at three weeks of age and sacrificed at 15 weeks of age. These rats were maintained under specific pathogen-free conditions, and had free access to a commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. All experimental procedures were carried out in accordance with the Regulations for Animal Experimentation of Shinshu University.

Hematologic and histological examination

Blood samples were collected from the jugular vein of rats at 15 weeks of age under ether anesthesia. In our previous study, we observed that the blood eosinophil count in (ACI × MES)F_1 × MES backcross rats homozygous for the mutant $Cyba^{mes}$ gene sacrificed at 12 weeks of age was significantly lower than in parental MES rats of the same age [14]. The reason for this difference was unclear. It was supposed that eosinophilia development was delayed in the heterogeneous genetic background of the backcross rats. Therefore, the rats were examined at 15 weeks of age in this study. The numbers of total leukocytes and eosinophils in the blood and bone marrow were counted with an automated cell counter (XT-2000iV,

Sysmex Co., Ltd., Kobe, Japan). After euthanasia by cervical dislocation, the liver was collected, conventionally processed, formalin-fixed, paraffin-embedded, sliced to 3 μm thickness and stained with hematoxylin-eosin (HE).

Statistic analysis

Statistical differences in the percentages of eosinophils in bone marrow and peripheral blood eosinophil counts in MES, BN, BN.MES-*Cyba*^{mes}, and (MES × BN.MES-*Cyba*^{mes})F_1 rats were evaluated by one-way analysis of variance (one-way ANOVA) followed by the Tukey-Kramer post hoc test. Probability values of $P<0.05$ were considered statistically significant. All statistic analyses were performed using StatView software (version 5.0; SAS Institute Inc., Cary, NC, USA).

Genetic association study

Genomic DNA was extracted from the liver specimens using the G NOME DNA kit (Quantum Biotechnologies, Carlsbad, CA, USA). Ninety-four microsatellite markers that are polymorphic between MES and BN/Ssn were used for linkage analysis. These markers included *D1Rat2* (3.5 cM), *D1Mit9* (23.8 cM), *D1Rat32* (53.8 cM), *D1Mit3* (76.6 cM), *D1Rat56* (85.7 cM), *D1Rat67* (95.9 cM), *D1Rat79* (106.1 cM), *D1Rat169* (122.1 cM), *D1Rat89* (145.9 cM), *D2Rat187* (0.0 cM), *D2Rat199* (18.5 