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CHAPTER 13

Tropomyosins in Human Diseases: Ulcerative Colitis

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Abstract

Ulcerative colitis (UC) is a form of chronic inflammatory bowel disease (IBD) that almost always affects the rectal mucosa and variable length of the colon in continuity and at times mucosa of the entire colon. It is not caused by any specific pathogen. Genetics, environmental factors and altered immune responses to dietary macromolecules, colonic bacteria and cellular proteins have been implicated in the pathogenesis of UC. Autoimmune response against cytoskeletal, microfilament protein tropomyosin (Tm) seems to play an important role in the pathogenesis of UC. The predominant colonic epithelial Tm isoform, hTm5, can induce both humoral (B-cells) and cellular (T-cells) response in patients with UC. Such responses are not seen in normal subjects and disease control subjects, such as patients with Crohn's disease (CD, another type of IBD) and patients with lupus. A novel observation that hTm5 is expressed on colon epithelial cell surface but not on small intestinal epithelial cells provides evidence for presentation to immune effector cells. This surface expression of hTm5 seems to be facilitated by a colon epithelial cell membrane associated protein, CEP, that acts as a chaperone for the trans-migration of hTm5 to the surface and both hTm5 and CEP are then released outside the cell. Both CEP and hTm5 expression are increased with pro-inflammatory cytokine, such as γ -interferon. hTm5 expression in UC mucosa is also significantly increased compared to normal. Finally, autoantibodies against hTm5 observed both in circulation and in the colon mucosa of patients with UC are pathogenic causing colon epithelial cell destruction by antibody and complement mediated cytotoxicity.

Introduction

IBD is a general term for a group of chronic inflammatory disorders of unknown etiology involving the gastrointestinal tract. UC is a form of IBD primarily associated with chronic inflammation of the large intestine (colon mucosa) starting from the rectum proximally up to the variable length of the colon. The other form of IBD is known as CD that involves the entire thickness of the bowel and can affect both small and large intestines. Symptoms of ulcerative colitis include rectal bleeding, mucus discharge and diarrhea. Depending on the state of the disease, endoscopy reveals reddening of the mucosa, increased friability, mucosal bleeding, ulcerations, pseudopolyps, granularity and loss of vascular architecture. UC is usually associated with recurrent attacks or flares with complete remission of symptoms in the interim. Drugs-Corticosteroids, aminosalicylates, immunomodulators and anti-TNF α preparations interacting at various levels along the immune and inflammatory cascades are available for the treatment of UC. They effectively induce remission and some of them are largely used to prevent disease recurrence.

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In patients with IBD, the immune system seems to be abnormally activated in genetically susceptible host by an unknown antigen (dietary, bacterial and colon epithelial) in the GI tract. Although UC and CD share several common anatomical locations such as colon and clinical features, the mucosal immune response in UC differs from that occurring in CD. A T-cell driven immune response indeed predominates in CD and most of the pathophysiological changes in CD inflamed tissue can be related to the effects of T helper cell type 1 (Th1) cytokines.^{1,3} Differently in UC, characteristics of pathological changes, including the epithelial damage, the occurrence of multiple (auto)antibodies and evidences from several immunopathological studies suggest that in UC an enhanced humoral immunity predominates. In UC also there is an increased number and state of activation of lamina propria mononuclear cells (LPMC) in the involved gut that gives rise to an increased mucosal release of soluble mediators. Although the cytokine profile in UC is less defined than for CD, the role of IL-5 in UC is of interest as this cytokine contributes to the development of a humoral-mediated immune response.³ The continued abnormal activation of the immune system results in chronic inflammation. UC is, however, a systemic disease that can affect other parts of the body outside the intestine, involving joints, skin, eyes and the bile ducts in up to 20% of the patients.

