Histol Histopathol (2024) 39: 41-47

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE Open Access

Colonic mucosa barrier defects in collagenous and ischemic colitis

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Summary. Aims. The subepithelial myofibroblasts (SEMFs) and the subepithelial band of macrophages (SEBM) are major components of the colonic mucosa barrier. Although their role in homeostasis is widely recognized, their contribution to disease states is largely unknown. Our aim was to explore histological characteristics of SEMFs and SEBM in collagenous and ischemic colitis in order to identify specific changes in distinct mucosa backgrounds lacking significant inflammation.

Methods. SEMFs, SEBM and lamina propria (LP) macrophages were identified immunohistochemically by alpha smooth muscle Actin and Cluster of Differentiation 68 respectively in 38 colonic biopsies [14 collagenous colitis (CC), 14 ischemic colitis (IC), 10 normal mucosa].

Results. In CC, SEMFs were rarely detectable in the collagenous band while aSMA-negative pericryptal fibroblast-like cells appeared. In lower LP interconnecting SEMFs processes were formed. SEBM was preserved in areas with a collagenous layer up to 20 μm. In thicker layers, it was fragmented and gradually disappeared in parallel with engulfment of enlarged macrophages. LP macrophages were usually increased. In IC, slight SEMFs changes preceded discernible epithelial alterations. Rounding, disintegration and extinction of SEMFs constituted successive alterations coinciding with crypt shrinkage and denudation. SEBM displayed total or almost total abolishment in areas with crypt damage but also in sites with minimal changes and in adjacent normal mucosa.

Conclusion. Our findings provide evidence of impairment of both mucosa barrier constituents in CC and IC. In CC, histological alterations are closely related to the collagenous layer which seems to affect SEMFs differentiation and migration as well as SEBM integrity. The early extinction of SEBM in IC is indicative of its high sensitivity to hypoxia and hypoperfusion.

Key words: Colonic mucosa barrier, Subepithelial myofibroblasts, Subepithelial band of macrophages, Collagenous colitis, Ischemic colitis

Introduction

The intestinal mucosa barrier is a complex, multilayered structure, whose integrity is crucial for the defense, repair and functional properties of the mucosa. Besides the epithelium, major components of the mucosa barrier also include the pericryptal and subsurface subepithelial sheath of fibroblasts/myofibroblasts and the subepithelial band of macrophages residing in the upper region of the lamina propria (LP) beneath the surface epithelium. The intricate role of these cell types is justified both by their topography and their specialized functions (Andoh et al., 2005; Rubio et al., 2018).

The subepithelial myofibroblasts (SEMFs) are pericryptal mesenchymal cells with a myofibroblast phenotype located underneath the basement membrane. They express α-smooth muscle actin (α-SMA) and vimentin and differ from pericytes by the absence of desmin expression (Mifflin et al., 2011; Roulis and Flavell, 2016). It is widely accepted that they belong to a syncytium together with the blood vessel-surrounding pericytes which extends throughout the LP (Joyce et al., 1987; Powell et al., 1999; Andoh et al., 2005). In ultrastructural studies in the 70's-90's, mesenchymal cells along the crypt and in the subepithelial area were

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www.hh.um.es. DOI: 10.14670/HH-18-623

Abbreviations. α-SMA, α-smooth muscle actin; CC, collagenous colitis; CD 68, Cluster of Differentiation 68; HPF, high power field; IBD, inflammatory bowel disease; IC, ischemic colitis; MCP, Monocyte chemoattractant protein 1; NLRs, NOD-like receptors; SEBM, subepithelial band of macrophages; SEMFs, subepithelial myofibroblasts; TLRs, Toll-like receptors

referred to as "pericryptal fibroblasts" and fibrocytes. Structural changes of "pericryptal fibroblasts" supported their migration from an undifferentiated cell at the lower part of the crypt to a fibroblast-like cell at the upper part. It is hypothesized that the subepithelial pericryptal cells, later recognized as myofibroblasts, lose α-SMA staining as they migrate in the aforementioned superficial mucosa regions and transform to fibroblasts (Widgren et al., 1988; Mifflin et al., 2011). These fibroblast-like cells are α-SMA negative, platelet-derived growth factor receptor-αþ positive and express weakly vimentin, while their specific functions are still a matter of investigation (Roulis and Flavell, 2016). The occasionally observed α-SMA positive subsurface cells represent pericytes being also present in the entire LP (Mifflin et al., 2011). There is evidence that myofibroblasts form the stem cell niche at the crypt base (Mifflin et al., 2011) and seem to be involved in intestinal epithelial cell differentiation (Halttunen et al., 1996; Powell et al., 1999).