cM), *D2Rat218* (44.6 cM), *D2Rat38* (60.5 cM), *D2Mgh12* (78.5 cM), *D2Rat106* (110.2 cM), *D3Rat54* (3.6 cM), *D3Rat42* (36.1 cM), *D3Rat164* (52.9 cM), *D3Rat4* (75.5 cM), *D3Rat1* (91.5 cM), *D4Rat3* (0.0 cM), *D4Rat9* (10.3 cM), *D4Rat13* (24.9 cM), *D4Rat103* (41.9 cM), *D4Mgh7* (71.2 cM), *D4Rat206* (96.6 cM), *D5Rat121* (3.6 cM), *D5Rat132* (23.8 cM), *D5Rat16* (43.4 cM), *D5Rat71* (54.6 cM), *D5Rat33* (74.0 cM), *D5Rat93* (85.3 cM), *D5Rat205* (94.3 cM), *D5Rat49* (105.7 cM), *D6Rat68* (13.3 cM), *D6Rat34* (33.0 cM), *D6Rat18* (53.4 cM), *D6Rat160* (76.0 cM), *D7Rat64* (4.6 cM), *D7Rat103* (25.4 cM), *D7Rat25* (36.7 cM), *D7Rat140* (52.3 cM), *D7Rat132* (62.4 cM), *D7Rat105* (88.5 cM), *D8Mit6* (10.3 cM), *D8Rat46* (32.5 cM), *D8Mgh4* (49.0 cM), *D8Rat11* (71.9 cM), *D9Rat44* (1.3 cM), *D9Rat135* (9.2 cM), *D9Rat131* (15.1 cM), *D9Rat130* (20.1 cM), *D9Rat10* (49.0 cM), *D9Rat106* (58.0 cM), *D9Rat110* (64.8 cM), *D9Rat1* (87.2 cM), *D10Rat96* (0.1 cM), *D10Rat42* (27.2 cM), *D10Rat25* (51.0 cM), *D10Rat84* (67.6 cM), *D10Rat108* (92.8 cM), *D11Rat19* (6.0 cM), *D11Rat5* (24.1 cM), *D11Rat1* (38.2 cM), *D12Rat57* (2.1 cM), *D12Rat28* (20.5 cM), *D12Rat46* (38.8 cM), *D13Rat59* (3.4 cM), *D13Rat126* (19.3 cM), *D13Mit3* (29.9 cM), *D14Rat54* (2.3 cM), *D14Rat36* (19.3 cM), *D14Kyo2* (46.9 cM), *D14Rat22* (66.2 cM), *D15Rat75* (9.6 cM), *D15Rat83* (25.6 cM), *D15Mgh8* (45.7 cM), *D15Rat106* (65.4 cM), *D16Mgh4* (9.5 cM), *D16Rat67* (18.1 cM), *D16Rat56* (36.3 cM), *D17Rat112* (11.6 cM), *D17Mgh5* (30.4 cM), *D18Rat30* (2.5 cM), *D18Rat95* (17.2 cM), *D18Rat45* (47.8 cM), *D19Rat15* (11.3 cM), *D19Rat46* (23.7 cM), *D19Rat59* (43.9 cM), *D20Rat48* (5.4 cM), *D20Rat10* (24.0 cM), *D20Mit1* (44.6 cM), *DXRat3* (0.0 cM), and *DXRat104* (44.6 cM). The genomic DNA was subjected to PCR amplification using 25-μl reaction volumes that contained 0.625 U *Taq* polymerase (Promega), 0.4 μM microsatellite primers, 125 ng template DNA, and 50 μM of each dNTP in a PCR buffer containing 1.5 mM $MgCl_2$. The conditions for PCR were as follows: 94°C for 1 min, 40 cycles of 94°C for 20 s, 50°C for 20 s, and 72°C for 1 min, followed by extension at 72°C for 1 min. The products were then separated by electrophoresis on 4% agarose gels stained with ethidium bromide and photographed with UV illumination using a digital camera.