Epidemiology

The disease is frequently seen among young adults in their twenties or thirties. It is more common in Caucasians than in Blacks or Orientals with an increased incidence (three to six folds) in Jewish ethnicity. Both sexes are equally affected. In Western Europe and in the USA, UC has an incidence of approximately 6 to 8 cases per 100,000 populations and an estimated prevalence of approximately 70 to 150 per 100,000 populations.

Factors Involved in the Pathogenesis of UC

Extrinsic Factors

Diet

No specific dietary factor has been shown to be involved in the pathogenesis of UC. Lactose intolerance, causing diarrhea, is a common problem in almost half of the world's adult population with some ethnic variation. It does not directly affect UC except that it can increase diarrhea.

Bacterial Infection

The bacteria that normally live in the colon also have an important role in the development of the disease, since animals at risk for developing colitis do not develop it when raised in a bacteria-free environment.⁴ Patients who are susceptible for UC may develop the chronic disease following an attack of bacterial colitis.

Environmental Factors

Cigarette smoking prevents flare-ups of colitis, particularly among the past smokers. The mechanism of this protection is unclear.

Stress

While stress can aggravate the symptoms, it does not cause the disease.

Intrinsic Factors

Genetic Susceptibility

Various factors are suspected of triggering UC in people who have a genetic susceptibility. However, no single factor has been consistently proven to be the primary trigger. In contrast to the identification of a definite susceptibility gene NOD/CARD15 in Crohn's disease no specific gene has yet been linked to UC. There is compelling evidence that ulcerative colitis tends to run in families, suggesting that genetics does have a role in this disease. About 10 to 25 percent of

affected people have a first-degree relative (either sibling or parent) with inflammatory bowel disease (either ulcerative colitis or CD). Linkage studies have associated colitis with the HLA classII alleles on chromosome 6P.^{6,7} Other gene families with potential yet differing population association are Interleukin 1 family of genes on chromosome 2q13^{6,7} and the multidrug resistance gene (MDR1) on chromosome 7.⁶

Autoimmune Etiology of Ulcerative Colitis

Autoimmunity has been emphasized in the pathogenesis of UC.⁹ Broberger and Perlmann (1959), from Sweden, were the first to report that ulcerative colitis might be an autoimmune disease. Using a phenol-water extract of fetal colon as antigen, they demonstrated high-titer haemagglutinating antibodies to an unknown colon antigen in children with ulcerative colitis.¹⁰ Using the indirect immunofluorescent technique, they showed that those antibodies reacted with colonic epithelial cells. Autoantibodies against colonic epithelial components have been subsequently confirmed by many other investigators.¹¹

Patients with UC appear to have a disturbance in oral tolerance. Peripheral blood lymphocytes (PBL), as well as lamina propria lymphocytes (LPL)-T-cells from patients with UC, are cytotoxic in vitro specifically for human colonic, including autologous, epithelial cells.¹² In an animal model of chronic colitis with trinitrobenzene sulfonic acid (TNBS), oral tolerance was demonstrated by feeding total colon extract as well as colon epithelial cell extract to the animals.¹³ Such tolerance lacked with small intestinal extract, suggesting organ specificity of the autoimmune process.

That a colon epithelial antigen is crucial in organ-specific inflammation in UC, is strongly supported by a model of UC in mice, transgenic for the human CD3e gene (Tge26), which displays early arrest in T-cell development.¹⁴ It appears that T-cells selected in an aberrant thymic micro environment contain a population of cells that can induce severe colitis, but that this can be prevented by T-cells that have undergone normal thymic development. Inoue et al¹⁵ reported that B-cell lines, established from LPL and PBL of UC patients that produce anti-colon epithelial cell antibody, expressed a restricted V_H3 family usage, strongly suggesting that a particular antigenic stimulus from colon epithelial cell contributed to the pathogenesis of UC.¹⁵

