Human Pathology (2017) 62, 126–133

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Immunohistochemical study of mucins in human intestinal spirochetosis☆

Sho Ogata MD, PhD[a,b,*], Ken Shimizu MD, PhD[b], Susumu Tominaga MT[c], Kuniaki Nakanishi MD, PhD[a,c]

[a]*Department of Laboratory Medicine, National Defense Medical College Hospital, Tokorozawa, Saitama 359-8513, Japan*
[b]*Department of Diagnostic Pathology, JCHO Saitama Medical Center, Saitama, Saitama 330-0074, Japan*
[c]*Department of Pathology and Laboratory Medicine, National Defense Medical College, Tokorozawa, Saitama 359-8513, Japan*

Received 2 December 2016; revised 11 January 2017; accepted 26 January 2017

Keywords:
Brachyspira;
Human intestinal spirochetosis;
Immunohistochemistry;
Mucin;
Spirochetosis

Summary Most patients with human intestinal spirochetosis (HIS; a colorectal bacterial infection caused by *Brachyspira* species) seem asymptomatic, and its pathogenicity remains unclear. Recently, alterations in mucin expression were reported in animal *Brachyspira* infection. The present question was "Is mucin expression altered in HIS?" Using antibodies for MUCs 1, 2, 4, 5AC, and 6, we immunohistochemically compared 215 specimens from 83 histology-confirmed HIS cases with 106 specimens from 26 non-HIS cases. Positive staining (which included even focal positive staining) was rated "high (+)" or "low (+)." Results were analyzed for 4 categories of lesions, and associations between MUC expression and spirochetal presence were also analyzed. In the "specimens without polyps or adenocarcinoma" category, high (+) MUC2 positivity was more frequent in HIS than in control. In the hyperplasia/serrated polyp category, in HIS (versus control), the MUC5AC positivity rate was lower, whereas high (+) MUC4 positivity was more frequent. In the conventional adenoma category, in HIS (versus control), the MUC1 positivity rate was lower, whereas both high (+) MUC2 positivity and high (+) MUC5AC positivity were less frequent. In the adenocarcinoma category, high (+) MUC2 positivity was more frequent in HIS than in control. Among the above mucins, only MUC1 positivity was significantly associated with an absence of the so-called fringe formation, an absence of spiral organisms within mucus, and an absence of strong immunopositive materials within the epithelial layer and within the subepithelial layer. The results suggest that *Brachyspira* infection or a related change in the microbiome may alter the large intestine mucin expression profile in humans.

1. Introduction

Mucus, which lines and protects the intestine, traps and transports debris and bacteria, and it also reduces the mechanical stress on the epithelium [1]. In the large intestine, mucus forms a double layer, with the inner layer—which is firmly attached to the surface epithelium of the mucosal layer—being devoid of commensal bacteria [2]. Mucins, major components of such mucus, are large glycoproteins that have more than 50% of their mass as *O*-glycans, and they are divided into 2 types, namely, gel-forming and transmembrane types [1]. Gel-forming mucins, which are dominant components of the mucus layer, are released from goblet cells. Of this type, mucin MUC2 is expressed in the normal large

☆ Disclosures: None declared.

* Corresponding author at: Department of Laboratory Medicine, National Defense Medical College Hospital, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan.
E-mail address: sogata@ndmc.ac.jp (S. Ogata).

http://dx.doi.org/10.1016/j.humpath.2017.01.013
0046-8177/

intestine. Transmembrane mucins cover the apical cell membrane of enterocytes, and of this type, mucin MUC4 is expressed in the normal large intestine [1-3].

Human intestinal spirochetosis (HIS), one of the zoonoses, is caused by colonization by *Brachyspira* spp bacteria within the human large intestine [4,5]. These species of *Brachyspira* have a peculiar capacity to penetrate through the thick inner layer of mucus to reach the surface epithelium [6], and a parallel arrangement of *Brachyspira* attaching to the cell surface forms a histologically diagnostic clue, the so-called fringe [4]. Most HIS patients seem asymptomatic, and some researchers regard *Brachyspira* colonization of the human intestine as harmless [4]. However, among 85 HIS cases in our previous study, 7.1% had gastrointestinal symptoms and 11.8% exhibited mucosal inflammation on endoscopic examination [7]. Moreover, an in vitro study inoculating the human colonic adenocarcinoma cell line Caco-2 with the weakly β-hemolytic *B pilosicoli* (one of the causative organisms for HIS) indicated a potential pathogenicity [8]. However, the precise pathogenicity of HIS remains unclear.