For quantitative trait locus (QTL) analysis, genotyping data were analyzed using Map Manager QTXb20 [16]. Critical threshold values of likelihood ratio statistics (LRS) for significance of association were determined by the permutation test.

Results

Phenotypes of F_1 and F_2 rats for eosinophilia

The average blood eosinophil count in the (MES × BN.MES-*Cyba*^{mes})F_1 rats was 875/μl, which is above the normal range, but below that of MES rats (Fig. 1A). The average percentage of eosinophils in the bone marrow of (MES × BN.MES-*Cyba*^{mes})F_1 rats was 18.5%. This value was higher than that in BN (7.9%), but significantly lower than those in the parental strains: MES (31.5%) and BN.MES-*Cyba*^{mes} (29.0%) (Fig. 1B).

We bred 134 (MES × BN.MES-*Cyba*^{mes})F_2 rats (65 females and 69 males). The blood eosinophil counts

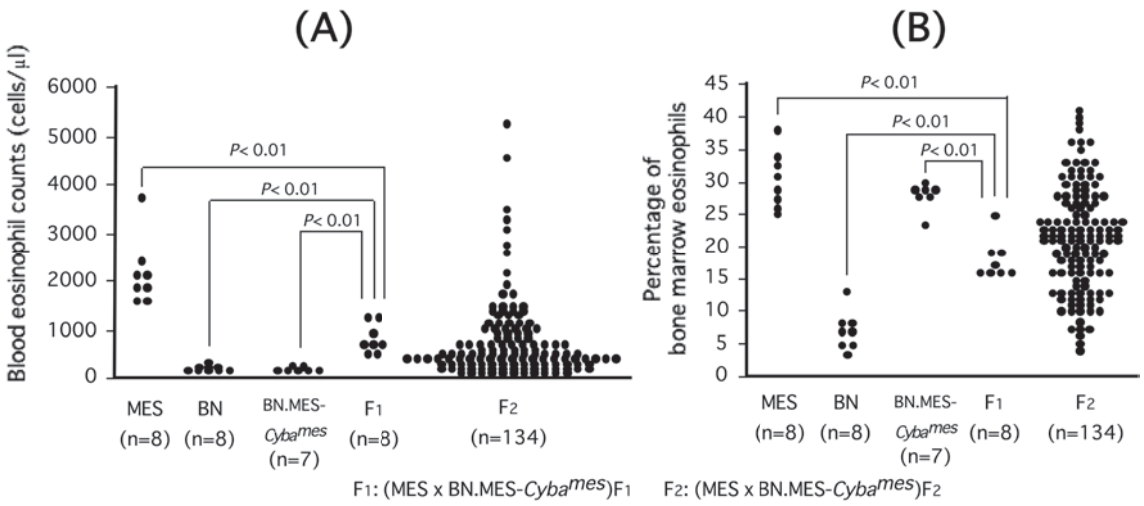

Fig. 1. Distribution of peripheral blood eosinophil counts (A) and percentages of eosinophils in bone marrow (B) in MES, BN, BN.MES-*Cyba*^{mes}, (MES × BN.MES-*Cyba*^{mes})F_1, and (MES × BN.MES-*Cyba*^{mes})F_2 rats at 15 weeks of age. Only the significant differences between (MES × BN.MES-*Cyba*^{mes})F_1 and parental strains are denoted.

(Fig. 1A) and percentages of eosinophils in the bone marrow (Fig. 1B) in the F_2 rats showed a broad distribution, suggesting that the traits were under the influence of multiple genes. Blood eosinophil counts in the F_2 rats ranged from 80 to 5,200/μl, with an average of 750/μl. There was no significant difference between the average values in females (781/μl) and males (723/μl) (P=0.69). The percentage of bone marrow eosinophils in the F_2 rats ranged from 4.1 to 41.2%, with an average of 21.4%. The average value for females was 24.7%, which was significantly higher than the average value in males (18.3%) (P<0.0001). As for the liver pathology, 79 F_2 rats had focal necrosis with eosinophilic infiltration with varying degrees of severity. Fifty-five F_2 rats were devoid of the lesions.

QTL analysis of blood eosinophil level

Segregation of genotypes of the 134 F_2 rats conformed to the expected ratio of BN/BN homozygote : BN/MES heterozygote : MES/MES homozygote = 1 : 2 : 1 for all autosomal marker loci examined (data not shown). For marker loci on chromosome X, segregation of genotypes conformed to the expected ratio of MES/MES homozygote : BN/MES heterozygote = 1 : 1 in female F_2 rats, and MES hemizygote : BN hemizygote = 1:1 in males (data not shown). Since the blood eosinophil counts and bone marrow eosinophil percentages in the F_2 rats showed a continuous distribution, we conducted QTL analysis of the traits. The analysis revealed significant and highly significant QTLs on chromosome 1, and suggestive QTLs on chromosomes 4, 3, 7, 8, and 5 (Table 1 and Fig. 2A). The QTL on chromosome 1 explained 15% of the total trait variance. Epistasis between these QTLs was not observed. The BN-derived allele was responsible for decreasing the blood eosinophil level for all of the QTLs.

QTL analysis of bone marrow eosinophil level

Because there was a sex difference in the percentage of bone marrow eosinophils in F_2 rats, female and male F_2 rats were separately analyzed for the trait. QTL analysis of females revealed significant QTLs on chromosomes 9 and 10, and suggestive QTLs on chromosomes 5, 2, and 7 (Table 2 and Fig. 2B). The BN-derived alleles on chromosomes 9, 5, 2, and 7 increased the proportion of bone marrow eosinophils, while the BN-derived alleles on chromosome 10 decreased the proportion. A highly significant QTL on chromosome 9 and significant QTL on chromosome 5 were also detected in males (Table 3 and Fig. 2B). The BN-derived allele on these chromosomes increased the proportion of bone marrow eosinophils. When the data for females and males were combined, the QTL on chromosome 9 explained 23% of the total trait variance. In males, sig-

