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BN.MES-*Cyba*^{mes} Congenic Rats Manifest Focal Necrosis with Eosinophilic Infiltration in the Liver without Blood Eosinophilia

Hiroshi TOMOZAWA[1)], Ayako NISHIO[1)], Yuji OKUHARA[2)], Keiichi HIGUCHI[3)], Kiyoshi MATSUMOTO[1)], and Masayuki MORI[3)]

[1)]*Division of Laboratory Animal Research, Research Center for Human and Environmental Sciences, Shinshu University, 3–1–1 Asahi, Matsumoto 390-8621, Japan,* [2)]*Toxicology Research Laboratory, R & D, Kissei Pharmaceutical Co., Ltd., 2320–1 Maki, Hotaka, Azumino 399-8305, Japan, and* [3)]*Department of Aging Biology, Institute on Aging and Adaptation, Shinshu University Graduate School of Medicine, 3–1–1 Asahi, Matsumoto 390-8621, Japan*

Abstract: The Matsumoto Eosinophilia Shinshu (MES) rat strain develops hereditary blood eosinophilia and eosinophil-related inflammatory lesions in organs due to the mutant *Cyba*^{mes} gene. We hypothesized that a new eosinophilia model with a different phenotype could be established by changing the genetic background of rats. We bred and characterized a congenic strain, in which the mutant *Cyba*^{mes} gene was introduced into the background of a BN strain (BN.MES-*Cyba*^{mes}). The congenic rats showed robust proliferation of eosinophils in the bone marrow. Nonetheless, blood eosinophil levels of the rats 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 the presence of genetic polymorphisms between MES and BN strains which modulate the mobilization of eosinophils to the peripheral circulation and organs. The newly established BN.MES-*Cyba*^{mes} congenic rat strain, together with the original MES strain, will provide useful models for elucidating the molecular genetic mechanisms involved in the development and trafficking of eosinophils.

Key words: congenic rat, *Cyba*, eosinophilia, necrosis, polymorphism

Introduction

The eosinophil is a type of white blood cell that plays an important role in the innate immune system [9]. Eosinophils proliferate and differentiate in the bone marrow and are subsequently mobilized into the peripheral circulation [1, 9]. Under steady-state conditions, circulating eosinophils are maintained at 40–400/μl of blood in humans and 15–250/μl in rats. Eosinophilia is a condition in which abnormally high numbers of eosinophils are found in either blood (600–1500/μl) or tissues [4]. Eosinophilia may occur in a number of disease conditions, which include parasitic infections, allergies, collagen vascular diseases, and neoplastic disorders. A morbid condition distinct from these secondary eosinophilias, is idiopathic hypereosinophilic syndrome (IHES)

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Address corresponding: M. Mori, Department of Aging Biology, Institute on Aging and Adaptation, Shinshu University Graduate School of Medicine, 3–1–1 Asahi, Matsumoto 390-8621, Japan

in which eosinophilia persists without any apparent etiology [4]. Progressive tissue damage caused by eosinophilic factors released from infiltrating eosinophils is clinically important both in secondary eosinophilia and IHES. Major tissue targets include the skin, heart, and nervous system, with more than 50% of patients presenting clinical complications in each of these sites. Great clinical heterogeneity in eosinophil target tissues is observed in IHES patients. Defining the molecular steps involved in determination of target tissues is fundamental to understanding these disease processes and providing targets for novel therapeutic intervention.

The Matsumoto Eosinophilia Shinshu (MES) rat strain genetically develops eosinophilia [12, 16–18, 23]. 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). The 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 and 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 [11, 14, 15].

As animal models for eosinophilia, three transgenic mouse strains overexpressing IL-5 with different genetic backgrounds have been developed [5, 10, 27]. All these strains manifest blood eosinophilia. Intriguingly, these strains vary in their tissue distributions of eosinophils. These observations underscore the importance of genetic background in determining the tissue distribution of eosinophilic lesions. Given the mouse strain-specific differences, we hypothesized that a new eosinophilia model, having different phenotypes for eosinophilia useful for the elucidation of the molecular genetic mechanisms involved in eosinophil homeostasis, could be established by changing the genetic background of rats. We bred a congenic strain in which the mutant $Cyba^{mes}$ gene of the MES rat was introduced into the background of a BN strain. BN was selected as the recipient strain based on specific features of the strain. First, BN is considered to be the most appropriate rat strain for investigating allergic airway disease and asthma because of its pro-Th2 and proeosinophilic phenotypes [24, 26]. Secondly, the nucleotide sequence of the entire genome is known for the strain [7]. Thirdly, it is genetically remote from the MES strain (http://www.anim.med.kyoto-u.ac.jp:80/NBR/phylo.aspx). Here, we report that the BN.MES-$Cyba^{mes}$ congenic rats do indeed show a distinct phenotype with regard to eosinophilia compared to the original MES rat strain.