Activated colonic SEMFs are known to have proinflammatory and repairing effects as indicated by upregulation of molecular pathways and secretion of growth factors, and extracellular matrix components (Powell et al., 1999; McKaig et al., 1999; Rogler et al., 2001; McKaig et al., 2002; Andoh et al., 2005). Moreover, SEMFs contribute to adaptive immune response acting as antigen presenting cells (Saada et al., 2006) and to the preservation of colonic mucosa tolerance (Pinchuk et al., 2011).

The subepithelial band of macrophages (SEBM) constitutes the second line of mucosa defense. Macrophages, members of the innate immune system, exert bactericidal functions as phagocytes and clear apoptotic cells, contributing to the maintenance of intestinal homeostasis (Ruder and Becker, 2020). These indigenous macrophages do not express certain innate receptors, but they possess several recognition receptors which help them to distinguish commensal from harmful bacteria (Zhou et al., 2016; Ruder and Becker, 2020). Although highly phagocytic, they express a rather anti-inflammatory profile, thus avoiding inflammatory reactions to bacterial intrusion in the intestine under normal conditions (Smythies et al., 2005; Ruder and Becker, 2020).

Although the role of both SEMFs and SEBM in homeostasis is widely recognized, their contribution to disease states is still largely unknown. Data regarding SEMF functional properties derive mainly from *in vitro* experiments on isolated cells. Their investigation in colonic injury has been the subject of a limited number of studies referring in particular to inflammatory bowel disease (IBD) (Lawrance et al., 2001; Andoh et al., 2002, 2005; McKaig et al., 2002) and collagenous colitis (CC) (Hwang et al., 1986; Balázs et al., 1988; Aigner et al., 1997; Powell et al., 1999) while studies addressing histopathological alterations in other colonic lesions are missing. Similarly, the study of the pathophysiological role of SEBM has been focused almost exclusively on IBD (Rubio et al., 2018).

The present study aims to explore the histological characteristics of the SEMFs and SEBM in collagenous and ischemic colitis (IC) in order to identify specific changes reflecting their behavior in distinct mucosa backgrounds lacking significant inflammation.

Materials and methods

The examined material consists of 38 colon biopsies from 38 adult patients. In 14 cases the histological diagnosis was CC (6 males, 8 females; median age 73 years with chronic watery diarrhea) and in the other 14 IC (8 males, 6 females; median age 77 years). An additional 10 cases with normal colonic mucosa served as controls (5 males, 5 females; median age 62 years). All cases were anonymized and permission for scientific use of patient data was obtained by the Research Ethics and Deontology Committee, NKUoA.

Biopsies were obtained both from right and left colon of each patient, fixed in buffered formalin, embedded in paraffin, cut in 4 μm sections and examined initially on histochemical stains, Hematoxylin-Eosin and Masson Trichrome for collagen depiction. The diagnosis of CC was based on segmental subepithelial collagen band thickness >10μm (Freeman 2005), assessed on camera captured images. Sections with diagnostic features of CC and IC were further processed for immunohistochemical analysis.

SEMFs were identified by immunohistochemical expression of α-SMA (clone 1A4, DAKO) and macrophages by Cluster of Differentiation 68 (CD68) immunostaining (clone PG-M1, DAKO). α-SMA positive SEMFs were assessed around the crypt wall (pericryptal) and beneath the surface epithelium. SEMFs processes with or without interconnections were also registered.

