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BASIC—ALIMENTARY TRACT

Mucin Gene Deficiency in Mice Impairs Host Resistance to an Enteric Parasitic Infection

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BACKGROUND & AIMS: Hyperplasia of mucin-secreting intestinal goblet cells accompanies a number of enteric infections, including infections by nematode parasites. Nevertheless, the precise role of mucins in host defense in nematode infection is not known. We investigated the role of the mucin (Muc2) in worm expulsion and host immunity in a model of nematode infection. **METHODS:** Resistant (BALB/c, C57BL/6), susceptible (AKR), and Muc2-deficient mouse strains were infected with the nematode, *Trichuris muris*, and worm expulsion, energy status of the whipworms, changes in mucus/mucins, and inflammatory and immune responses were investigated after infection. **RESULTS:** The increase in Muc2 production, observed exclusively in resistant mice, correlated with worm expulsion. Moreover, expulsion of the worms from the intestine was significantly delayed in the Muc2-deficient mice. Although a marked impairment in the development of periodic acid Schiff (PAS)–stained intestinal goblet cells was observed in Muc2-deficient mice, as infection progressed a significant increase in the number of PAS-positive goblet cells was observed in these mice. Surprisingly, an increase in Muc5ac, a mucin normally expressed in the airways and stomach, was observed after infection of only the resistant animals. Overall, the mucus barrier in the resistant mice was less permeable than that of susceptible mice. Furthermore, the worms isolated from the resistant mice had a lower energy status. **CONCLUSIONS: Mucins are an important component of innate defense in enteric infection; this is the first demonstration of the important functional contribution of mucins to host protection from nematode infection.**

Keywords: Muc2; Goblet Cell; Enteric Infection; Host Resistance; Innate Immunity.

The mucus barrier is an essential part of the innate immune system which hydrates and protects the underlying epithelia. The gel-like properties of the barrier are mainly due to the polymeric mucins that are the main secretory products of epithelial goblet cells.[1–3] The colonic epithelium expresses mainly MUC2/Muc2 in large amounts which is stored in bulky apical granules of the goblet cells and is the most important factor determining the goblet cell morphology.[4–6] Muc2 forms a heterogeneous mucus barrier that is proposed to contain 2 distinct layers; a "loose" outer layer that bacteria can penetrate and an adherent inner layer that excludes bacteria from direct contact with the underlying epithelia.[7]

Alterations or absence of MUC2 production can lead to many common human disorders such as colon carcinoma,[8] ulcerative colitis,[9] and celiac disease.[10] A role for Muc2 in the suppression of colorectal carcinoma has also been suggested because Muc2 knockout (KO) mice spontaneously develop colitis and adenomas that progress to invasive adenocarcinoma,[11] suggesting an important function for this mucin in colonic protection.[6] Furthermore, missense mutations in the *Muc2* gene results in aberrant Muc2 oligomerization, leading to endoplasmic reticulum stress and subsequently increased susceptibility to colitis.[12]

Hyperplasia of mucin-producing goblet cells has been described in a number of parasitic infections, including *Nippostrongylus brasiliensis, Hymenolepis diminuta*, *Trichinella spiralis*, and *Trichuris muris*.[13–17] Putative mechanisms underlying the protective role of mucins against infectious agents include the demonstration of trapping of *Hymenolepis diminuta*[17] and *Trichinella spiralis*[18] in the mucus and inhibition of parasite motility and feeding capacity.[18–20] Goblet cell response, in all 4 of these nematode models, is thought to be under the control of a T helper (T_H)

Abbreviations used in this paper: ATP, adenosine triphosphate; BrdU, bromodeoxyuridine; IL-4, interleukin-4; KO, knockout; mMuc2, murine Muc2; PAS, periodic acid Schiff; Relm, resistin-like molecule; RT-PCR, reverse transcription–polymerase chain reaction; SCID, severe combined immunodeficient; Tff3, trefoil factor 3; T_H, T helper; WT, wild-type.

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2-type immune response and is considered as a potential effector mechanism.[21–23] A number of goblet cell bioactive factors such as resistin-like molecule-β (Relm-β), intelectin, and calcium-activated chloride channel-3 have been suggested to play an important role in nematode infection.[24,25] However, a definitive and precise role of mucins, the main secreted product of goblet cells, in host defense in intestinal nematode infection remains to be elucidated.