Materials and Methods

Breeding the BN.MES-$Cyba^{mes}$ congenic rat strain

The MES rats were maintained at the Institute of Experimental Animals, Shinshu University. The BN/Ssn (BN) rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan). A congenic strain BN.MES-$Cyba^{mes}$ that had the mutant $Cyba^{mes}$ gene in the BN background, was developed by standard procedures with seven consecutive backcrossings to BN. At each generation, rats heterozygous for the mutant $Cyba^{mes}$ allele were discriminated from homozygotes for the normal allele by PCR-genotyping [11], and used for breeding of the next generation. After seven generations of backcrossing, BN.MES-$Cyba^{mes}$ congenic rats homozygous for the mutant $Cyba^{mes}$ gene were obtained by intercross between heterozygous rats. Subsequently, it was found that female congenic rats are sterile. The reason for the sterility remains unclear. Accordingly, congenic rats have been maintained by mating homozygous males with heterozygous females. All the rats were maintained under specific pathogen-free conditions and were fed a commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*. All experimental procedures were carried out in accordance with the Regulations for Animal Experimentation of Shinshu University.

Determination of the congenic interval

BN.MES-$Cyba^{mes}$ congenic rats were genotyped for microsatellite DNA sequence polymorphisms in genes on chromosome 19: hydroxysteroid (17-beta) dehydro-

genase 2 (*Hsd17b2*), phospholipase C, gamma 2 (*Plcg2*), zinc finger, DHHC domain containing 7 (*Zdhhc7*), ankyrin repeat domain 11 (*Ankrd11*), and spastic paraplegia 7 homolog (*Spg7*). The nucleotide sequences of the primers were as follows:
Zdhhc7-1: 5'-GCAGAGGCAAGTATCACATG-3',
Zdhhc7-2: 5'-CACACGACTCTGGTCTTTCCAAAGTTG-3',
Ankrd11-1: 5'-CTGCCAGTTTCACAGTTACTGTC-3',
Ankrd11-2: 5'-TCCTGAGACCATAGGACCTG-3',
Spg7-1: 5'-TGCTACCAGGCCTAGACTTC-3', and
Spg7-2: 5'-CTGCCAAGCTTGATGACCTG-3'.

Rats were genotyped for microsatellite DNA sequence polymorphisms on other chromosomes: *D1Mit9* (23.8 cM), *D1Rat32* (53.8 cM), *D1Mit3* (76.6 cM), *D1Rat218* (101.5 cM), *D1Rat89* (145.9 cM), *D2Rat199* (18.5 cM), *D2Rat218* (44.6 cM), *D2Mgh12* (78.5 cM), *D2Rat106* (110.2 cM), *D3Rat54* (3.5 cM), *D3Rat40* (36.4 cM), *D3Rat4* (75.5 cM), *D4Rat133* (0.0 cM), *D4Rat13* (24.9 cM), *D4Rat103* (41.9 cM), *D4Rat61* (73.6 cM), *D5Rat121* (3.6 cM), *D5Rat132* (23.8 cM), *D5Rat71* (54.6 cM), *D5Rat33* (74.0 cM), *D5Rat49* (105.7 cM), *D6Rat68* (13.3 cM), *D6Rat34* (33.0 cM), *D6Rat18* (53.4 cM), *D6Rat160* (76.0 cM), *D7Rat35* (6.8 cM), *D7Rat103* (25.4 cM), *D7Rat15* (57.9 cM), *D7Rat105* (88.5 cM), *D8Mit6* (10.3 cM), *Thy1* (36.7 cM), *D8Mgh4* (49.0 cM), *D8Rat11* (71.9 cM), *D9Rat135* (9.2 cM), *D9Rat121* (41.0 cM), *D9Rat106* (58.0 cM), *D9Rat1* (87.2 cM), *D10Rat42* (27.2 cM), *D10Rat25* (51.0 cM), *D10Rat84* (67.6 cM), *D10Rat2* (93.9 cM), *D11Rat19* (6.0 cM), *D11Rat5* (24.1 cM), *D11Rat1* (38.2 cM), *D12Rat28* (20.5 cM), *D12Rat46* (38.8 cM), *D13Rat59* (3.4 cM), *D13Mit3* (29.9 cM), *D14Rat36* (19.3 cM), *D14Kyo2* (46.9 cM), *D14Rat22* (66.2 cM), *D15Rat69* (6.2 cM), *D15Rat83* (25.6 cM), *D15Rat22* (43.7 cM), *D15Rat29* (66.5 cM), *D16Mgh4* (9.5 cM), *D16Rat56* (36.3 cM), *D17Rat112* (11.6 cM), *D17Mgh5* (30.4 cM), *D18Rat30* (2.5 cM), *D18Rat95* (17.2 cM), *D18Rat58* (44.4 cM), *D20Rat59* (11.5 cM), *D20Rat10* (24.0 cM), *D20Mit1* (44.6 cM), *DXRat8* (7.9 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, Madison, WI, USA), 0.1 μM each primer, 125 ng template DNA, and 50 μmol of each dNTP in a PCR buffer containing 1.5 mM $MgCl_2$. The conditions for PCR were 94°C for 1 min, 40 cycles of 94°C for 20 s, 50°C for 20 s, and 72°C for 40 s, followed by 72°C for 1 min. The products were then separated by electrophoresis on 4% agarose gels stained with ethidium bromide and imaged under UV illumination using a digital camera.