PG-M1 positive SEBM was recorded as continuous or fragmented. Fragmentation was further estimated as focal, patchy or extensive. Concerning its width, SEBM was assessed as normal, thickened, attenuated or abolished. Dispersed macrophages in the rest LP were counted and the number >40 macrophahes per high power field (HPF) was considered increased.

Results

Histology

In the CC group the width of the collagenous layer ranged between 13 and 60 μm. Entrapped capillaries and inflammatory cells were seen, while a small number of intraepithelial lymphocytes were occasionally present.

In IC group, 7 cases showed mild and 7 moderate changes, occasionally with foci of severe damage. Cases showing total mucosa necrosis were not included in the study. In 8 IC cases, collagen deposition was seen in the LP, namely 4 in the upper third, 3 in the upper half and 1 in the whole mucosa.

Immunohistochemical evaluation of normal mucosa

SEMFs surrounded 4/5 of the crypt length up to a distance of 10 μm from the mucosa surface. Directly beneath the surface epithelium solitary α-SMA-positive cells were rarely encountered and only in 2 cases were focally in close contact. The pericryptal rim of SEMFs was complete in 6 and incomplete in 4 cases. Cell processes were usually not discernible except very few in the lower mucosa.

The SEBM was invariably continuous between 20 and 40 μm in width while in 2 cases it was focally or segmentally attenuated. Low numbers of macrophages (<40/HPF) were seen in all other regions of the LP.

Immunohistochemical findings in normal mucosa are depicted in Figure 1.

Immunohistochemical evaluation of CC

Pericryptal α-SMA-positive SEMFs almost totally disappeared in the region of the collagenous band while the few residual cells were usually enlarged. In lower crypt segments surrounded by non-collagenized stroma, SEMFs were often enlarged and characterized by the formation of cell processes interconnecting neighboring crypts and capillaries. The absence of pericryptal SEMFs was considered reliable, only when the collagenous band exceeded in depth the upper 1/5 of the crypt taking into consideration that in normal mucosa this part of the crypt may be devoid of α-SMA-positive pericryptal cells. Transition crypt zones exhibited partial loss and enlargement of SEMFs, while prominent cell processes with interconnections of crypts and vessels and occasional network formation were also seen. In crypt parts with diminished or missing SEMFs, α-SMA-negative fusiform pericryptal cells, most probably fibroblasts, were visible. SEMFs changes in CC are depicted in Figure 2A.

SEBM was preserved in all cases in areas with a collagenous layer up to 20μm width, showing

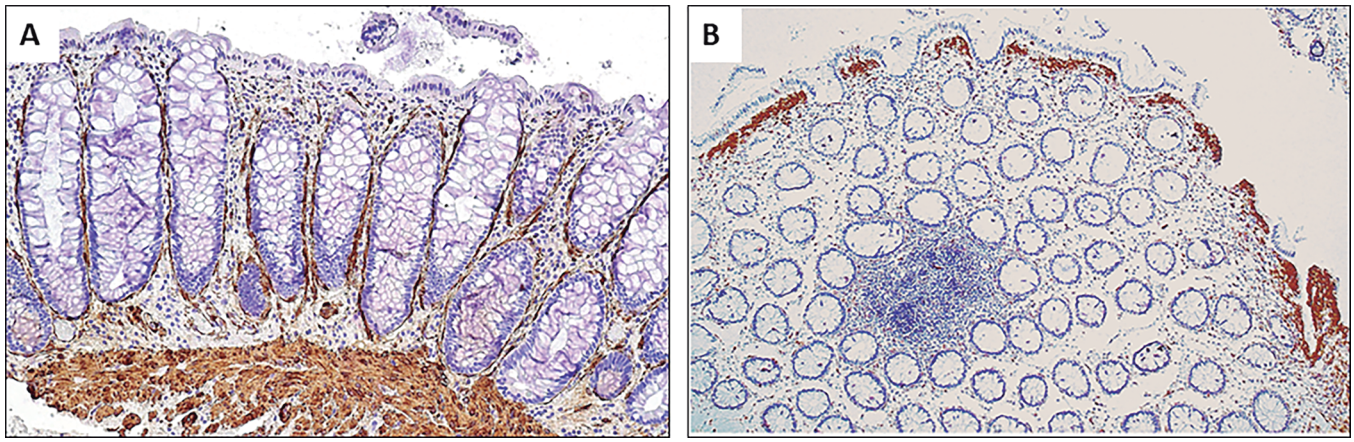

Fig. 1. Normal mucosa. **A.** pericryptal rim of α-SMA positive SEMFs. α-SMA. **B.** SEBM. PGM1. A, x 100; B, x 40.

