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# Dysfunction in ABCB1A Has Only a Weak Effect on Susceptibility to Dextran Sulfate Sodium-Induced Colitis in SAM Strains

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**Abstract:** Genetic alterations in the gene for ATP-binding cassette, sub-family B (MDR/TAP), member 1A (ABCB1A) determine susceptibility to colitis in mice and humans. We investigated the influence of ABCB1A dysfunction on susceptibility to dextran sulfate sodium (DSS)-induced colitis by using Senescence-Accelerated Mouse (SAM) strains with a loss-of-function mutation in the *Abcb1a* gene (SAMR1, SAMP1, and SAMP6). Susceptibility to DSS colitis was different among SAM strains but on the whole was not different from other mouse strains with normal ABCB1A function. Thus, genetic factors other than loss of ABCB1A are more crucial in determining susceptibility to DSS colitis in SAM strains.

**Key words:** ABCB1A, dextran sulfate sodium-induced colitis, Senescence-Accelerated Mouse

The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis (UC), are characterized by chronic inflammation of the gastrointestinal tract [for review, see 22]. While the etiology of these disorders remains unclear, both genetic and environmental factors are associated with susceptibility to IBD. The importance of a genetic component was corroborated by identification of several distinct gene mutations or alleles in IBD patients. One of these genes, a variant of which may be associated with increased risk of UC, is ATP-binding cassette, sub-family B (MDR/TAP), member 1A (*ABCB1A*) [6, 13, 14]. ABCB1A is highly expressed in various tissues, including the epithelial surfaces of the intestine, where it can actively pump orally administered toxic compounds out of the cells into the intestinal lumen [5]. It has been assumed that alterations in the *ABCB1A* gene would compromise the intestinal epithelial barrier function; however, controversy still exists regarding the significance of the *ABCB1A* gene polymorphisms for IBD. Extrinsic factors such as a dietary component or commensal bacterial microflora may modulate the incidence and severity of IBD. Thus, identification of these factors is the key to understanding the etiology of IBD.

Mouse models for colitis have greatly contributed to understanding the etiology of this disease [21]. *Abcb1a* knockout mice with an FBV genetic background (FVB-

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*Abcb1a*$^{-/-}$) spontaneously develop colitis with characteristics including dysregulated epithelial cell growth and leukocytic infiltration into the lamina propria of the large intestine; 20 weeks is the average age at onset [1, 2, 4, 7, 10, 15, 16, 20]. In a previous study, we found that a series of Senescence-Accelerated Mouse (SAM) strains, including SAMR1, SAMP1, and SAMP6, have a spontaneous loss-of-function mutation in the *Abcb1a* gene [24]. The SAMR1 and SAMP6 mice also develop spontaneous colitis [19], but the age at onset is much later (>1 year) than FVB-*Abcb1a*$^{-/-}$ mice. Furthermore, the incidence was different between strains. Approximately 90% of both male and female SAMP6 mice show spontaneous colitis, whereas SAMR1 mice develop colitis at a much lower incidence (67% in males and 50% in females). These observations suggest that genetic and environmental factors play important roles in colitis incidence in ABCB1A dysfunctional mice as well as in humans. Indeed, genetic profiles other than the mutant *Abcb1a* gene are different in the SAMR1, SAMP1, SAMP6, and FVB strains [18, 23, our unpublished results]. Thus, SAM mice should be a useful model for revealing the factors associated with the incidence of UC.

Dextran sulfate sodium (DSS) is a chemical agent that can induce colitis in mice when orally administered. Feeding mice for several days with DSS in drinking water reproducibly induces acute colitis characterized by bloody diarrhea, ulceration, and infiltrations with granulocytes in the colon [12]. The exact mechanism of colitis induction in this model is largely unknown. The most widely accepted hypothesis is that DSS is directly toxic to colon epithelial cells and therefore disrupts the integrity of the mucosal barrier and increases mucosal permeability [3, 12]. Excessive permeation of luminal bacteria or bacterial products into colon mucosa then gives rise to inflammatory infiltration of immune cells. Thus, both oral DSS administration and loss of ABCB1A seem to accelerate the development of colitis by disrupting the colon mucosal barrier function. FVB-*Abcb1a*$^{-/-}$ mice show accelerated colitis development following exposure to DSS [20]. FVB-*Abcb1a*$^{-/-}$ mice required only 2% DSS, whereas FVB wild-type mice required 8% to achieve a similar disease state. Meanwhile, susceptibility to DSS colitis varies among other inbred mouse strains [8], and this strain difference is determined by multiple uncharacterized genes or genetic background [9]. It is not known, however, if difference in genetic background still exerts a major effect on DSS colitis in combination with ABCB1A dysfunction. We addressed this issue using the SAM mouse strains.