Tropomyosin (Tm) as an Autoantigen in UC

Humoral Response to Tm, Particularly to hTm5, in UC

We reported a disease specific IgG antibody bound to colon mucosa (colitis colon bound IgG antibody or CCA-IgG) in UC and not in CD.¹⁶ Using the CCA-IgG, by Western blot assay we identified a 40 kDa protein termed "p40" that is present in colon mucosal extract and specifically binds with CCA-IgG.¹⁷ IgG antibodies in UC, capable of binding to colon epithelial cell targets and causing antibody dependent cell mediated cytotoxicity (ADCC) and a significant increase in IgG1 producing cells in the mucosa was found in UC when compared to CD, where all IgG subclasses (particularly IgG2) predominated.^{18,19} Furthermore, in UC and not in CD, this IgG1 deposition on colonic epithelium was associated with C₃b and terminal complement complex deposition against the "p40".²⁰

P40 was subsequently purified to homogeneity and sequence analysis demonstrated 93-100% identity with Tm.²¹ Tms are cytoskeletal microfilament-associated proteins present in all eukaryotic cells, with organ specific isoform(s) (molecular weight ranging from Mr 30 to 40K) and with distinct functions (see chapters 4, 15 and 16).²² hTm5 coresponds to the mammalian isoform Tm5nml (see chapters 2 and 16). The majority of UC sera, as well as IgG (mostly IgG1) synthesized in vitro by lamina propria lymphocytes (LPL)-B-cells from UC, but not from CD, recognized hTms, particularly isoform 5 (hTm5),²³ the isoform most abundant in colon epithelial cells.²⁴ Plasma cells isolated from the involved intestinal mucosa of both UC and CD patients produce high immunoglobulins G (IgG) levels. However, up to 42% of mucosal B-cells in UC were found to be committed to produce IgG antibody against hTm5.²⁵ Such an anti-hTm5 immune response was absent in CD, suggesting disease specificity of hTm5 as an autoantigen in UC. While in CD

mucosa the IgG1/IgG2 subclass ratio is similar to controls, in UC-diseased gut there is a marked increase of IgG1 subclass antibodies, capable of complement activation.²⁶

IgG antibodies against Tm isoform 5 (Tm5) and to a lesser extent hTm1 have been shown to be spontaneously released by LPMC infiltrating UC tissues.^{23,24} The major hTm isoforms present in colonic and jejunal epithelial cells are hTm5 and hTm4, whereas intestinal smooth muscles contain hTm1, hTm2 and hTm3 isoforms.²⁴ IgG, particularly IgG1, synthesized *in vivo* by LPMCs from UC recognized hTm5 and hTm1, more significantly ($p < 0.04$ and $p < 0.001$) when compared with CD and controls, IgG produced by LPMCs from CD did not show such anti-hTm reactivity. In UC non IBD colon epithelium, deposits of activated complement and IgG1 antibodies have been detected by immunofluorescence analysis to be colocalized with the expression of "p40" antigen that was subsequently found to be hTm5.^{20,21} These observations suggest that in UC colon, the *in situ* immune-recognition of a putative autoantigen "p40", by specific IgG1 antibodies, is followed by complement activation causing cell destruction. This mechanism of cytolysis of colon epithelial cells may represent one of the important pathogenic processes capable of amplifying and perpetuating the acute and chronic inflammatory process in UC.

Cellular Immune Response to hTm5 in UC

Subsequently, we further demonstrated that hTm5 can induce T-cell response with release of γ -IFN in UC.²⁷ Such response was not seen in CD, indicating disease specificity. This study demonstrated, for the first time, that a defined colon epithelial cell antigen, hTm5, is capable of inducing a significant T-cell response in UC but not in CD.²⁷

Other Studies Supporting the Role of Tm in UC

In an independent study from Japan, the anti-Tm antibody was detected frequently in UC but not in CD.²⁸ Furthermore, antibody dependent cell mediated cytolytic (ADCC) activity of UC sera was associated with the antibody against a synthetic Tm peptide.²⁸