Hematological and histological examination

Blood samples were collected from the jugular vein of rats at 15 weeks of age under ether anesthesia. After euthanasia by cervical dislocation, the liver and the mesenteric lymph nodes were collected for histology observation. 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). The tissues were conventionally processed, formalin-fixed, paraffin-embedded, sliced to 3 μm and stained with hematoxylin-eosin (HE). Serum aspartate aminotransferase and alanine aminotransferase levels were measured with an auto analyzer (Model 7150, Hitachi, Tokyo, Japan).

Semi-quantitative measurement of transcripts for chemokine (C-C motif) ligands

Three MES rats and three BN.MES-*Cyba*^{mes} rats were examined. mRNA was extracted from the bone marrow, spleen, mesenteric lymph nodes, liver, and colon using the QuickPrep Micro mRNA Purification Kit (Amersham Biosciences, Buckinghamshire, England). First-strand cDNA was synthesized using a First-Strand cDNA Synthesis Kit (Amersham Biosciences), and cDNA fragments containing a part of the coding sequence for three genes of chemokine (C-C motif) ligands, chemokine (C-C motif) ligand 11 (*Ccl11*, also known as eotaxin 1), *Ccl24* (eotaxin 2), and *Ccl26* (eotaxin 3) were PCR-amplified. Expression of *Gapdh* was used as an internal control. The nucleotide sequences of the primers were as follows:
Ccl11-1: 5'-CACAGCACTTCTATTCCTGC-3',
Ccl11-2: 5'-TGCCGATATTCTCCCATAGC-3',
Ccl24-1: 5'-TGAACCCTGAGCTGTACCTG-3',
Ccl24-2: 5'-AAGGCAGCCTGGTAAAGCGT-3',
Ccl26-1: 5'-AGACAAACCAGGAGGAGCTG-3',
Ccl26-2: 5'-GCAATGCACCAGATTCCATG-3',
Gapdh-1: 5'-ACCACAGTCCATGCCATCAC-3', and

Gapdh-2: 5'-TCCACCACCCTGTTGCTGTA-3'.

The reaction volume of PCR was 25 μl. The cycling parameters for PCR were initial denaturation of 1 min at 94°C, followed by 40 cycles of 20 s at 94°C, 20 s at 50°C, and 40 s at 72°C. Five-microliter aliquots of each PCR product were then subjected to 1.5% agarose gel electrophoresis.