All experimental procedures were carried out in accordance with the Regulations for Animal Experimentation of Shinshu University. Specific pathogen-free 8- to 10-week-old male SAMR1, SAMP1, SAMP6, C57BL/6J, C3H/He, and DBA/2J mice were purchased from JAPAN SLC (Hamamatsu, Japan). Mice were acclimated to a clean conventional condition and a commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) for 1 week before use. Colitis was induced in the mice by replacing their drinking water with bottled DSS solution. DSS (mol wt 36,000–50,000; MP Biomedicals Inc., Tokyo, Japan) was dissolved in tap water. Mice had free access to the DSS solution for 8 days and subsequently had access to regular tap water without DSS for 7 days. One experimental group consisted of 5 mice housed in one cage. Consumption of DSS solution was not measured for each mouse; however, consumption was not different between cages or strains (data not shown). During this experimental period, mice were weighed daily, and monitored for physical signs of disease including bloody stool or diarrhea. Live mice at the end point were sacrificed by cervical dislocation and the cecum and whole colon were removed. Tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Microscopic evidence of inflammation and disease severity was assessed using a scoring system described previously [8]. Four main grading criteria (depth of inflammatory cell accumulation, ulceration, hyperplasia, and area involved), with four different subgrades of severity (0–3) for each, were used. The difference in combined score (maximum=12) was evaluated by Mann-Whitney's U test.

The dose response of mice was examined by administering DSS solutions ranging in concentration from 0.5 to 3.0%. The incidence of colitis in the 6 mouse strains at the different DSS concentrations is summarized in Table 1. Among the 3 SAM strains with ABCB1A dysfunction, SAMR1 developed colitis at a DSS concentrations of as low as 0.5%; SAMP1 and SAMP6 did not

**Table 1.** Incidence of colitis in mouse strains after administration of DSS

| Mouse strain | DSS concentration (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| SAMR1 | 2 | 5 | ND | ND | ND | ND |
| SAMP1 | 0 | 0 | 0 | 5 | 5 | ND |
| SAMP6 | 0 | 0 | 0 | 0 | 5 | 5 |
| C57BL/6J | 2 | 5 | 5 | 5 | 5 | 5 |
| C3H/He | ND | 0 | 1 | 5 | ND | ND |
| DBA/2J | ND | 0 | 0 | 0 | 5 | 5 |

Data are number of colitis mice in 5 subjects. ND: not determined.