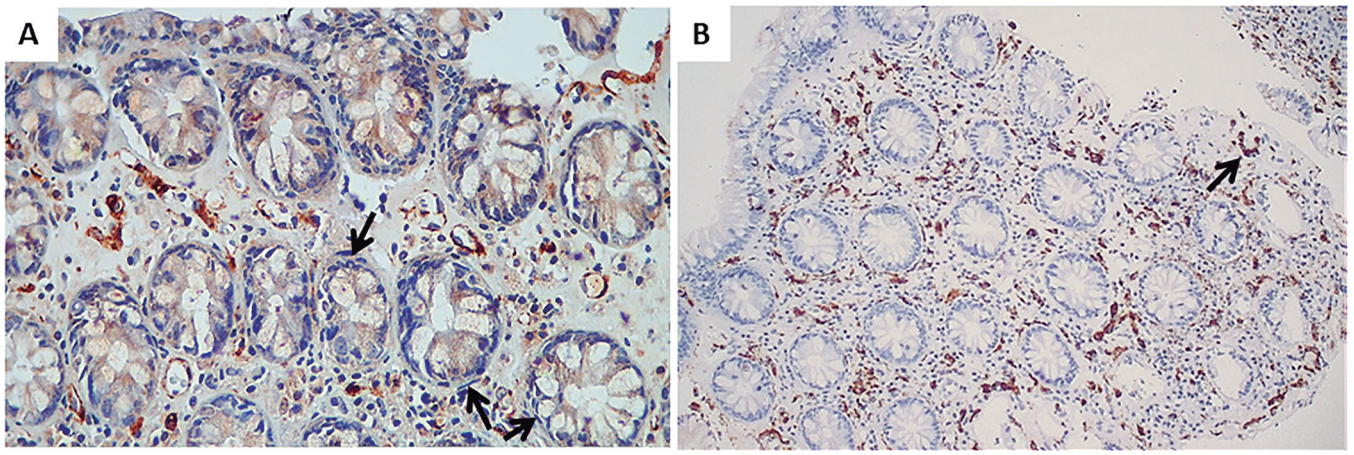

Fig. 2. Collagenous colitis. **A.** Collagen band: Total loss of α-SMA positive SEMFs. Few α-SMA negative fibroblast-like cells (arrows). α-SMA. **B.** Collagen band >20μm: SEBM disintegration with incorporation of a small group of macrophages in the collagen deposits (arrow). Enlarged macrophages in LP. PGM1. A, x 200; B x 100.

segmental thickening or attenuation. It was localized underneath the collagenous band most often retaining its continuity. In areas with thicker collagenous layer (>20μm), the band of macrophages was fragmented showing gradual incorporation and disintegration within the collagen deposits in the form of enlarged macrophages arranged in small groups or randomly distributed (Fig. 2B). In the rest LP macrophages were numerically increased (>40/HPF) in the majority of the cases (11/14) and often enlarged (9/14).

Immunohistochemical evaluation of IC

In all cases, prominent configuration of pericryptal SEMFs was an initial change in crypts without epithelial changes. Shrinkage and rounding in a bead-like manner constituted successive alterations in parallel with mucin depletion and narrowing of the crypt epithelium and stroma fibrosis (Fig. 3A). Partial or total disintegration was seen together with further narrowing and flattening of the epithelium while extinction was encountered in partially or totally denuded crypts. Cell processes with interconnections were rarely seen. Inflammatory infiltrates were mild with participation of neutrophils.