Table 1. Marker loci associated with blood eosinophil level in the (MES × BN.MES-*Cyba*^{mes})F_2 progeny

Marker locus	Effect[a]	LRS[b]	%[c]	Blood eosinophil level (/μl; mean ± SD) (no. of rat)		
				BN/BN[d]	BN/MES[d]	MES/MES[d]
D1Rat67	(↓) additive	21.2***	15	440 ± 394 (39)	688 ± 812 (66)	1,308 ± 970 (29)
D1Rat32	(↓) additive	14.9**	11	513 ± 529 (41)	697 ± 626 (67)	1,261 ± 1,293 (26)
D4Rat3	(↓) dominant	10.9*	8	564 ± 443 (32)	652 ± 613 (70)	1,151 ± 1,259 (32)
D3Rat1	(↓) dominant	8.2*	6	597 ± 438 (26)	630 ± 532 (68)	1,053 ± 1,231 (40)
D7Rat25	(↓) additive	7.3*	5	499 ± 331 (41)	791 ± 911 (64)	1,014 ± 978 (29)
D8Rat46	(↓) dominant	6.8*	5	642 ± 421 (25)	626 ± 708 (68)	1,022 ± 1,072 (41)
D5Rat33	(↓) additive	6.6*	5	514 ± 304 (37)	755 ± 770 (64)	1,006 ± 1,162 (33)

[a]Effect of the BN allele on bone marrow eosinophil ratio: to decrease (↓) in either an additive or dominant manner. [b]Likely ratio statistic with *suggestive, **significant, and ***highly significant association. [c]The amount of the total trait variance which would be explained by a QTL at this locus, as a percent. [d]Genotype of rats, either homozygous for BN allele (BN/BN), heterozygous (BN/MES), or homozygous for MES allele (MES/MES). Only one locus with the highest LRS value is listed for each chromosome, except for chromosome 1, where two peaks in the LRS plot were observed.

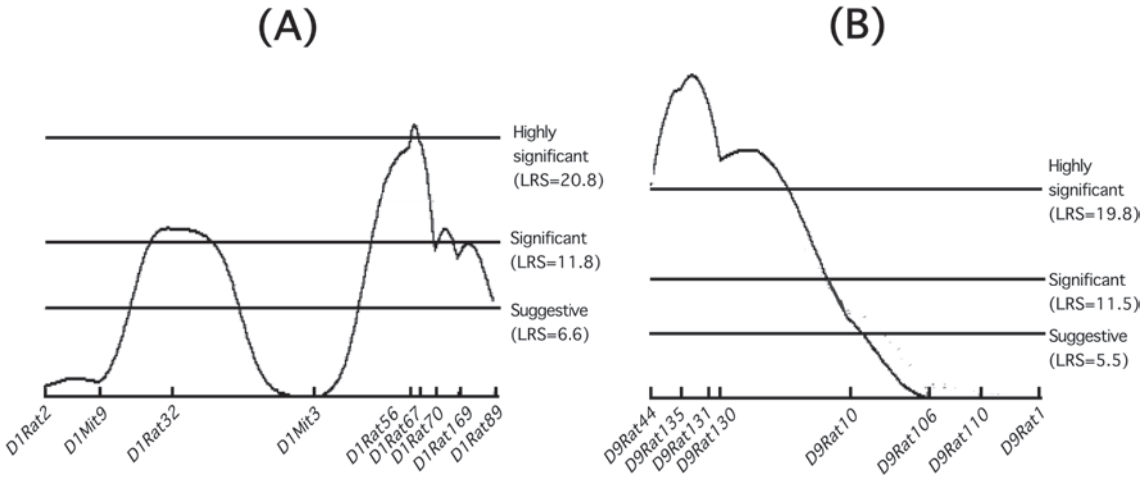

Fig. 2. LRS statistical score plot along chromosome 1 for blood eosinophil count (A) and along chromosome 9 for bone marrow eosinophil percentage (B) in (MES × BN.MES-*Cyba*^{mes})F_2 rats. The plots were drawn with the combined data for females and males. The horizontal lines indicate highly significant, significant, and suggestive linkage levels as determined by genome-wide permutation testing with MapManager QTX software.

nificant QTLs were revealed on chromosomes 1 and 19, and suggestive QTLs on chromosomes 6, X, 7, and 12. Epistasis between these QTLs was not observed.