Nucleotide sequence analysis of integrin and chemokine (C-C motif) receptor 3 transcripts

Oligonucleotide primer pairs for PCR amplification of transcripts were designed based on the reported nucleotide sequences of the following rat genes: integrin alpha 4 (*Itga4*; GenBank Accession No. NM_001107737), integrin alpha M (*Itgam*; NM_012711), integrin beta 1 (*Itgb1*; NM_017022), integrin beta 2 (*Itgb2*; NM_001037780), and chemokine (C-C motif) receptor 3 (*Ccr3*; NM_053958). Bone marrow mRNA was subjected to RT-PCR amplification with the oligonucleotide primers. The PCR products were purified with Ultra-Clean PCR Clean-up DNA Purification Kit (MO BIO Laboratories, Solana Beach, CA, USA) and were sequenced. Sequencing reactions were performed using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Japan, Tokyo, Japan) and were run on an ABI 310 automated sequencer (Applied Biosystems).

Genetic association between blood eosinophilia and polymorphisms in the Itga4 and Ccr3 genes

Male BN.MES-*Cyba*^{mes} rats were crossed to female MES rats to obtain (MES × BN.MES-*Cyba*^{mes})F_1 rats. (MES × BN.MES-*Cyba*^{mes})F_2 rat progeny were then bred by intercross of F_1 rats. At 15 weeks of age, blood, femoral bone marrow and liver of rats were examined as described above. Genomic DNA was extracted from the liver specimens using the G NOME DNA kit (Quantum Biotechnologies, Carlsbad, CA, USA). Genotypes for single nucleotide polymorphisms in *Itga4* and *Ccr3* genes were determined by PCR amplification from genomic DNA and direct sequencing of the PCR products. The nucleotide sequences of the primers were as follows:

Itga4-g9: 5'-GTAGGAAATGTTAACGCTACCGCT-3',
Itga4-g10: 5'-GATGTGTACTGATAGGGTAGTCAAC-3',

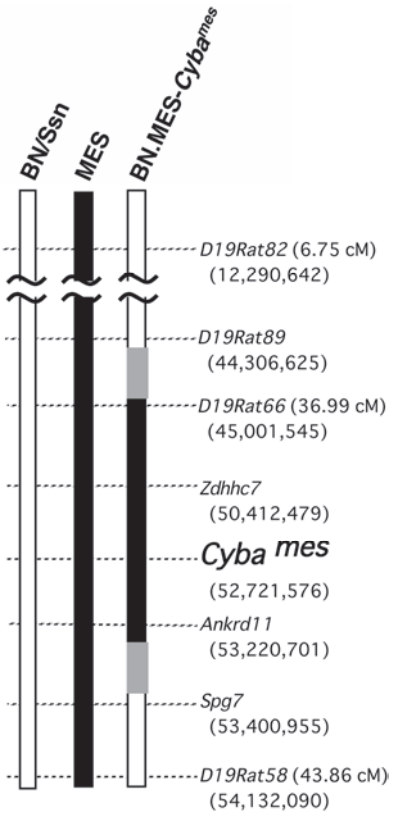

Fig. 1. Schematic representation of chromosome 19 of the BN.MES-*Cyba*^{mes} congenic rat strain. White and black bars indicate chromosomal segments in BN/Ssn and MES rats, respectively. Segments in gray indicate regions of ambiguous origin, corresponding to cross-over breakpoints between markers. The location of the marker loci in cM and coordinates shown in parentheses are based on the Rat Genome Database (http://rgd.mcw.edu/) and the Genome Browser (http://genome.ucsc.edu), respectively.

Ccr3-g3: 5'-CTCCTGGGCAACATGATGGT-3', and
Ccr3-g4: 5'-TCTGGATAGCGAGGACTGCA-3'.

Results

Genetic profile of a BN.MES-Cyba^{mes} *congenic strain*

The congenic interval of the BN.MES-*Cyba*^{mes} rats was determined by using polymorphic microsatellite markers. Congenic rats were homozygous for the MES-derived allele at *D19Rat66*, *Zdhhc7*, *Cyba*, and *Ankrd11* genes (Fig. 1). The rats were homozygous for the BN-derived allele at the *D19Rat82*, *D19Rat89*, *Spg7*, and *D19Rat58*

