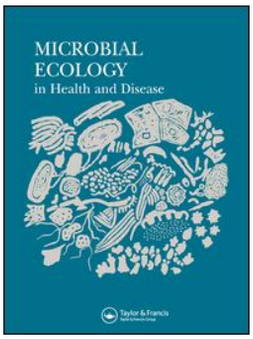

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ORIGINAL ARTICLE

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Abnormal Intestinal Environment in Rats with Spontaneous Eosinophilia (MES Rat): A Possible New Model for Studying Intestinal Putrefaction

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Intestinal putrefactive products appear to be harmful substances to the host. However, there are few good animal models with elevated production of intestinal putrefactive substances. Recently, an MES rat developing spontaneous eosinophilia with eosinophil-related inflammatory lesions especially in the gastrointestinal tract was introduced. In the present study, haematology, macropathology, composition of faecal flora and caecal concentration of putrefactive products of MES and normal rats at different ages were investigated. An apparent increase in blood leukocytes and eosinophils was observed in the MES rats at 10 weeks of age and became more severe with age. Polypoid lesions in the stomach and thickening of the intestinal walls were associated with the development of eosinophilia. At 18–19 weeks of age, the numbers of total bacteria, Bacteroidaceae, clostridia and lactobacilli and percentage of cultured bacteria to direct microscopic counts were significantly higher in the MES rats than in normal rats. The concentrations of caecal putrefactive products increased remarkably with age in the MES rats and were significantly higher than in normal rats at 18–19 weeks of age. As the abnormal intestinal environment was developed with age accompanying abnormal gastrointestinal structure, the MES rat may be a good model for studying the mechanism of production and methods of controlling intestinal putrefactive products accompanying aging and gastrointestinal disorders. *Key words:* animal model, eosinophilia, faecal flora, indole, intestinal putrefaction, MES rat.

INTRODUCTION

Eosinophilia is observed in a variety of disorders including allergic reactions, parasitic inflammation, chronic inflammation and neoplastic diseases (1–5). Eosinophilia is accompanied by a wide variety of inflammatory lesions because toxic inflammatory mediators are released from eosinophils (1, 5, 6). Matsumoto *et al*. (7) have found and maintained a colony of the mutant rat with spontaneous eosinophilia named Matsumoto Eosinophilia Shinshu (MES) rat. MES rats have haematological features resembling human idiopathic hypereosinophilic syndrome (HES) (8) and apparent increase in blood eosinophils is first found at 8 weeks of age. Eosinophilia becomes more severe with age. The MES rats develop eosinophil-related inflammatory lesions in various organs including gastroenteritis, mesenteric lymphadenitis and aortitis (9, 10). Among the lesions, diffuse fibrosis with inflammatory response is quite frequently observed in the gastrointestinal tract. Macroscopic lesions in the stomach and small intestine are frequently found after 10 weeks of age and, histopathologically, eosinophilic infiltration in the gastrointestinal tract is found in some rats initially at 8 weeks of age and more severely after 10 weeks of age (9).

In the present study, the intestinal environment of the MES rats, i.e. composition of intestinal flora and concentration of caecal putrefactive products, was investigated at different ages in relation to the development of eosinophilia and accompanying gastrointestinal lesions.

MATERIALS AND METHODS

Animals

The MES and normal Slc:SD rats were bred and maintained in the Institute of Experimental Animals, Shinshu University School of Medicine. The animals were kept in specific pathogen-free facilities controlled at $24 \pm 2^{\circ}C$ and relative humidity of $55 \pm 10\%$, with a complete ventilatory exchange of fresh air 15 times/h and a 12-h light-dark cycle. The rats were housed in polycarbonate cages and fed a commercial diet (MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum*. This study was carried out in accordance with the Guidelines for Animal Care and

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Experimentation of the University of Tokyo and Shinshu University School of Medicine.

Sampling

At 6–7, 10 and 18–19 weeks of age, blood samples for haematology were collected from the jugular vein. The animals were then transported to the Laboratory of Veterinary Public Health, University of Tokyo. After fresh faeces were collected individually, the animals were killed with carbon dioxide and whole gastrointestinal tracts were removed. Faeces were processed for bacteriological investigation immediately. Caecal contents were stored at −80°C until the measurement of putrefactive products and the rest of the gastrointestinal tracts were fixed with 10% neutral buffered formalin for macropathologic observation.