SEBM was totally or almost totally abolished in areas with conspicuous crypt damage with or without collagen deposition (Fig. 3B). In mucosa with mild ischemic injury, there was total or subtotal loss while in sites showing minimal changes as well as in neighboring normal-looking mucosa SEBM was thinned, rarefied or absent. The number of macrophages in LP was <40/HPF while in the most severe cases they almost disappeared.

Due to similar findings among the cases of each diagnostic category, the results are pooled together in Table 1.

Table 1. Immunohistochemical findings in Collagenous and Ischemic colitis regarding SEMFs and SEMB.

		SEMFs	SEBM
Collagenous Colitis	Collagen band ≤20 μm	Almost total disappearance Enlargement of few residual	Preserved underneath collagen band Segmental thickening or attenuation
	Collagen band >20μm		Fragmented Gradual incorporation in the collagen band
	Transition zone	Partial loss Often enlargement Cell processes formation	Not applicable
	Lower crypt segments	Often enlargement Cell processes formation	Not applicable
Ischemic Colitis	Minimal	Prominent configuration	Thinned, rarefied or absent
	Mild	Shrinkage and bead-like rounding	Total or subtotal loss
	Moderate-Severe	Partial or total disintegration	Total loss

SEMFs subepithelial myofibroblasts; SEBM subepithelial band of macrophages.

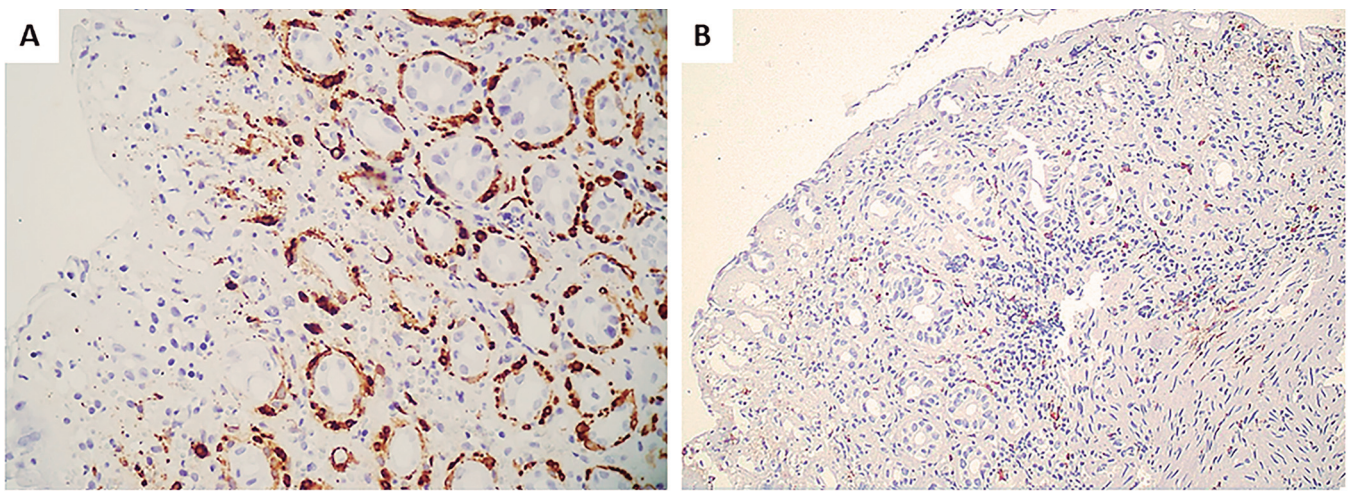

Fig. 3. Ischemic colitis. Moderate mucosal damage. **A.** SEMFs shrinkage and bead-like rounding, partial or total disintegration and loss. α-SMA. **B.** Abolishment of SEBM. Few macrophages in LP. PGM1. x 100.

Discussion

Our study focused on the investigation of integrity and behavior of the subepithelial barrier components, SEMFs and SEBM, in the colonic mucosa of patients with CC and IC, in search of early and advanced histological alterations.