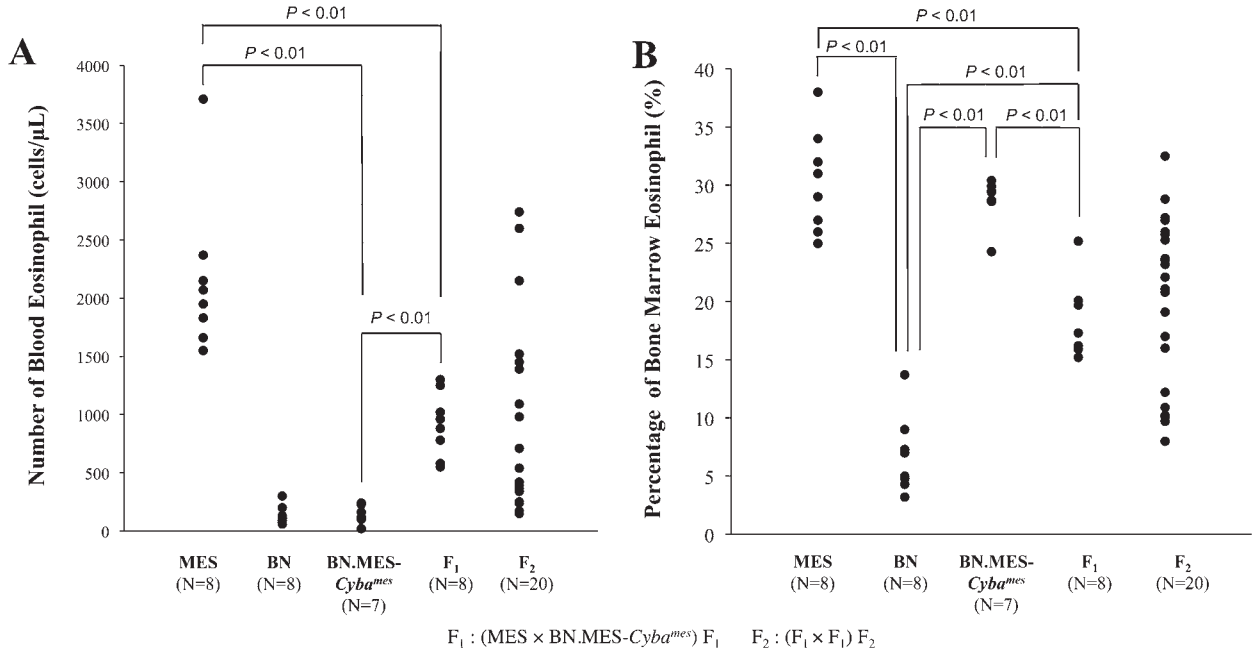

Fig. 2. Bone marrow but not blood eosinophilia in the BN.MES-*Cyba*^{mes} congenic rat. Distribution of blood eosinophil counts (A) and bone marrow eosinophil ratio (B) in MES, BN, BN.MES-*Cyba*^{mes}, (MES × BN.MES-*Cyba*^{mes})F_1, and (MES × BN.MES-*Cyba*^{mes})F_2 rats at 15 weeks of age.

loci. The rats were also homozygous for the BN-derived allele at microsatellites on other chromosomes (Figure not shown). These data indicate that even though backcrossing was discontinued at the 7th generation, only a small chromosomal segment of approximately 9 Mb including the mutant *Cyba*^{mes} gene was present in the BN/Ssn strain, and the genetic background has been successfully replaced by that derived from the BN/Ssn strain.

BN.MES-Cyba^{mes} *congenic rats have normal blood eosinophil levels despite robust eosinophil proliferation in the bone marrow*

The average blood eosinophil count in the BN.MES-*Cyba*^{mes} congenic rats was 150 ± 59 (SD)/μl in females and 127 ± 110/μl in males, which was within the normal range, and far below that in the original MES strain (2403 ± 1149/μl in females and 2016 ± 277/μl in males; P<0.01) (Fig. 2A). The proportion of eosinophils in the bone marrow was over 25% (25.2% in females and 29.4% in males), which was above the normal value in BN (6.8%; Fig. 2B) and SD rats (3.0%). Thus, the robust proliferation of eosinophils in the bone marrow was similar to that seen in MES.

