Muc2-Deficient Mice Spontaneously Develop Colitis, Indicating That Muc2 Is Critical for Colonic Protection

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Background & Aims: **Expression of mucin MUC2, the structural component of the colonic mucus layer, is lowered in inflammatory bowel disease. Our aim was to obtain insight in the role of Muc2 in epithelial protection.** ***Methods:*** **Muc2 knockout ($Muc2^{-/-}$) and Muc2 heterozygous ($Muc2^{+/-}$) mice were characterized and challenged by a colitis-inducing agent, dextran sulfate sodium (DSS). We monitored clinical symptoms, intestinal morphology, and differences in intestine-specific protein and messenger RNA levels.** ***Results:*** **The $Muc2^{-/-}$ mice showed clinical signs of colitis (as of 5 weeks), aggravating as the mice aged. Microscopic analysis of the colon of $Muc2^{-/-}$ mice showed mucosal thickening, increased proliferation, and superficial erosions. Colonic goblet cells in the $Muc2^{-/-}$ mice were negative for Muc2, but trefoil factor 3 was still detectable. In $Muc2^{-/-}$ mice, transient de novo expression of *Muc6* messenger RNA was observed in the distal colon. On day 2 of DSS treatment, the histologic damage was more severe in $Muc2^{+/-}$ versus wild-type ($Muc2^{+/+}$) mice, but the disease activity index was not yet different. By day 7, the disease activity index and histologic score were significantly elevated in $Muc2^{+/-}$ versus $Muc2^{+/+}$ mice. The disease activity index of the $Muc2^{-/-}$ mice was higher (versus both $Muc2^{+/+}$ and $Muc2^{+/-}$ mice) throughout DSS treatment. The histologic damage in the DSS-treated $Muc2^{-/-}$ mice was different compared with $Muc2^{+/+}$ and $Muc2^{+/-}$ mice, with many crypt abscesses instead of mucosal ulcerations.** ***Conclusions:*** **This study shows that Muc2 deficiency leads to inflammation of the colon and contributes to the onset and perpetuation of experimental colitis.**

Ulcerative colitis and Crohn's disease are two of the most important inflammatory bowel diseases (IBD), characterized by chronic inflammation and mucosal tissue damage of parts of the gastrointestinal tract. The etiology of these inflammatory disorders remains unknown, but research so far has shown that these diseases are caused by a combination of genetic, environmental, immunoregulatory, and epithelial factors.[1–6]

The epithelium and mucus layer in the intestinal tract form a physical barrier between the potential toxic and noxious agents present in the gut lumen and the underlying tissues. Goblet cells exert a vital role since they secrete molecules, which serve protective roles in the gut, like mucins and trefoil factors. Mucins are the building blocks of the mucus layer, and previous studies have shown that human, rat, and mouse colonic epithelium expresses mainly one secretory mucin in high amounts, MUC2.[7–11] The secretory mucin MUC2 is stored in bulky apical granules of the goblet cells and is the most important factor determining the goblet cell morphology.[12,13] Trefoil factor 3 (Tff3) is a protein also expressed by goblet cells in the intestine and has been shown to play an essential role in the maintenance and repair of the intestinal mucosa.[3] Damage to the epithelium, in particular those events affecting the protective properties as offered by the secretory products of the goblet cells, is a likely cause of the inflammation.

Histologic analysis for patients with ulcerative colitis often shows depletion of recognizable goblet cells in the colonic epithelium.[14] Defects in the intestine, such as loss of E-cadherin expression, multidrug-resistant protein, or Tff3 deficiency, have been shown to lead to higher susceptibility to chronic inflammation that can progressively deteriorate the epithelial barrier func-

Abbreviations used in this paper: BrdU, bromodeoxyuridine; DAI, disease activity index; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; IL, interleukin; mRNA, messenger RNA; Muc, Mucin; PCR, polymerase chain reaction; Tff3, trefoil factor 3; TNF, tumor necrosis factor.

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Table 1. DAI Score

Score	% weight loss	Stool consistency	Blood loss	Appearance
0	None (none)	Normal droppings	None	Lively/normal
1	0–17 (0–10)	Loose droppings	Hemoccult positive	Hunched
2	18–35 (10–20)	Diarrhea	Gross bleeding	Starey coat
3	>35 (>20)			Lethargic

NOTE. Criteria were obtained by pooling all data and calculating quartiles. For the initial phenotyping experiment, the percentage weight loss was calculated by comparing the weight of the mice with corresponding wild-type littermates. For the experiment in which the animals were treated with DSS, percentage weight loss was calculated by using the initial body weight of the individual animals. The classification of the weight loss in the DAI as applied in the DSS experiment appears in parentheses.

tion.[3,4,6] Furthermore, it has been shown that in patients with ulcerative colitis, the activity of the mucosal inflammation correlates significantly with a decrease in MUC2 synthesis[15] and secretion,[16] again implying that the mucosal barrier plays a key role in the course of the disease.