In normal colonic mucosa SEMFs formed a pericryptal rim which generally did not reach the crypt orifice. This finding is consistent with the proposed model of SEMF differentiation towards a fibroblast phenotype as they migrate to the upper region of the crypt (Kaye et al., 1968; Mifflin et al., 2011). Moreover, the recorded attenuated and fenestrated fibroblastic sheath beneath the surface epithelium justifies the lack of a subsurface myofibroblast layer noted in our cases while the detected solitary α-SMA-positive cells under the surface epithelium probably represent pericytes (Mifflin et al., 2011). The SEBM was well preserved, occasionally showing focal or segmental attenuation as has already been described (Rubio et al., 2018).

In CC pericryptal SEMFs invariably disappeared in the site of the collagenous band whereas in the crypt segments in the lower collagen free mucosa, they were partially missing and often enlarged. In contrast to normal mucosa, multiple α-SMA-positive cell processes were discernible forming interconnections between crypts and intercryptal vessels. To the best of our knowledge, the absence of SEMFs in relation to the collagenous band and the prominence of interconnecting cell processes have not been mentioned before. Most studies in CC have dealt with the synthetic and degradation activities of SEMFs as well as with the composition of the collagen band (Widgren et al., 1988; Aigner et al., 1997; Günther et al., 1999; Powell et al., 1999; Pardi and Kelly, 2011; Münch et al., 2012). Only in one report, a decreased number and increased size of "pericryptal fibroblasts" was noted on light microscopy (Hwang et al., 1986). The disappearance of pericryptal myofibroblasts has also been described in Crohn's disease raising the possibility of a trans-differentiation phenomenon towards a fibroblastic phenotype in response to proinflammatory cytokines (Francoeur et al., 2009). Our findings provide further evidence of SEMF differentiation and migration derangement indicated by loss of α-SMA expression when they abut the collagenous layer, and by the concomitant appearance of pericryptal fibroblast-like cells. The frequent enlargement of preserved SEMFs and the formation of interconnecting processes may represent reactive changes.

In regard to SEBM, histological evaluation revealed its preservation in mucosa areas with a collagenous layer up to 20 μm in width showing variation in thickness as in normal conditions. In regions with thicker collagenous layer, SEBM was fragmented whereas engulfment and gradual disintegration could be observed within the collagen deposits in parallel with macrophage enlargement. LP histiocytes were in almost all cases numerically increased and often enlarged. Literature data regarding the relationship of SEBM with the thickness of the collagenous band are missing. In the survey of Nishida et al no significant quantitative differences were found regarding CD68 positive macrophages in the upper part of the LP between IBD patients, CC patients and controls, having disregarded the existence of SEBM (Nishida et al., 2002). In IBD, the number of macrophages was found to be both increased and reduced while fragmentation or total abolishment of SEBM was observed in patients with ulcerative colitis and Crohn's disease in remission (Rubio et al., 2018).

In IC, slight SEMFs changes preceded discernible epithelial alterations implying higher sensitivity to hypoxia. Shrinkage and rounding constituted successive alterations while disintegration and extinction were seen in parallel with crypt shrinkage and denudation. In addition, a striking sensitivity of SEBM to ischemia was demonstrated as we observed total or almost total extinction not only in areas with established crypt damage but also in sites with minimal changes and in adjacent normal looking mucosa.

A negative impact on mucosa defense is excepted considering the early disappearance of SEBM. Intestinal macrophages under normal conditions are constantly replenished from peripheral-blood monocytes recruited from the bone marrow (Wang et al., 2019). It is reasonable to suggest that in ischemic states migration of blood monocytes to gut mucosa is impeded due to reduced blood flow. Moreover, the reduction or cessation of chemokine production, such as MCP-1 (Monocyte chemoattractant protein-1), one of the key chemokines that regulate migration and infiltration of monocytes/macrophages by damaged SEMFs should be considered a synergistic factor (Roulis and Flavell, 2016).

However, further evidence should be gained by future studies focusing on SEMFs and SEBM structural and functional alterations, their relation to the microenvironment and their impact on epithelial barrier function. The investigation of adhesion molecules, chemokines and growth factors could potentially provide more specific information. Finally, the identified histological differences between CC and IC may bear significance for the differential diagnosis, especially in cases of CC with markedly thickened collagenous band and surface epithelium detachment.