Haematology

The leukocytes were counted with an automated cell counter (System F-820, Toa Medical Electronics Co. Ltd, Kobe, Japan) and eosinophil counts (%) were obtained on Wright stained blood films.

Macropathology

The stomach was cut open and the lesions in the stomach were observed macroscopically. The central part of the small intestine (jejunum) and upper part of the colon (approximately 5 cm below the caecum) were excised and the thickness of the intestinal wall was measured with the cross-section.

Bacteriological procedures

Faeces were weighed and introduced into an anaerobic chamber. Bacteriological procedures were essentially the same as those described elsewhere (11). Bacteria were identified at the levels of genus or family based on colony form, Gram's stain, cell morphology and growth under aerobic conditions. Faecal dilutions were also smeared in a 1×1 cm square on slides and stained by Gram's stain for direct microscopic counts. Bacterial cells and spores were then counted microscopically. Bacterial numbers were expressed as $\log_{10}$ number of bacteria per gram wet weight of faeces.

Measurement of caecal putrefactive products

Caecal indole, skatole, *p*-cresol and phenol were measured by high-performance liquid chromatography (HPLC) (11). Samples were added to methanol and *p*-isopropylphenol as an internal standard. The mixtures were left to stand at −30°C for 30 min and centrifuged for 10 min at 14,000 rpm. The supernates were filtered with 0.45-μm filters and analysed by HPLC (655A-11, Hitachiseisakusho Ltd, Tokyo, Japan) with a Superspher 100 RP-18(e) (4 μm) column (250×4 mm, Merck, Darmstadt, Germany) and a LiChrospher 100 RP-18(e) (5 μm) guard column (4×4 mm, Merck). Samples were eluted with 1.0 ml/min of 50 mM ammonium acetate buffer (pH 4.9)/acetonitrile (70/30). The column was maintained at 40°C and putrefactive products were detected with a UV detector (655A, Hitachiseisakusho) at a wavelength of 265 nm.

Statistical analysis

Non-parametric evaluation was performed with StatView (SAS Institute Inc., NC, USA) to compare the median of the numbers of bacteria in faeces. The significance of differences of the mean values among groups was determined by ANOVA with StatView.

RESULTS

General conditions and haematology

Body weight, leukocyte count and percentage of eosinophils of the MES and normal rats at different ages are shown in Table I. The body weight was essentially the same in the MES and normal rats except that the body weight of the MES rats was lower at 6–7 weeks of age. As regards their general condition, both the MES and normal rats were healthy.

An apparent increase in blood leukocytes was found at 10 weeks of age in the MES rats and subsequently blood leukocytes increased with age. The percentage of eosinophils in leukocytes was significantly higher at 10 weeks of age in the MES rats than in normal rats. The increase in blood eosinophils in the MES rats became more severe at 18–19 weeks of age.

Macropathology

Polypoid lesions were observed in the stomach of four of five MES rats at 10 weeks of age. Lesions were more severe in the MES rats at 18–19 weeks of age and three of five animals showed huge lesions which covered more than half of the inner surface of the stomach (Fig. 1A, left). No lesions were observed in the MES rats at 6–7 weeks of age, nor in any of the normal rats (Fig. 1A, right). The thickness of the intestinal wall significantly increased with age in the MES rats. The jejunal wall was significantly thicker in the MES rats than in normal rats at 10 and 18–19 weeks of age. The colonic wall was also significantly thicker in the MES rats than in normal rats at 18–19 weeks of age (Fig. 1B and C, Table I).