Genetic association study of formation of focal necrosis with eosinophilic infiltration in the liver

We undertook a genetic association study of formation of focal necrosis with eosinophilic infiltration in the liver. The F_2 population was split into two groups according to the presence (n=79) or absence (n=55) of the lesion. Genetic association was evaluated by a χ^2 test of the segregation ratio of genotypes at the marker loci. In the F_2 rats with the lesions, significant deviation to the BN-derived allele was observed at loci on chromosomes 5 and 9 (Table 4). In the F_2 rats devoid of the lesions, significant deviation to the MES-derived allele was observed at these loci.

Discussion

This study of eosinophilic diseases in MES and BN.MES-*Cyba*^{mes} rat strains has shown that the pathol-

Table 2. Marker loci associated with bone marrow eosinophil percentage in the female (MES × BN.MES-*Cyba*^{mes})F_2 progeny

Marker locus	Effect[a)]	LRS[b)]	%[c)]	Bone marrow eosinophil ratio (%; mean ± SD) (no. of rat)		
				BN/BN[d)]	BN/MES[d)]	MES/MES[d)]
D9Rat131	(↑) additive	19.7**	26	28.7 ± 7.1 (18)	26.2 ± 6.6 (25)	19.9 ± 3.8 (22)
D10Rat84	(↓) dominant	13.2**	18	22.7 ± 5.7 (16)	23.1 ± 6.7 (31)	29.5 ± 6.3 (18)
D5Rat205	(↑) dominant	10.4*	15	27.2 ± 7.3 (18)	26.3 ± 7.0 (25)	21.1 ± 5.0 (22)
D2Rat218	(↑) dominant	9.8*	14	27.3 ± 8.1 (19)	25.9 ± 5.9 (25)	21.0 ± 5.6 (21)
D7Rat140	(↑) additive	6.9*	10	27.1 ± 7.5 (19)	24.8 ± 6.4 (36)	20.0 ± 5.4 (10)

[a)]Effect of the BN allele on bone marrow eosinophil percntage: increase (↑) or decrease (↓) in either additive or dominant manner. [b)]Likely ratio statistic with *suggestive and **significant association. [c)]The amount of the total trait variance which would be explained by a QTL at this locus, as a percent. [d)]Genotype of rats, either homozygous for BN allele (BN/BN), heterozygous (BN/MES), or homozygous for MES allele (MES/MES). Only one locus with the highest LRS value is listed for each chromosome.

Table 3. Marker loci associated with bone marrow eosinophil percentage in the male (MES × BN.MES-*Cyba*^{mes})F_2 progeny

Marker locus	Effect[a)]	LRS[b)]	%[c)]	Bone marrow eosinophil ratio (%; mean ± SD) (no. of rat)		
				BN/BN[d)]	BN/MES[d)]	MES/MES[d)]
D9Rat131	(↑) additive	28.9***	34	24.0 ± 5.3 (20)	18.2 ± 7.1 (33)	11.5 ± 5.6 (16)
D1Rat56	(↓) recessive	19.4**	25	14.1 ± 7.9 (22)	19.7 ± 7.1 (33)	21.6 ± 6.0 (14)
D5Rat93	(↑) recessive	12.8**	17	23.8 ± 6.8 (17)	16.6 ± 7.6 (39)	16.1 ± 5.7 (13)
D19Rat59	(↑) recessive	12.1**	16	23.3 ± 8.1 (19)	16.9 ± 6.8 (35)	15.3 ± 6.4 (15)
D6Rat68	(↑) recessive	10.7*	14	24.0 ± 7.1 (16)	17.2 ± 7.0 (33)	15.5 ± 7.1 (20)
DXRat104	(↓)	7.4*	10	15.5 ± 6.6 (30)	NA (0)	20.4 ± 7.8 (39)
D7Rat64	(↑) dominant	6.0*	8	20.5 ± 6.8 (16)	19.3 ± 8.2 (34)	14.8 ± 6.4 (19)
D12Rat57	(↑) recessive	5.5*	8	22.9 ± 7.4 (12)	17.6 ± 7.9 (39)	16.7 ± 6.3 (18)