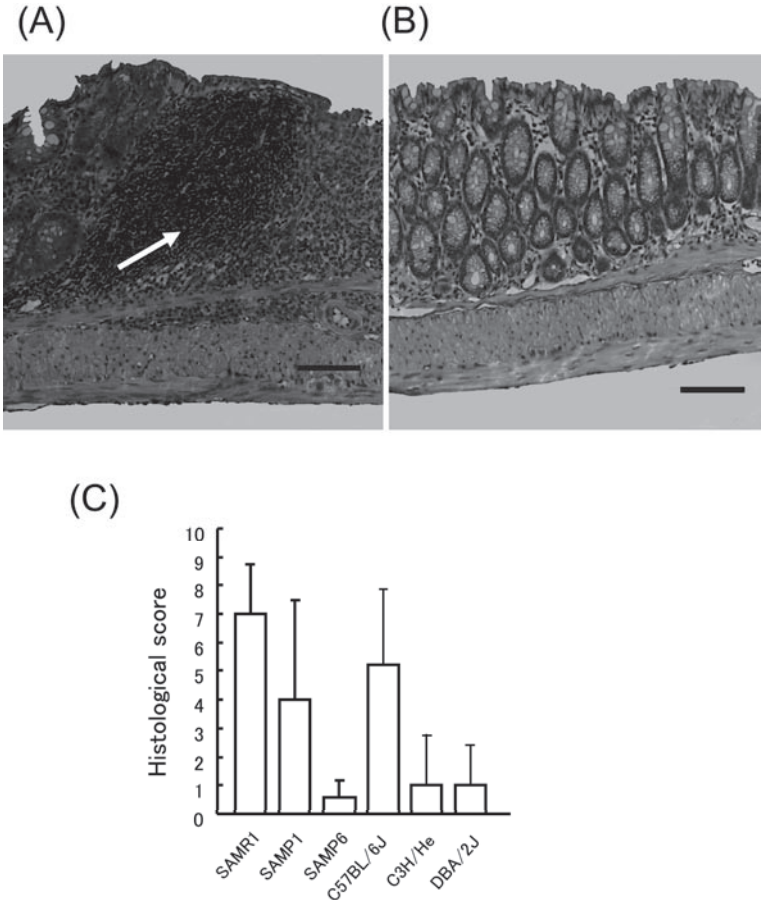


**Fig. 1.** Evaluation of colitis in SAM strains following treatment with DSS. Representative colonic histology of SAMR1 with a score of 7.0 (A) and SAMP6 with a score of 1.0 (B) at sacrifice. An arrow indicates massive leukocytic infiltration into the lamina propria. Scale bar, 200 $\mu$m. (C) Histological scores (mean ± SD) of mice treated with 2.0% DSS.

develop colitis at a DSS concentration lower than 2.0 and 2.5%, respectively. These threshold values were not particularly low compared with those of the standard mouse strains C57BL/6J (1.0%), C3H/He (2.0%), and DBA/2J (2.5%). The disease severity of the mice treated with 2.0% DSS solution was significantly higher in SAMR1 (score=7.0) than SAMP1 (4.0) and SAMP6 (0.6) (Fig. 1). However, it was not particularly different

from C57BL/6J (5.2), C3H/He (1.0), and DBA/2J (1.0). If dysfunction in ABCB1A has a major role in the determination of susceptibility to DSS colitis, the SAM strains should have shown higher disease severity and/or lower dose response to DSS. However, the present results indicate that the 3 SAM strains with ABCB1A dysfunction were not particularly susceptible to DSS colitis compared to the 3 strains with normal ABCB1A function.

These observations suggest that dysfunction in ABCB1A has only a weak effect, if any, on susceptibility to DSS-induced colitis in SAM strains. Rather, the difference among SAM strains in susceptibility to DSS colitis appears to be largely determined by differences in genetic backgrounds other than the *Abcb1a* gene. Indeed, inbred strains of mice show major differences in genetic susceptibility to DSS-induced colitis [8, 11]. This differential colitis susceptibility was genetically studied by utilizing a (C3He/HeJ × C57BL/6J)$F_2$ progeny [9]. Two quantitative loci (QTL) associated with colitis susceptibility (*Dssc1* and *Dssc2*) and several other potential genomic regions which modified susceptibility were identified. These results indicate that DSS-induced colitis in mice is controlled by multiple genes. The genes for *Dssc1* and *Dssc2* loci have not been identified yet. SAMP6 show a higher incidence of spontaneous colitis than SAMR1 [19]. In contrast, susceptibility to DSS colitis is higher in SAMR1 than in SAMP6, suggesting there is a substantial difference in the genetic pathway leading to the two colitis forms in these SAM strains. Similarly, differential susceptibility to spontaneous colitis dependent on genetic background was observed for FVB.129P2-*Abcb1a*$^{-/-}$ (with severe colitis) and C57BL/6J-*Abcb1a*$^{-/-}$ (free of colitis) [17]. These observations of variability in colitis incidence in the *Abcb1a*-deficient mouse strains accentuate the importance of genetic background in the development of the disease, and might help explain the conflicting data on the association of *ABCB1A* polymorphisms and colitis incidence in human populations [13]. Consequently, SAM strains provide an opportunity to identify the susceptible/resistance loci to spontaneous as well as DSS-induced colitis.

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