Mizoguchi et al²⁹ demonstrated IgG antibodies against Tm in the animal model of TCR $\alpha^{-/-}$ mice that develop spontaneous colitis. They further reported a positive correlation of anti-Tm antibody titer to severity of colitis.³⁰ That Tm has potent antigenic potential is supported by a structural analysis of 109 autoantigens involved in various autoimmune diseases that showed a majority of antigenic peptides have significantly charged coiled-coiled helices. Interestingly, α -Tm has the highest potential as an autoantigen.³¹ Autoimmunity to alpha-tropomyosin was also found in Behcet's syndrome and Tm was found to be pathogenic as Tm induced lesions in the uveal tract and skin with features of Behcet's disease.³² It has been shown that Tm extracted from human cardiac muscle has immunogenic epitopes that cross react with group A streptococcal M protein that causes autoimmune myocarditis.³³

Family Study

Using a large number of IBD patients and their first degree blood relatives, Biancone, et al reported that in UC, anti-hTm5 IgG was higher in sera from UC probands were more frequently seropositive for hTm5 IgG, while sera from UC relatives were more frequently seropositive against both hTm1 and hTm5, suggesting genetic susceptibility to immune recognition of hTm isoforms in UC.³⁴

Surface Expression of hTm5 in Colon Epithelial Cells

We further demonstrated a novel observation that hTm5 physically binds with a colon epithelial membrane associated glycoprotein (Mr > 200K), termed CEP that reacts with the monoclonal antibody 7E12H12.^{35,36} CEP acts as a chaperone and appears to form CEP+hTm5 complex and both are expressed on cell surface and spontaneously released from the cells in the environment.³⁶ CEP is expressed specifically in colon epithelial cells and not with any other parts of the gastrointestinal tract, including small intestine.³⁵ This may explain why hTm5 is not expressed on the surface of small intestinal enterocytes.³⁷ The novel observation, that hTm5 is expressed on colon epithelial cell surface and not on small intestinal enterocytes, provides the possibility that the putative autoantigen may be accessible to the hyper-responsive mucosal immune effector cells,

both humoral and cellular response. This may also be important to explain why UC is restricted to colon. In a colon cancer cell line model, we observed that both hTm5 and CEP expression on the surface is increased when cultured with interferon- γ .^{38,39} Expression of hTm5 in the colonic mucosa is significantly increased in patients with UC.^{40,41}

Extraintestinal Manifestations of UC

Up to 20% of patients with UC demonstrate extraintestinal inflammation including the biliary epithelium (primary sclerosing cholangitis), skin (Pyoderma gangrenosum), eyes (uveitis) and joints (arthritis). As mentioned above, CEP is specific to colon epithelial cells, however, the corresponding epitope reactive to the 7E12H12 mAb has been detected in biliary epithelium, keratinocytes, the nonpigmented ciliary epithelium of the eyes and chondrocytes.^{42,43} More recently, hTm5 has also been localized at these extraintestinal sites where CEP is also expressed (Fig. 1).³⁷ Such a selective reactivity matches well with established extracolonic complications in UC involving the eyes, skin, joints and biliary epithelium.⁴⁴

The pathogenesis of UC and of the most common extraintestinal manifestations such as primary sclerosing cholangitis is also immunologically mediated and appears to be mainly due to an autoimmune-related process.^{28,45-47} Antibodies against biliary epithelial cells (BEC) have been reported in patients with PSC.^{45,28} The anti-hTm autoantibody was found in 100% of patients with primary sclerosing cholangitis.²⁸ Furthermore, the specific IgG antibodies to BEC were capable of initiating ERK1/2 signaling and up-regulation of TLR and production of cytokines and chemokines leading to recruitment of inflammatory cells.⁴⁵ These results suggest that in PSC the BECs are not only targets of the immune attack, but may also act as inflammatory mediators.