Table 1. Comparison of eosinophil-related lesions of MES and BN.MES-*Cyba*^{mes} congenic rats

	MES	BN.MES-*Cyba*^{mes}
Hypereosinophilia		
Bone marrow	+	+
Blood	+	–
Inflammation		
Gastroenteritis	+	–
Aorta, thickening of intima	+	+
Granuloma		
Mesenteric lymph nodes	+	+
Focal necrosis		
Liver	–	+

BN.MES-Cyba^{mes} *congenic rats manifest focal necrosis with eosinophilic infiltration in the liver*

Similar to MES rats, BN.MES-*Cyba*^{mes} congenic rats had enlarged mesenteric lymph nodes. Histology revealed granulomatous lesions with eosinophilic infiltration (data not shown). In contrast to MES rats, gastroenteritis was not observed in the congenic rats (Table 1). Rather, severe focal necrosis was observed in the livers of congenic rats, which has never been observed in the

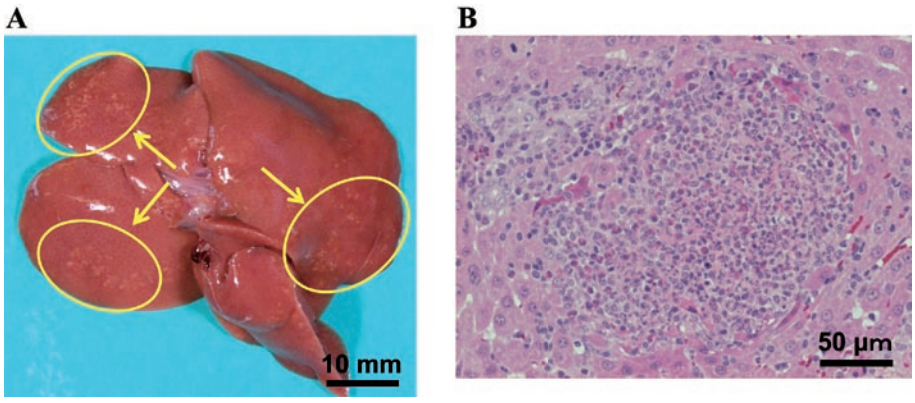

Fig. 3. Hepatic eosinophilic infiltration in the BN.MES-*Cyba*^{mes} congenic rats. (A) Macroscopic image of the liver of a congenic rat. Necrotic foci are visible as white spots. (B) HE-stained section of the liver including focal necrosis with eosinophil infiltration.

original MES strain. Foci were macroscopically visible as randomly distributed white patches or nodules on the surface of the liver (Fig. 3A). Histopathological examination indicated that the lesions arose from focal necrosis with eosinophil infiltration (Fig. 3B). Serum aspartate aminotransferase levels were significantly elevated in male BN.MES-*Cyba*^{mes} congenic rats (n=9; 150.3 ± 65.6 IU/l) compared with male BN rats [n=10; 61.3 ± 9.8 IU/l (P<0.001)]. Also, serum alanine aminotransferase levels were significantly elevated in male BN.MES-*Cyba*^{mes} congenic rats (61.6 ± 21.0 IU/l) compared with male BN rats [22.0 ± 5.1 IU/l (P<0.001)]. These results indicate liver damage occurred in the congenic strain.

MES and BN.MES-Cyba^{mes} *have similar tissue transcription profiles for chemokine (C-C motif) ligands*

Eotaxin [chemokine (C-C motif) ligand] produced by epithelial cells mediates organ-specific attraction of eosinophils [9]. The mammalian eotaxin family includes eotaxin 1 (CCL11), eotaxin 2 (CCL24), and eotaxin 3 (CCL26). Expression of transcripts of these three chemokine (C-C motif) ligands was compared between MES and BN.MES-*Cyba*^{mes} congenic rats by semi-quantitative RT-PCR analysis. Transcripts for *Ccl11* were observed in all of the four organs examined (Fig. 4). Weak expressions of *Ccl24* and *Ccl26* were observed in the spleen and liver. Overall, the analysis did not reveal any marked strain differences in the transcription profiles in organs, including the liver.

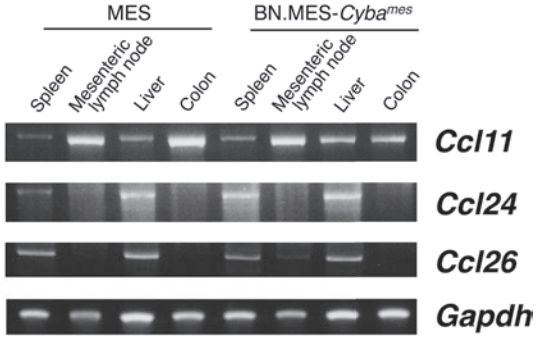

Fig. 4. Comparison of transcription profiles for chemokine (C-C motif) ligands in MES and BN.MES-*Cyba*^{mes} congenic rats. Agarose gel electrophoresis of RT-PCR products for chemokine (C-C motif) ligands is shown. Representative results from one rat for each strain are shown.