[a)]Effect of the BN allele on bone marrow eosinophil percntage: increase (↑) or decrease (↓) in either an additive, dominant, or recessive manner. [b)]Likely ratio statistic with *suggestive, **significant, and ***highly significant association. [c)]The amount of the total trait variance which would be explained by a QTL at this locus, as a percent. [d)]Genotype of rats, either homozygous for BN allele (BN/BN), heterozygous (BN/MES), or homozygous for MES allele (MES/MES). Only one locus with the highest LRS value is listed for each chromosome.

Table 4. Segregation ratio of (MES × BN.MES-*Cyba*^{mes})F_2 rats with or without liver lesion for marker loci on chromosomes 5 and 9

Marker locus	Position	Rats with liver legion (n=79)			Rats without liver legion (n=55)			χ^2 value
	(cM)	BN/BN	BN/MES	MES/MES	BN/BN	BN/MES	MES/MES	
D5Rat121	3.6	23	39	17	18	18	19	7.76
D5Rat132	23.8	26	35	18	10	23	22	9.36
D5Rat16	43.4	25	35	19	8	29	18	5.72
D5Rat71	54.6	26	37	16	13	21	21	8.25
D5Rat33	74.0	28	41	10	9	23	23	16.91**
D5Rat93	85.3	31	41	7	2	25	28	39.36***
D5Rat205	94.4	30	41	8	3	26	26	31.76***
D5Rat49	105.7	27	42	10	3	23	29	33.67***
D9Rat44	1.3	31	36	12	5	23	27	28.83***
D9Rat135	9.2	33	34	12	7	25	23	22.46***
D9Rat131	15.1	33	32	14	5	26	24	25.27***
D9Rat130	20.1	31	34	14	6	25	24	21.09***
D9Rat10	49.0	22	38	19	3	31	21	13.02*
D9Rat106	58.0	21	42	16	10	29	16	2.42
D9Rat110	64.8	20	40	19	11	26	18	1.98
D9Rat1	87.2	23	34	22	16	23	16	3.12

*P<0.05; **P<0.01; ***P<0.001.

Table 5. Effect of BN-derived alleles on chromosomes 9, 5, and 1 in the (MES × BN.MES-*Cyba*^{mes})F_2 progeny

Chromosome	Trait		
	Blood eosinophil level	Bone marrow eosinophil percentage	Eosinophil infiltration in the iver
1	↓[a)]	↓[b)]	No effects
5	↓	↑	↑
9	No effects	↑	↑

[a)]Effect of the BN allele: decrease (↓) or increase (↑) in the level. [b)]This effect was observed only in males.

ogy is regulated in a complex fashion by multiple genes. The bone marrow eosinophil level in (MES × BN.MES-*Cyba*^{mes})F_1 rats was lower than those in both parental strains (Fig. 1B). This observation suggests the presence of different recessive genes in MES and BN strains that increase bone marrow eosinophil percentages in the condition of homozygous state of the mutant *Cyba*^{mes} gene. The blood eosinophil levels were reduced in F_1 rats because they were heterozygous for the recessive genes. Accordingly, the blood eosinophil levels in F_2 rats showed a broad distribution as they had different genotype combinations of the genes. This hypothesis is supported by the fact that both MES-derived and BN-derived genes were revealed in the QTL analysis, and the homozygosity of each of these genes was associated with increase in bone marrow eosinophil percentage in F_2 rats. However, the effects to reduce blood eosinophil level in F_2 rats were always associated with the BN-derived alleles. Thus, it appears that genes suppressing the blood eosinophil level were possessed exclusively by BN rats.