Aberrant homing of mucosal T-cells has also been suggested to be responsible for extraintestinal manifestations of IBD.⁴⁶ Various inflammatory cytokines also have an important role in the recruitment of inflammatory cells and tissue damage. The autoimmune basis of extraintestinal manifestations is also supported by several clinical observations including a higher prevalence of autoimmune disease among patients with UC with extraintestinal inflammations compared to control populations,⁴⁸ improvement of disease activity following immunomodulator and anti-TNF therapies. Pyoderma gangrenosum was remarkably improved with anti-TNF treatment.⁴⁹

A reasonable hypothesis is that a central process to the occurrence of extraintestinal manifestations is the development of self-reactive B-cells, which are triggered to produce IgG autoantibodies directed against self antigen(s) such as hTm5+CEP related epitopes in UC that is present in the target colon epithelial cells and biliary epithelial cells.^{20,24,28,37,47} The specific antibodies are capable of inducing inflammatory response.^{45,50} This process is facilitated by CD4⁺ T-cells, which may cause enhanced antigen presentation, aberrant homing in various organs,⁴⁶ thereby facilitating an immune response, particularly in genetically susceptible individuals. In addition, autoreactive T-cells (CD4⁺ or CD8⁺) may be primed by microbial antigens that are crossreactive to autoantigens restricted to specific extracolonic organs, the concentrations of which are normally too low to permit recognition by naïve T-cells.⁵¹

Differential Expression of "P40 (Tm)" in the Colon Mucosa and Influence of Cytokines on hTm5 Expression

Using two- and three-color immunofluorescence assay, Halstensen et al reported colocalization of IgG1 autoantibody with p40, along with activated complement products, in UC and not in CD.²⁰ They further reported that the distribution of "p40" in the colon was intriguing, with increasing expression caudally from cecum to left colon and the rectum showing intense staining of all colonocytes. Such an expression correlates with the clinical distribution of UC. These results may explain the location of the disease, severity of disease and why the rectum is the last site to heal following treatment. Expression of hTm5 has been recently shown to be upregulated in the colon epithelium of patients with UC.⁴¹