Composition of faecal flora

The composition of faecal flora of the MES and normal rats is shown in Table II. In normal rats, the number of Bacteroidaceae, clostridia, lactobacilli and streptococci decreased significantly at 18–19 weeks of age compared with 6–7 weeks of age. The number of total cultivable bacteria was also significantly lower at 18–19 weeks of age

Table I

Body weight, leukocyte count, percentage of eosinophils and thickness of jejunal and colonic walls of the MES and normal rats at different ages

Parameter	6–7 weeks	10 weeks	18–19 weeks
Normal rats	($n=5$)	($n=4$)	($n=4$)
Body weight (g)	168.9 ± 3.9[a]	195.7 ± 3.7[b]	259.4 ± 10.1[b,c]
Leukocytes (/μl)	8400 ± 1093	7425 ± 1287	7100 ± 1606
Eosinophils (%)	1 ± 0	1 ± 0	1 ± 1
Jejunum (mm)	0.72 ± 0.22	0.73 ± 0.16	0.75 ± 0.12
Colon (mm)	0.24 ± 0.05	0.28 ± 0.06	0.35 ± 0.13
MES rats	($n=4$)	($n=5$)	($n=5$)
Body weight (g)	91.5 ± 11.7[d]	203.0 ± 18.4[b]	254.0 ± 26.7[b,c]
Leukocytes (/μl)	9225 ± 1929	18860 ± 3233[b,d]	33020 ± 9836[b,c,d]
Eosinophils (%)	3 ± 1	6 ± 2[b,d]	12 ± 3[b,c,d]
Jejunum (mm)	0.75 ± 0.17	1.08 ± 0.18[b,d]	1.39 ± 0.08[b,c,d]
Colon (mm)	0.22 ± 0.06	0.39 ± 0.14	0.57 ± 0.24[b,c,d]

[a]Mean ± SD.
[b]Significant difference against rats aged 6–7 weeks.
[c]Significant difference against rats aged 10 weeks.
[d]Significant difference against normal rats of the same age.