The data presented here show several genes were responsible for the differences in eosinophilic phenotype between MES and BN.MES-*Cyba*^{mes} rats. It is noteworthy that genes on chromosomes 1, 5, and 9 were associated with more than two traits, and explained a relatively large amount of the total trait variance. The genes on chromosomes 5 and 9 of the BN suppressed blood eosinophil levels, retained eosinophils in the bone marrow, and induced focal necrosis with eosinophilic infiltration in the liver (Table 5). The gene on chromosome 1 of the BN rats was responsible for decreases in both blood and bone marrow eosinophil levels. In our previous study, a QTL for blood eosinophil count (eosinophilia 3; *eos3*) was revealed on chromosome 1 in (MES × ACI) × MES backcross progeny [19]. The allele derived from MES had an additive effect, increasing the blood eosinophil level. The location of the QTL revealed in this study overlaps the *eos3* region. It is possible that the 2 QTLs are actually identical.

Chromosomal regions associated with blood eosinophil level [7, 8, 17], familial eosinophilia [30], and other eosinophil-associated diseases [2, 32] have been identified in humans. In a few cases, polymorphisms have been demonstrated in candidate genes for these traits [3, 8, 38]. In most cases, however, the molecular genetic basis of these traits has not been revealed. The chromosomal segments revealed in these reports for humans do not show syntenic conservation with chromosomes 9 and 5 of rats.

The data obtained in the present study will form the basis for positional cloning studies of the genes which regulate eosinophil mobilization in the bone marrow and recruitment to the organs. In order to confirm the existence and more precisely define the locations of the genes, we are now breeding reciprocal congenic strains for the three chromosomal regions, in which, for example, a segment of chromosome 9 from the MES rat has been introduced into the background of a BN.MES-*Cyba*^{mes} strain (and conversely from BN.MES-*Cyba*^{mes} to MES). There are a lot of candidate genes on chromosomes 1, 5, and 9, which are reportedly associated with eosinophil biology. Genes on chromosome 1 include Janus kinase 2 (*Jak2*), G protein-coupled receptor 44 (*Gpr44*), aldehyde dehydrogenase 1 family, member A1 (*Aldh1a1*), annexin A1 (*Anxa1*), and interleukin-33 (*Il33*). The JAK2 signaling pathway possibly participates in the IL5-induced survival of rat eosinophils [11].

It has been suggested that GPR44 mediate mobilization of eosinophils from bone marrow [33, 35]. ALDH1A1 can perturb murine hematopoiesis by promoting myeloid differentiation at the expense of lymphopoiesis [29]. Glucocorticoid-induced surface expression of ANXA1 blocks beta 2-integrin adhesion of human eosinophils to intercellular adhesion molecule 1 surrogate protein [15]. IL33 potently induces effector functions and cytokine production, enhances adhesion, and prolongs survival of eosinophils [4, 5, 26, 36]. Candidate genes on chromosome 5 include CD52 antigen (*Cd52*) and group V phospholipase A2 (*Pla2g5*). The function of CD52 antigen is not well understood. However, it is expressed on eosinophils, and expression of CD52 is enhanced in eosinophilic rhinosinusitis patients. In addition, treatment with an antibody against CD52 has been shown to completely deplete circulating eosinophils in renal recipients [39]. PLA2G5 induces beta 2-integrin-mediated adhesion of eosinophils [21]. Candidate genes on chromosome 9 include interleukin-17F (*Il17f*) and signal transducer and activator of transcription 1 (*Stat1*). IL17F has been shown to have a pro-inflammatory role in asthma. Moreover, IL17F induces several cytokines, chemokines and adhesion molecules in bronchial epithelial cells, vein endothelial cells, fibroblasts and eosinophils [13]. Phosphorylation of STAT1 causes enhanced ICAM1 surface expression and eosinophil adhesion [28]. Also, STAT1 plays a role in promoting the infiltration of leukocytes in experimental hepatitis by stimulating hepatocytes, sinusoidal endothelial cells, and Kupffer cells to produce multiple chemokines and adhesive molecules [12]. Thus, it is conceivable that polymorphisms in these genes could influence blood or bone marrow eosinophil levels, or eosinophil infiltration in the liver. Further investigation of candidate genes in the congenic regions will enable us to identify the responsible genes, advance our understanding of the mechanisms involved in eosinophil mobilization from the bone marrow and recruitment to the organs, and hopefully lead to therapeutic control of diseases associated with eosinophils.

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