MES and BN.MES-Cyba^{mes} *have missense nucleotide substitutions in Itga4 and Ccr3 genes*

Eosinophils express a receptor for eotaxins, chemokine (C-C motif) receptor 3 (CCR3), through which eotaxin signaling is transmitted. In addition, eosinophils express seven integrin heteromeric adhesion molecules: $\alpha4\beta1$, $\alpha6\beta1$, $\alpha L\beta2$, $\alpha M\beta2$, $\alpha X\beta2$, $\alpha D\beta2$, and $\alpha4\beta7$ [2]. It is likely that $\alpha4\beta1$ and $\alpha M\beta2$ are the most important integrins mediating eosinophil adhesion and movement. Infusion of a $\beta2$ integrin-blocking antibody prevented IL-5-mediated bone marrow release of eosinophils, while

Table 2. Nucleotide substitutions between MES and BN.MES-*Cyba*^{mes} rats in the genes for integrin and chemokine (C-C motif) receptor 3

Gene name	Gene symbol	Position*	Codon change (MES/BN)	Amino acid substitution
Integrin alpha 4	*Itga4*	451	auC/auU	No
		2,356	agU/agC	No
		2,458	agA/agG	No
		2,677	caC/caU	No
		2,833	gcC/gcA	No
		3,020	Guc/Auc	**V929I**
Integrin alpha M	*Itgam*	2,592	gaC/gaU	No
Integrin beta 2	*Itgb2*	314	Uug/Cug	No
		2,482	aaA/aaG	No
		2,614	C/G	3' UTR
Chemokine (C-C motif) receptor 3	*Ccr3*	704	Uuu/Cuu	**F164S**

*Position in the reported nucleotide sequences for *Itga4* (NM_001107737), *Itgam* (NM_012711), *Itgb2* (NM_001037780), and *Ccr3* (NM_053958).

Table 3. Comparison of blood eosinophil counts in (MES × BN.MES-*Cyba*^{mes})F_2 rats for genotype at the *Itga4* and *Ccr3* genes

Genotype at *Itga4*			Genotype at *Ccr3*		
MES/MES (n=5)	MES/BN (n=9)	BN/BN (n=6)	MES/MES (n=6)	MES/BN (n=10)	BN/BN (n=4)
758 ± 802	979 ± 797	905 ± 1,003	832 ± 696	880 ± 860	1,060 ± 1,100

Values are mean ± standard deviation.

an antibody to α4 integrin enhanced release in guinea pigs [19].

The possibility that MES and BN.MES-*Cyba*^{mes} congenic rats have functional differences in these key molecules modulating eosinophil motility was examined. Nucleotide differences between the MES and BN.MES-*Cyba*^{mes} strains were found in the transcripts for three integrin genes (Table 2). All (except for one in *Itga4*) were silent nucleotide substitutions. A G to A nucleotide substitution in *Itga4* resulted in a substitution of a valine (MES) for an isoleucine (BN) at codon 929 (V929I). A T to C nucleotide substitution was identified in the *Ccr3* gene. This led to substitution of a phenylalanine (MES) for a serine (BN) at codon 164 (F164S).

Polymorphisms in Itga4 and Ccr3 genes are not associated with blood eosinophilia

Itga4 (chromosome 3) and *Ccr3* (chromosome 8) lie outside the congenic region. A genetic association study between nucleotide substitutions in *Itga4* and *Ccr3* genes and blood eosinophilia was performed by breeding (MES × BN.MES-*Cyba*^{mes})F_2 rats. The average ratio 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 of both parental lines of MES (31.5%) and BN.MES-*Cyba*^{mes} (29.0%) (Fig. 2B). The reason for the reduction is unclear. The average blood eosinophil counts in the (MES × BN.MES-*Cyba*^{mes})F_1 rats were 792 ± 263/μl in females and 958 ± 226/μl in males, which were above the normal ranges, but below that of MES rats (Fig. 2A). In contrast, blood eosinophil counts in the F_2 rats had a broad distribution (Fig. 2A). Blood eosinophil counts in the F_2 rats were not different between the genotypes of *Itga4* and *Ccr3* genes (Table 3), negating a causal link of the polymorphisms to blood eosinophilia.