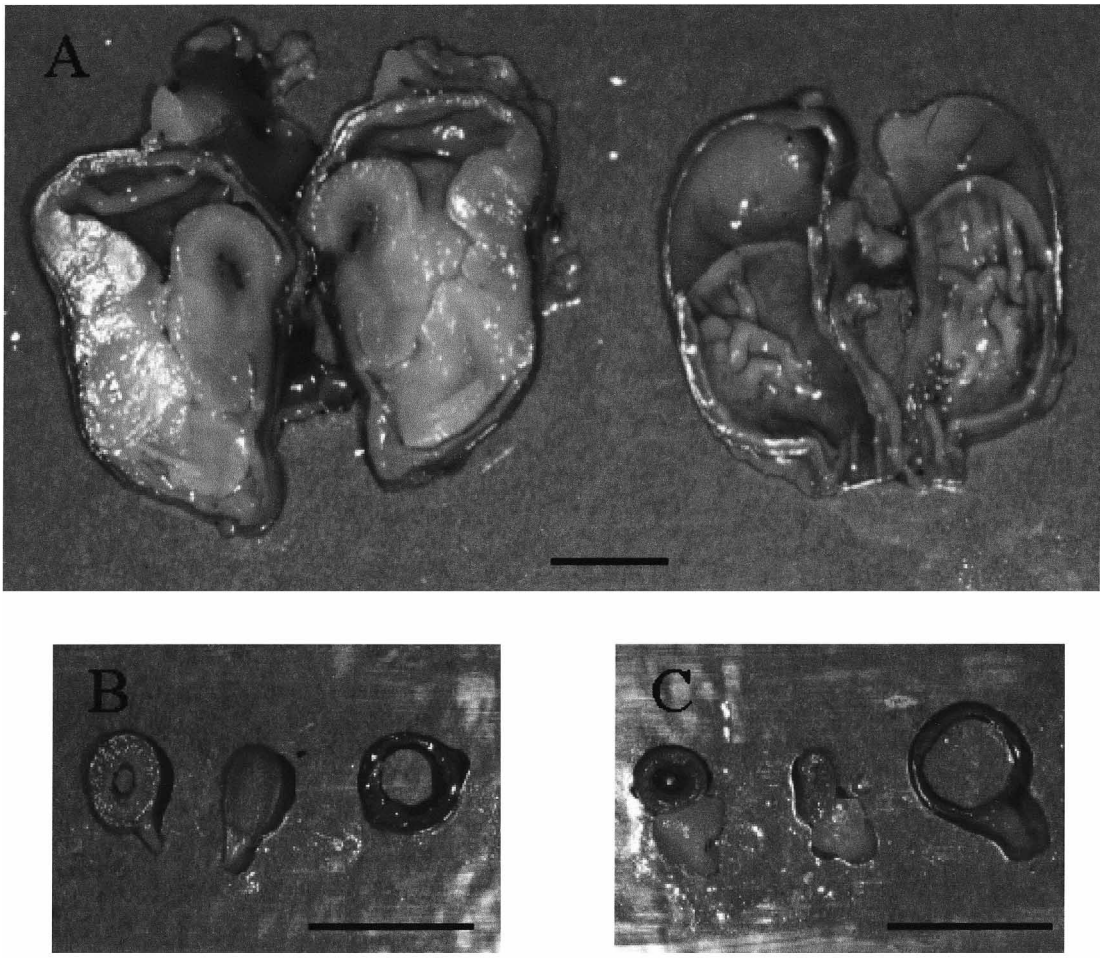

Fig. 1. Macroscopic findings in the gastrointestinal tract of MES and normal rats at 18–19 weeks of age. (A) Longitudinal section of stomach of MES (left) and normal (right) rats. (B) Transverse section of jejunum (left and middle) and colon (right) of MES rat. (C) Transverse section of jejunum (left and middle) and colon (right) of normal rat. Bars correspond to 1 cm.

Table II

Composition and direct microscopic count of faecal flora of the MES and normal rats at different ages

Parameter	6–7 weeks	10 weeks	18–19 weeks
Normal rats	($n = 5$)	($n = 4$)	($n = 4$)
Total cultivable bacteria	10.3 (10.2–10.6)[a]	10.3 (10.2–10.3)	9.8 (9.7–10.2[b,c])
Bacteroidaceae	10.0 (9.9–10.3; 5)	10.0 (9.7–10.1; 4)	9.4 (9.0–9.9[b,c]; 4)
Bifidobacteria	6.5 (6.5–6.5; 1)	8.1 (8.0–8.5; 3)	6.9 (6.2–8.4; 4)
Eubacteria	9.2 (8.7–9.8; 5)	9.0 (9.0–9.3; 4)	9.1 (8.8–9.5; 4)
Clostridia	9.1 (8.7–9.3; 4)	8.0 (7.7–8.7; 3)	8.2 (8.0–8.8[b]; 4)
Veillonella	7.7 (6.9–8.0; 5)	7.7 (6.5–8.3; 4)	7.0 (6.0–7.9; 4)
Fusiform-shaped bacteria	9.0 (8.0–9.4; 4)	8.7 (8.0–9.0; 4)	8.6 (7.7–9.0; 3)
Lactobacilli	9.8 (9.6–10.1; 5)	9.9 (9.4–10.1; 4)	9.3 (9.1–9.4[b,c]; 4)
Enterobacteriaceae	8.3 (7.5–9.4; 5)	8.7 (7.3–9.5; 4)	7.9 (6.1–8.0; 4)
Streptococci	8.4 (7.9–8.8; 5)	8.0 (7.7–8.3; 4)	7.7 (7.1–8.0[b]; 4)
Staphylococci	5.1 (4.2–6.2; 5)	4.5 (3.9–5.3; 4)	5.2 (5.0–5.5; 4)
Direct count	11.2 (11.2–11.3)	11.4 (11.3–11.5[b])	11.4 (11.3–11.4[b])
Percentage cultured	14.4 (8.3–21.6)	8.2 (6.9–12.2)	2.8 (1.9–7.2[b,c])
MES rats	($n = 4$)	($n = 5$)	($n = 5$)
Total cultivable bacteria	10.0 (9.9–10.3)	10.5 (10.2–10.6[b])	10.6 (10.5–10.7[b,d])
Bacteroidaceae	9.8 (9.5–10.2; 4)	9.9 (9.7–10.2; 5)	10.2 (9.7–10.4[d]; 5)
Bifidobacteria	7.0 (7.0–7.0; 2)	8.3 (8.0–9.0; 4)	7.9 (7.6–8.4; 4)
Eubacteria	9.0 (8.8–9.0; 4)	9.9 (9.2–10.1[b,d]; 5)	9.8 (9.5–10.0[b]; 5)
Clostridia	8.9 (8.7–9.0; 4)	8.9 (8.3–9.0; 4)	9.0 (8.7–9.7[d]; 5)
Veillonella	7.1 (6.8–7.6; 4)	8.4 (7.6–9.0[b]; 5)	9.0 (6.6–9.6; 5)
Fusiform-shaped bacteria	8.7 (8.5–9.4; 3)	9.0 (8.4–9.3; 5)	9.2 (8.7–9.3; 5)
Lactobacilli	9.2 (9.2–9.5[d]; 4)	9.8 (9.6–10.3[b]; 5)	9.9 (9.3–10.2[b,d]; 5)
Enterobacteriaceae	7.0 (6.4–7.6[d]; 4)	8.3 (6.5–9.8; 5)	8.6 (6.4–10.0; 5)
Streptococci	7.5 (5.8–8.7; 4)	7.8 (7.1–8.6; 5)	7.6 (6.4–8.6; 5)
Staphylococci	4.6 (4.5–5.1; 3)	6.0 (3.6–6.3; 5)	5.6 (3.9–6.1; 5)
Direct count	11.3 (11.3–11.3)	11.4 (11.3–11.6)	11.3 (11.2–11.3[c,d])
Percentage cultured	6.3 (3.3–10.8)	9.6 (7.8–17.2)	21.4 (16.3–29.8[b,c,d])