In conclusion, our findings suggest impairment of both mucosa barrier constituents in CC and IC. In CC histological alterations are closely related to the collagenous layer, which seems to affect SEMFs differentiation and migration as well as SEBM integrity. The early extinction of SEBM in IC is indicative of its high sensitivity to hypoxia and hypoperfusion.

Acknowledgements. Not applicable.

List of contributors. SS: histological evaluation, interpretation of histological findings, literature review, writing part of the manuscript. MP: histological evaluation, interpretation of histological findings. GB: clinical responsibility review of the data, critical review of the final manuscript.

ID: study concept central clinicopathological review, writing of the Discussion.

Statements and Declarations. Competing Interests: All authors declare no competing interest.

Funding. None

Availability of data and material. All research data and material will be made available upon request.

Consent for publication. All authors have approved the final version of the manuscript and have given their consent for publication.

Compliance with Ethical Standards. The study was approved by the Research Ethics and Deontology Committee, National and Kapodistrian University of Athens and complied with the ethical principles for medical research involving human subjects according to the Declaration of Helsinki (as amended in 2008).

References

Aigner T., Neureiter D., Müller S., Küspert G., Belke J. and Kirchner T. (1997). Extracellular matrix composition and gene expression in collagenous colitis. Gastroenterology 113, 136-143.

Andoh A., Fujino S., Okuno T., Fujiyama Y. and Bamba T. (2002). Intestinal subepithelial myofibroblasts in inflammatory bowel diseases. J. Gastroenterol. 14, 33-37.

Andoh A., Bamba S., Fujiyama Y., Brittan M. and Wright N.A. (2005). Colonic subepithelial myofibroblasts in mucosal inflammation and repair: contribution of bone marrow-derived stem cells to the gut regenerative response. J. Gastroenterol. 40, 1089-1099.

Balázs M., Egerszegi P., Vadász G. and Kovács A. (1988). Collagenous colitis: an electron microscopic study including comparison with the chronic fibrotic stage of ulcerative colitis. Histopathology 13, 319-328.

Francoeur C., Bouatrouss Y., Seltana A., Pinchuk I.V., Vachon P.H., Powell D.W., Sawan B., Seidman E.G. and Beaulieu J.F. (2009). Degeneration of the pericryptal myofibroblast sheath by proinflammatory cytokines in inflammatory bowel diseases. Gastroenterology 136, 268-277.e3.

Freeman H.J. (2005). Collagenous mucosal inflammatory diseases of the gastrointestinal tract. Gastroenterology 129, 338-350.

Günther U., Schuppan D., Bauer M., Matthes H., Stallmach A., Schmitt-Gräff A., Riecken E.O. and Herbst H. (1999). Fibrogenesis and fibrolysis in collagenous colitis. Patterns of procollagen types I and IV, matrix-metalloproteinase-1 and -13, and TIMP-1 gene expression. Am. J. Pathol. 155, 493-503.

Halttunen T., Marttinen A., Rantala I., Kainulainen H. and Mäki M. (1996). Fibroblasts and transforming growth factor beta induce organization and differentiation of T84 human epithelial cells. Gastroenterology 111, 1252-1262.

Hwang W.S., Kelly J.K., Shaffer E.A. and Hershfield N.B. (1986). Collagenous colitis: a disease of pericryptal fibroblast sheath? J. Pathol. 149, 33-40.

Joyce N.C., Haire M.F. and Palade G.E. (1987). Morphologic and biochemical evidence for a contractile cell network within the rat intestinal mucosa. Gastroenterology 92, 68-81.

Kaye G.I., Lane N. and Pascal R.R. (1968). Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. II. Fine structural aspects of normal rabbit and human colon. Gastroenterology 54, 852-865.

Lawrance I.C., Maxwell L. and Doe W. (2001). Altered response of intestinal mucosal fibroblasts to profibrogenic cytokines in inflammatory bowel disease. Inflamm. Bowel Dis. 7, 226-236.