Discussion

BN.MES-*Cyba*^{mes} congenic rats had normal blood eosinophil levels despite having robust eosinophil proliferation in the bone marrow. The absence of blood

eosinophilia was in sharp contrast to severe blood eosinophilia in the original MES rat strain. This observation was contrary to our expectation that BN.MES-*Cyba*^{mes} congenic rats would show more serious blood eosinophilia because BN has pro-Th2 and proeosinophilic phenotypes [24, 26]. Also, BN.MES-*Cyba*^{mes} congenic rats manifested severe focal necrosis with eosinophilic infiltration in the liver, a phenotype not seen in the original MES strain. Instead, MES rats manifest gastroenteritis. These observations suggest the existence of genetic polymorphism(s) between MES and BN strains, which are distinct from *Cyba* and which influence overall eosinophil homeostasis. A strain difference in tissue distributions of eosinophils was also observed in eosinophilia model mice [5, 10, 27]. Also, eosinophil-induced chronic active hepatitis has been occasionally reported in IHES patients [6, 28]. However, the mechanisms for selective eosinophil infiltration of the liver in these cases are not understood.

To the best of our knowledge, this is the first demonstration of a polymorphism for mobilization of eosinophils from the bone marrow to the peripheral circulation. The presence of mild blood eosinophilia in (MES × BN.MES-*Cyba*^{mes})F_1 rats indicates that suppression of blood eosinophilia is genetically semidominant. In addition, broad distribution of blood eosinophil counts in the F_2 rats suggests that the trait is under the influence of multiple genes. One possible explanation for the absence of blood eosinophilia in the BN.MES-*Cyba*^{mes} congenic rats is that the strain expresses factor(s) which potently suppress or impede mobilization of eosinophils from the bone marrow to the peripheral circulation. The emigration of eosinophils from the bone marrow is a multi-step process. These steps may include release of mature eosinophils attached to bone marrow stromal cells and extracellular matrix, migration across the sinus endothelium, and release from the luminal surface of the endothelium. The molecular mechanisms involved in these steps are not well understood. Integrin $\alpha4\beta1$ on eosinophils mediates adhesion to the bone marrow sinus endothelium by attaching to VCAM1 on the endothelium [19]. On the other hand, interleukin (IL)-5 and eotaxin 1 facilitate detachment of eosinophils from the endothelium [20, 25].

Under baseline conditions, circulating eosinophils interact with the vascular endothelium by processes involving rolling, adhesion, and diapedesis, and traffic into the thymus, mammary gland, uterus, and most prominently into the gastrointestinal tract [8, 13, 22]. Depending on the target organ, eosinophils cross the vascular endothelium into tissues by a regulated process involving coordinated interaction between networks involving eotaxin 1, eosinophil integrins, and adhesion receptors on the endothelium (e.g., MAdCAM-1, VCAM-1, and ICAM-1) [1, 3, 9]. The mechanisms by which eosinophils are recruited to specific tissue sites remain poorly understood. Based primarily on observations of allergic inflammation models, recruitment of eosinophils to 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 to facilitate their preferential migration [21]. BN.MES-*Cyba*^{mes} congenic liver might have potent attractants for eosinophils which are missing from the liver of the MES strain.

The genetic basis for the differences in phenotypes between MES and BN.MES-*Cyba*^{mes} congenic rats remains to be elucidated. It might reside in the eosinophils themselves, or in other cell types, such as bone marrow mesenchymal cells, or tissues outside of the bone. We concede that the F_2 population is currently too small, but we are now expanding the (MES × BN.MES-*Cyba*^{mes})F_2 population in order to perform chromosomal mapping and subsequent positional cloning of the gene(s) for the phenotypes. In our previous study, quantitative trait loci for blood eosinophil counts were revealed on chromosome 2 (eosinophilia 2; *eos2*) [11] and chromosome 1 (eosinophilia 3; *eos3*) [14] in (MES × ACI) × MES backcross progeny. The alleles derived from MES on both loci had an additive effect, increasing the blood eosinophil level. There remains a possibility that these loci are responsible for a polymorphism which mobilizes the eosinophils from the bone marrow to the peripheral circulation.

In summary, we have established a new congenic rat strain, BN.MES-*Cyba*^{mes}. Further study of this new rat strain and the original MES strain will shed new light on the molecular genetic mechanisms involved in the development and trafficking of eosinophils.

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