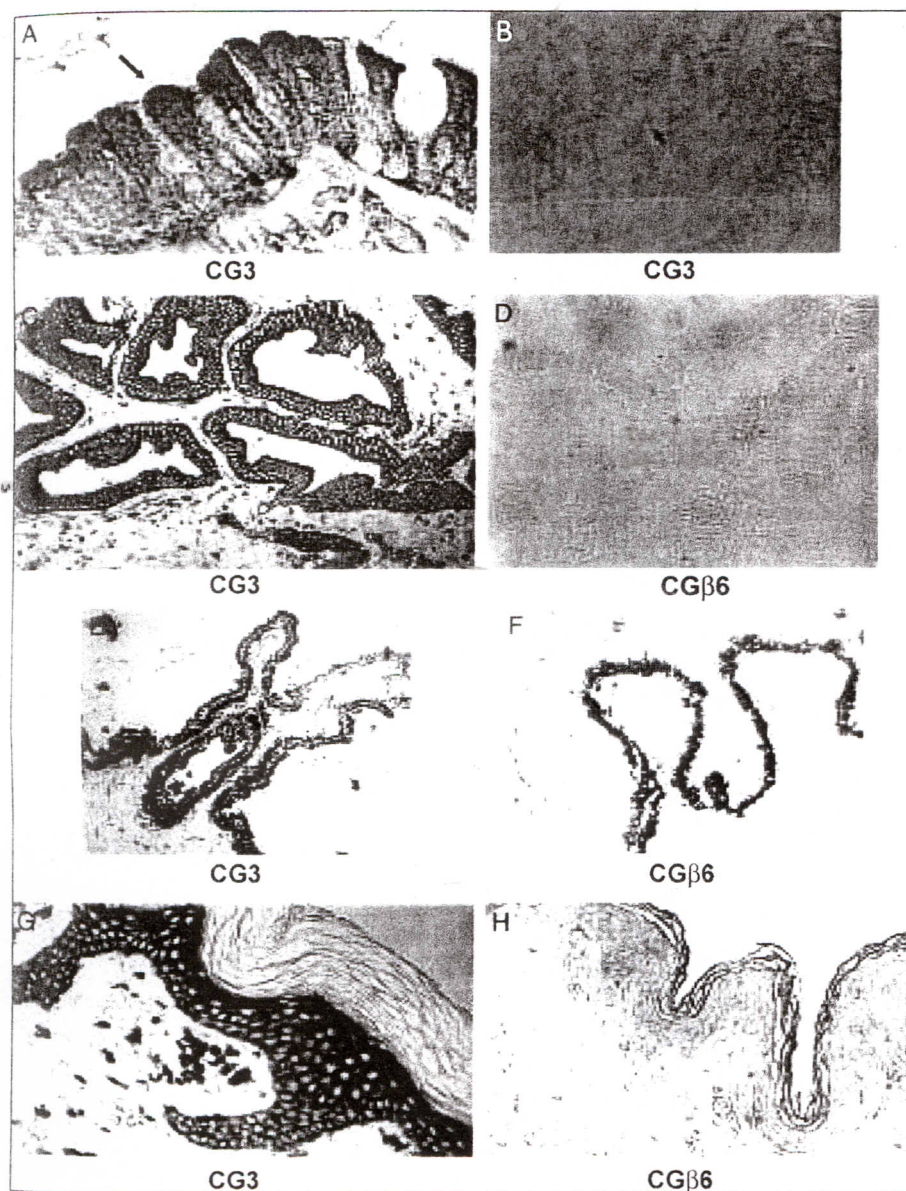


Figure 1. hTm5 expression in normal colon (A), ileum (B), gallbladder (C), ciliary body in the eye (E) and skin (G). 37 hTm5 expression in the colonic mucosa is intense and cytoplasmic, with even stronger reactivity (dense bandlike) evident along the brush border area (arrow) and basal areas. The reactivity in the ileum is faint cytoplasmic and no reactivity is evident along the brush border (arrow). Strong reactivity with CG3 monoclonal antibody (anti-hTm5) is evident in the biliary epithelium from a gallbladder mucosa, nonpigmented ciliary epithelium (outer layer) in the eye and keratinocytes in the skin. CG6 (anti-hTm2/3) reacted with neither the epithelium of the colon and ileum (not shown) nor gallbladder (D), eye (F) and skin (H). Pigmented layer (inner layer) of ciliary process is seen in both (E) and (F).