McKaig B.C., Makh S.S., Hawkey C.J., Podolsky D.K. and Mahida Y.R. (1999). Normal human colonic subepithelial myofibroblasts enhance epithelial migration (restitution) via TGF-beta3. Am. J. Physiol. 276, G1087-1093.

McKaig B.C., Hughes K., Tighe P.J. and Mahida Y.R. (2002). Differential expression of TGF-beta isoforms by normal and inflammatory bowel disease intestinal myofibroblasts. Am. J. Physiol. Cell Physiol. 282, C172-182.

Mifflin R.C., Pinchuk I.V., Saada J.I. and Powell D.W. (2011). Intestinal myofibroblasts: targets for stem cell therapy. Am. J. Physiol. Gastrointest. Liver Physiol. 300, G684-696.

Münch A., Aust D., Bohr J., Bonderup O., Fernández Bañares F., Hjortswang H., Madisch A., Munck LK., Ström M., Tysk C. and Miehlke S. (2012). European Microscopic Colitis Group (EMCG). Microscopic colitis: Current status, present and future challenges: statements of the European Microscopic Colitis Group. J. Crohns Colitis 6, 932-945.

Nishida Y., Murase K., Isomoto H., Furusu H., Mizuta Y., Riddell R.H. and Kohno S.I. (2002). Different distribution of mast cells and macrophages in colonic mucosa of patients with collagenous colitis and inflammatory bowel disease. Hepatogastroenterology 49, 678-682.

Pardi D.S. and Kelly C.P. (2011). Microscopic colitis. Gastroenterology 140, 1155- 1165.

Pinchuk I.V., Beswick E.J., Saada J.I., Boya G., Schmitt D., Raju G.S., Brenmoehl J, Rogler G., Reyes V.E. and Powell D.W. (2011). Human colonic myofibroblasts promote expansion of $CD4^+$ $CD25^{high}$ $Foxp3^+$ regulatory T cells. Gastroenterology 140, 2019-2030.

Powell D.W., Mifflin R.C., Valentich J.D., Crowe SE., Saada J.I. and West A.B. (1999). Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am. J. Physiol. 277, C183-201.

Rogler G., Gelbmann C.M., Vogl D., Brunner M., Scholmerich J., Falk W, Andus T, and Brand K. (2001). Differential activation of cytokine secretion in primary human colonic fibroblast/myofibroblast cultures. Scand. J. Gastroenterol. 36, 389-398.

Roulis M. and Flavell R.A. (2016). Fibroblasts and myofibroblasts of the intestinal lamina propria in physiology and disease. Differentiation 92, 116-131.

Rubio C.A., Langner C. and Schmidt P.T. (2018). Partial to complete abrogation of the subepithelial macrophage barrier against the gut microbiota in patients with ulcerative colitis and Crohn's colitis. Histopathology 72, 580-587.

Ruder B. and Becker C. (2020). At the forefront of the mucosal barrier: The role of macrophages in the intestine. Cells 9, 2162.

Saada J.I., Pinchuk I.V., Barrera C.A., Adegboyega P.A., Suarez G., Mifflin R.C., Di Mari J.F., Reyes V.E. and Powell D.W. (2006). Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa. J. Immunol. 177, 5968-5979.

Smythies L.E., Sellers M., Clements R.H., Mosteller-Barnum M., Meng G., Benjamin WH., Orenstein J.M. and Smith PD. (2005). Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J. Clin. Invest.115, 66-75.

Wang S., Ye Q., Zeng X. and Qiao S. (2019). Functions of macrophages in the maintenance of intestinal homeostasis. J. Immunol. Res. 2019, 1512969.

Widgren S., Jlidi R. and Cox J.N. (1988). Collagenous colitis: histologic,

morphometric, immunohistochemical and ultrastructural studies. Report of 21 cases. Virchows Arch. (A) 413, 287-296.

Zhou Z., Ding M., Huang L., Gilkeson G., Lang R. and Jiang W. (2016). Toll-like receptor-mediated immune responses in intestinal macrophages; implications for mucosal immunity and autoimmune diseases. Clin. Immunol. 173, 81-86.

Accepted April 17, 2023