Recently, increased MUCs 2 and 5AC expressions and decreased MUC4 expression in the mucosa of the large intestine were reported in animal *Brachyspira* infections [9,10]. In the human intestine, however, the mucosal condition as regard mucin expression in the *Brachyspira*-infected state has not been elucidated. The aim of this study was to examine whether or not mucin expressions are altered in HIS.

2. Materials and methods

The present study focused on a total of 83 HIS cases at the JCHO Saitama Medical Center (in Saitama, which is close to Tokyo, Japan) [7,11]. This medical center is a general hospital at which most of the patients are considered immunocompetent. Pathologic samples were also provided by hospitals or medical clinics within the same prefecture. The male-female ratio was 71:12, and the median age was 57 years (between 28 and 77). The study specimens were taken under colonoscopy, and most of the patients seemed to be immunocompetent to judge from pathology request forms. Cases providing one or more specimens that histologically exhibited a *distinct*, hematoxylinophilic fringe formation on the luminal surface of the colorectal surface epithelium in hematoxylin-eosin–stained glass slides were considered to have HIS. Moreover, these findings were confirmed by the presence of immunohistochemical cross-reactivity to a polyclonal antibody for *Treponema pallidum* (*TP*; Abcam, Cambridge, UK) [11]. Control specimens (ie, cases not showing obvious fringes) were taken endoscopically and operatively at the medical center.

Two hundred and fifteen specimens from the 83 histology-confirmed HIS cases were used. These specimens were placed in 1 of 4 categories: 24 specimens in the "specimens without polyps or adenocarcinoma" (w/o PAC) category, 36 in hyperplasia/serrated polyp (HP/SP), 146 in conventional (tubular, tubulovillous, and villous) adenoma (C-adenoma), and 9 in adenocarcinoma (AC). As controls, 106 specimens were obtained from the non-HIS large intestines of 26 cases (male-female ratio = 21:5; median 61 years [37-84 years]). Of these controls, 28 were placed in w/o PAC, 41 in HP/SP, 27 in C-adenoma, and 10 in AC. Information about the colorectal regions providing specimens was obtained from pathology request forms. For this analysis, large intestines were divided into 2 regions (right side: from cecum to transverse colon; left side: from the splenic flexure to rectum).

For immunohistochemistry, we used the polymer-peroxidase method (EnVision+/HRP; Dako Cytomation, Glostrup, Denmark) on deparaffinized sections from the HIS and control cases using antibodies for the gel-forming mucins MUCs 2, 5AC, and 6 and the transmembrane mucins MUCs 1 and 4. All antibodies were incubated overnight at 4°C. The monoclonal antibodies used are listed in Table 1, together with the antigen retrieval conditions.

For the evaluation of MUCs, even focal positive staining was considered positive. If present, the degree of positive staining was rated as either "high (+)" or "low (+)" depending on whether (or not) 50% or more of the epithelial cells (including goblet cells) of the whole surface and crypts in the lesion exhibited positivity. Cytoplasmic or luminal surface staining and even a weak staining intensity were considered positive. The results of the mucin immunohistochemistry were analyzed in relation to the 4 types of lesions mentioned above and also to colorectal location.

Moreover, the association between positivity for each MUC and the immunohistochemical results obtained using anti-*TP* antibody was analyzed. The specimens from HIS

Table 1 Monoclonal antibodies used for various mucins together with the retrieval conditions

Mucins	Clones	Manufacturers	Antigen retrieval conditions
MUC1	Ma695	Novocastra Laboratories, Ltd (Newcastle, UK)	10 mmol/L Tris base, 1 mmol/L EDTA (pH 9)
MUC2	Ccp58		10 mmol/L Tris base (pH 10)
MUC4	8G7	Santa Cruz Biotechnology (Dallas, TX)	10 mmol/L Tris base, 1 mmol/L EDTA (pH 9)
MUC5AC	CLH2	Novocastra Laboratories, Ltd (Newcastle, UK)	10 mmol/L Tris base, 1 mmol/L EDTA (pH 9)
MUC6	CLH5		10 mmol/L Tris base (pH 10)

NOTE. Dilution rate was ×100 for each antibody. All antigen retrievals were performed at 98°C for 1 hour.
Abbreviation: MUC, mucin.