To more specifically address the importance of MUC2 in epithelial protection, Muc2-deficient ($Muc2^{-/-}$) mice were generated through genetic inactivation of the murine *Muc2* gene.[12] In these $Muc2^{-/-}$ mice, the intestinal goblet cells were seemingly absent. Interestingly, mice lacking Muc2 developed adenomas as of 6 months of age, which progressed to invasive adenocarcinoma in the small intestine as well as rectal tumors at an older age. Therefore, Muc2 seems to play a role in the suppression of intestinal cancer,[12] but the exact mechanism whereby Muc2 suppresses tumorigenesis remains unclear.

To obtain insight in the role of Muc2 in epithelial barrier function, and thus its possible role in IBD, we characterized the Muc2 knockout ($Muc2^{-/-}$) and heterozygous ($Muc2^{+/-}$) mouse from birth to 16 weeks of age, before the development of adenomas, and challenged these mice with the colitis-inducing agent dextran sulfate sodium (DSS). This animal model is the first to provide the possibility to investigate the physiologic function of Muc2 in the intestine of the mouse under unchallenged and luminally challenged conditions.

Materials and Methods

Animals

The previously described $Muc2^{-/-}$ mice on mixed genetic background[12] were backcrossed onto a 129SV (Charles River, Maastricht, The Netherlands) genetic background for 9 generations followed by intercrosses to generate mice homozygous for the Muc2 disruption. To obtain experimental groups with a minimal age difference between the different litters, all mice were generated from $Muc2^{+/-}$ breedings. Throughout the backcrossing procedure, the targeted *Muc2* gene was monitored via polymerase chain reaction (PCR) assays performed on genomic DNA isolated from tail clips as described by Velcich et al.[12] All the mice were housed in the same specific pathogen–free environment with free access to standard rodent pellets (Special Diets Services, Witham, Essex, England) and acidified tap water in a 12-hour light/dark cycle. All animal care and procedures were conducted according to institutional guidelines (Erasmus MC Animal Ethics Committee, Rotterdam, The Netherlands).

Experimental Setup

Wild-type ($Muc2^{+/+}$), $Muc2^{+/-}$, and $Muc2^{-/-}$ littermates were scored weekly until the age of 16 weeks to obtain a disease activity index (DAI) as described by Cooper et al[17] (Table 1). Briefly, they were scored for the following: weight, softness of the stool, occult fecal blood,[18] and general appearance of the mice. One hour before the mice were killed, bromodeoxyuridine (BrdU) 30 mg/kg body wt (Sigma Chemical Co, St Louis, MO) was injected intraperitoneally to be able to study epithelial proliferation.[19] The time curve was started with 16 mice of each genotype: $Muc2^{+/+}$, $Muc2^{+/-}$, and $Muc2^{-/-}$. At the ages of 5, 8, 12, and 16 weeks, groups of 4 male mice per genotype were killed. Small intestine and colon were excised immediately and either fixed in 4% (wt/vol) paraformaldehyde in phosphate-buffered saline (PBS), stored in RNAlater (Qiagen, Venlo, The Netherlands) at −20°C, or frozen in liquid nitrogen and stored at −80°C.

Histology and Histologic Grading

Tissue fixed in 4% (wt/vol) paraformaldehyde in PBS was prepared for light microscopy, and 5-μm-thick sections were stained with H&E to study histologic changes as described previously.[20] Grading of intestinal inflammation was determined as described by Rath et al[21] with slight modifications (Table 2). In particular, the scoring of both the percentage of the total ulcer area per section and the number of crypt abscesses for each section was modified. To detect differences in mucosal and epithelial thickness in the colon, 10 well-oriented crypts or epithelial cells were chosen per intestinal segment and measured using calibrated Leica Image Manager 500 software (Leica Microsystems BV, Rijswijk, The Netherlands). All scores were obtained in a blinded fashion by 2 independent investigators.

Immunohistochemistry

Five-micrometer-thick sections were cut and prepared for immunohistochemistry as described previously[22] using the