[a]Median (range of $\log_{10}$ number per gram wet weight of faeces when the organism was present; number of samples in which the organism was detected).
[b]Significant difference against rats aged 6–7 weeks.
[c]Significant difference against rats aged 10 weeks.
[d]Significant difference against normal rats of the same age.

than in younger rats. On the other hand, the direct microscopic count increased from 10 weeks of age and the percentage of total number of cultivated bacteria to direct microscopic count decreased markedly with age. In contrast, the numbers of lactobacilli, eubacteria and total cultivable bacteria were significantly higher at 10 and 18–19 weeks of age than at 6–7 weeks of age, while the direct microscopic count was rather constant. Then the percentages of cultivable bacteria to direct microscopic count were significantly higher at 10 and 18–19 weeks of age than at 6–7 weeks of age. At 18–19 weeks of age, the numbers of total cultivable bacteria, Bacteroidaceae, clostridia and lactobacilli and the percentage of cultured bacteria to direct microscopic count were significantly higher in the MES rats than in normal rats, while the composition of faecal flora in the MES rats at 6–7 and 10 weeks of age was similar to that in normal rats except for the numbers of lactobacilli and Enterobacteriaceae at 6–7 weeks and eubacteria at 10 weeks of age.

Concentrations of caecal putrefactive products

The concentrations of caecal putrefactive products increased significantly with age in the MES rats (Table III). Caecal indole and phenol concentrations were significantly higher at 18–19 weeks of age than those at 6–7 weeks and 10 weeks of age in the MES rats. *p*-Cresol was detected only in the MES rats at 10 and 18–19 weeks of age. At 18–19 weeks of age, the concentrations of putrefactive products were significantly higher in the MES rats than in normal rats.

DISCUSSION

The MES rat was first found as a mutant rat with spontaneous eosinophilia in 1997 (7). This colony was maintained by brother–sister matings with the criterion of > 850 eosinophils/μl at 10 weeks of age (7). Diffuse fibrosis with inflammatory response is quite frequently found in the gastrointestinal tract of the MES rat (9), while cardiac

Table III

Concentrations of caecal putrefactive products of the MES and normal rats at different ages

Parameter	6–7 weeks	10 weeks	18–19 weeks
Normal rats	($n=5$)	($n=4$)	($n=4$)
Phenol	1.8±1.6[a]	3.6±4.3	1.7±2.2
p-Cresol	0.0±0.0	0.0±0.0	0.0±0.0
Indole	18.9±3.1	13.5±5.2	8.8±6.8
Skatole	0.8±1.1	0.4±0.8	0.4±0.8
MES rats	($n=4$)	($n=5$)	($n=5$)
Phenol	0.6±1.3	3.1±3.1	8.1±5.6[b,c,d]
p-Cresol	0.0±0.0	6.3±3.6[b,d]	6.7±6.3[b,d]
Indole	6.6±2.3[d]	14.5±8.0	31.6±9.8[b,c,d]
Skatole	0.5±1.0	0.4±1.0	0.2±0.5

[a]Mean±SD (μg/g).
[b]Significant difference against rats aged 6–7 weeks.
[c]Significant difference against rats aged 10 weeks.
[d]Significant difference against normal rats of the same age.

lesions are the most common feature of human chronic hypereosinophilia (1, 6). In the present study, the thickening of the small intestinal wall and polypoid lesions in the stomach were first found at 10 weeks of age in the MES rats and became more severe at 18–19 weeks of age when peripheral eosinophils exceeded the level for diagnosis of human HES (> 1500 eosinophils/μl) (1, 6).