The nematode *T muris* inhabits the cecum of mice and is closely related at the morphologic, physiologic, and antigenic levels to *Trichuris trichuria,* the causative agent of chronic trichuriasis in human beings.[26] In this parasitic infection, strains resistant to chronic infection (BALB/c, C57BL/6) expel the parasites through the generation of a T_H2-type immune response, whereas susceptible strains (AKR), which do not expel the worms, develop a T_H1-type immune response.[22,27] In this study, we demonstrated that the increase in Muc2, the main determinant of mucus barrier properties, correlates with worm expulsion. In the absence of Muc2 there is a delay in worm expulsion, but interestingly Muc5ac is up-regulated in the Muc2-deficient mice before expulsion. Moreover, Muc5ac is up-regulated in the wild-type (WT) mice that are resistant to infection, but not in those unable to expel. The physical properties of the mucus barrier are also altered during infection, resulting in a less-porous network, with overall changes having a direct effect on the viability of the whipworm. Collectively, these data show for the first time a protective role for mucins in nematode infection.

Materials and Methods

Animals

Breeding pairs of Muc2-KO mice originally produced by gene mutation[11] and their WT (C57BL/6) littermates (Albert Einstein Medical College, New York, NY) were kept at the animal facilities of McMaster University (Hamilton, ON, Canada). AKR, BALB/c (Harlan, UK), and severe combined immunodeficient (SCID) mice were maintained in the Biological Services Unit at Manchester University. The protocols used were in accordance with guidelines by the McMaster University Animal Care Committee, Canadian Council on the Use of Laboratory Animals, and the Home Office Scientific Procedures Act (1986). All mice were kept in sterilized, filter-topped cages, and fed autoclaved food in the animal facilities. Only 6- to 10-week-old male mice were used.

Parasitologic Techniques

The techniques used for *T muris* maintenance and infection were described previously.[28] Mice were orally infected with approximately 100–300 eggs for a high-dose infection and <15 eggs for a low-dose infection. Worm burdens were assessed by counting the number of worms present in the cecum as described previously.[28]

Histology, Immunohistochemistry, and Immunofluorescence

A 1-cm segment or the whole cecum (rolled) was fixed in 10% neutral buffered formalin or 95% ethanol and processed with the use of standard histologic techniques. Sections were treated with 0.1 mol/L KOH for 30 minutes before staining with periodic acid Schiff (PAS) reaction.[29] Slides were counterstained with either H&E or 1% fast-green. Standard immunohistochemical and immunofluorescent staining methods[29,30] were used to determine the levels of Muc2, Muc5ac, Relm-β, and trefoil factor 3 (Tff3).

Antibodies

Immunodetection was carried out with the use of a polyclonal antibody raised against a murine Muc2 (mMuc2).[12] Commercially available 45M1 antibody was used for the detection of mouse Muc5ac.[31] The mouse Muc5b-specific antibody[32] was a kind gift from Dr Camille Ehre (University of North Carolina, Chapel Hill). Commercially available mRelm-β (Abcam, Cambridge, UK) and mITF (Santa Cruz Biotechnology Inc, Santa Cruz, CA) antibodies were used to detect Relm-β and Tff3, respectively. Detection of bromodeoxyuridine (BrdU) incorporated into nuclei was carried out with the use of a monoclonal anti-BrdU antibody (AbD Serotec, Oxford, UK).[33]

Mucus Extraction and Agarose Gel Electrophoresis

The cecum was gently flushed with phosphate-buffered saline and scraped, and mucus was solubilized in 8 mol/L guanidium chloride. Subsequently, extracted mucus samples were reduced with 50 mmol/L dithiothreitol and carboxylmethylated with 0.125 mol/L iodoacetamide before electrophoresis on a 1% (wt/vol) agarose gel. Mucins were detected after Western blotting with mucin-specific antisera.[34]

Analysis of Mucus Network Properties

Cecal tissue isolated from BALB/c and AKR mice was cut longitudinally, washed with phosphate-buffered saline, and kept hydrated in a 6-well plate. Blue fluorescently labeled polymer microspheres (0.1 μm; Dukes Scientific, Dorchester, United Kingdom) were placed on top of the luminal surface of the cecum (set as a reference) and their position was analyzed with the use of the Nikon (Melville, NY) C1 Upright confocal microscope. Three-dimensional optical stacks were taken every 5 μm and combined to obtain a z-stack at the time points stated.

Energy Status of Worms

The CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI) was carried out according to manufacturer's instructions. Relative light units were cal-