The numbers of lactobacilli, Bacteroidaceae and clostridia were significantly higher in the MES rats than in age-matched normal rats at 18–19 weeks of age. The number of total cultivable bacteria was also significantly higher in the MES rats. As the direct microscopic counts of faecal bacteria were similar in both rat groups or rather fewer in the MES rats (Table II), the ratio of cultivable bacteria in the faecal flora was higher in the MES rats than in normal rats at 18–19 weeks of age, while there was no significant difference in younger rats. In adult mice and rats, the large bowels contain bacteria intimately associated with mucosal epithelia, fusiform-shaped bacteria and spiral-shaped organisms (12, 13). These microorganisms are highly fastidious as regards culture, and are considered to play an important role in the host's physiology as the most predominant organisms (14, 15). In the present study, most of the fusiform-shaped bacteria observed in direct smears of faecal suspension were not cultivated (data not shown). The high cultivability of faecal flora found in the MES rats indicates that the normal composition of the intestinal flora was disturbed in the MES rats by 18–19 weeks of age. The present study suggests that the normal development of the faecal flora of MES rats is disturbed, in association with the development of eosinophilia and accompanying intestinal lesions.

Lactobacilli are the predominant bacteria in the stomach and are detected in the upper part of the small intestine of rats, where other bacterial species are rarely detected (16, 17). It is reported that miscellaneous unidentified anaerobic bacteria can also be designated as resident, autochthonous microflora of the stomach of rats (16). In human studies, it is reported that – unlike normal stomachs, which contain few bacteria – the stomachs of patients with hypochlorhydria or achlorhydria maintain high bacterial counts (18, 19). Microbial titres in gastric aspirates are significantly higher in subjects receiving either antacids or cimetidine (an H_2 receptor antagonist), as compared with the titres before starting antacid or cimetidine regimens (20). In the MES rat, fibrosis accompanied by infiltration of numerous eosinophils is prominent in the lamina propria of the stomach and the muscularis in the jejunum and ileum (9). Eosinophilic infiltration often disrupts the normal architecture, and atrophy and compensatory metaplasia of the gastric glands, and ulceration is observed in the severe cases. These findings suggest that the disruption of the gastrointestinal tract, especially of the stomach and the upper part of small intestine, influenced the development of the intestinal flora in the MES rats.

Intestinal putrefactive products, e.g. indoles and phenols, are produced by intestinal bacteria as metabolites from dietary amino acids (21, 22). In the present study, concentrations of caecal putrefactive products significantly increased in the MES rats at 18–19 weeks of age, when the changes in faecal flora and peripheral eosinophilia with gastrointestinal lesions became obvious. The disruption of the intestinal flora and/or dysfunction of the gastrointestinal tract by eosinophilic infiltration may cause the elevated production of putrefactive substances in the MES rat intestine. Intestinal putrefactive products are an important factor in the malodour of flatus and faeces (23). They are also thought to injure the intestinal epithelia directly, and to be partially absorbed and potentially contributing to aging and geriatric diseases throughout the host's life (24). For example, it is reported that indole acts as a promoter in carcinogenesis (25). Thus, it is now well accepted that it is important to improve the intestinal environment by dietary components such as probiotics and prebiotics in reducing the risk for various diseases. However, there are few good animal models with elevated production of intestinal putrefactive substances. As the MES rat can be kept for quite a long time in good health even after eosinophilia has developed and production of putrefactive substances has increased, the MES rat may be a good model for studying the mechanism of production of intestinal putrefactive products accompanying aging and gastrointestinal disorders, and controlling intestinal putrefaction by manipulating dietary components.

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