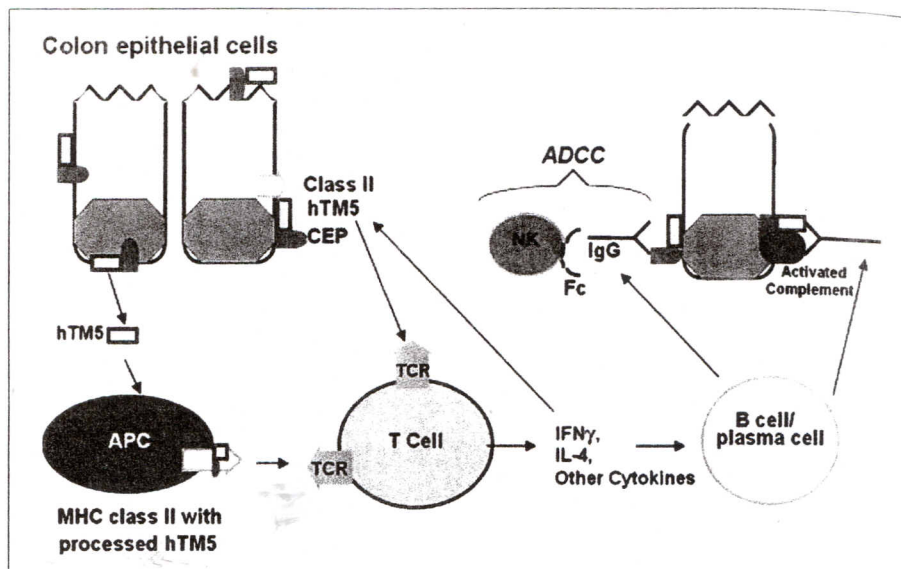


Figure 2. On the basis of current information, this is a model for colon epithelial cell destruction in UC through an autoimmune response to human tropomyosin isoform 5 (hTm5).⁵⁰ The hTm5 is closely associated with colonic epithelial cell protein (CEP) and is presented to T-cells through MHC Class II. The T-cells, stimulated by hTm5, produce cytokines, such as IFN γ which up-regulates expression of hTm5 and MHC Class II on the epithelial cells. T-cells may produce B-cell stimulatory factors that promote the production of hTm5-specific antibodies. These antibodies may induce disease by triggering antibody-dependent cellular cytotoxicity (ADCC) and complement mediated lysis.

Expression of hTm5 in Pouchitis

Patients with UC who do not respond to medical therapy may need to undergo surgery. The common operation that is performed is total colectomy and ileo-anal pull through operation to create an artificial rectum (pouch) prepared from the distal ileum. Twenty to 40% of these patients develop pouchitis with similar symptoms as in UC. In these patients with pouchitis, the ileal epithelium in the pouch expresses hTm5 although in normal ileal epithelium and in the absence of pouchitis, hTm5 can hardly be detected except in occasional goblet cells. There was a significant correlation between pouchitis disease activity index and hTm5 score.⁵²

Cytoskeletal Proteins Involved in the Immune Responses to Bacteria-Host Interactions

The involvement of cytoskeletal proteins (including Tms, vinculin, actin, villin) in the mucosal immune response in UC gut is also supported by recent evidences showing a rearrangement of the cytoskeletal protein expression in human enterocytes induced by apoptosis and bacteria-host interactions. Cytoskeletal proteins include a number of proteins (α -actinin, talin, vinculin, villin myosin II, ezrin, calpactin, gelsolin, laminin, tropomyosin) which modulate cells structure, shape and motility.⁵³ Evidences indicate that bacterial-host interactions and apoptosis may induce the expression of cryptic cytoskeletal proteins on the cells surface.⁵⁴ The Enteropathogenic Escherichia Coli (EPEC) may indeed bind to enterocytes by injecting a Translocated Intimin Receptor (TIR) in the host cells membrane, linking to the intimin receptor of the bacterium itself. This mechanism is followed by a rearrangement of the cytoskeletal proteins present in the cytoplasm of the enterocytes (α -actinin, talin, ezrin, villin, F-actin, myosin II, tropomyosin), thus forming pedestals linking the bacterium to colonic epithelial cells.⁵⁴ Vinculin, a cytoskeletal protein which modulates

the extracellular matrix interactions may be expressed "ex novo" on the surface of apoptotic cells, thus leading to tolerance or autoimmunity.⁵⁵ These observations suggest that several mechanisms may induce the expression of cryptic cytoskeletal proteins on the cells surface, with possible development of specific local immune response, both humoral and cellular, towards these antigens.

Autoantibodies against hTm5 Are Pathogenic

That anti-hTm5 auto-antibodies are pathogenic in UC has recently been reported for the first time to cause antibody mediated and complement mediated cytolysis.⁵⁰ Auto-antibody against a specific hTm peptide that causes ADCC was reported in UC sera.²⁸ The antibody titer in the sera against the hTm correlated with ADCC. With more recent data using recombinant hTm5, we demonstrated that colon epithelial cell lysis mediated by autoantibody present in UC could be blocked by hTm5.⁵⁰ This further confirms a direct role of hTm in this pathogenic process. Figure 2 shows the model for colon epithelial cell destruction in UC through an autoimmune response to hTm5.⁵⁰

Conclusion

Many studies, during the last 4 decades since the original landmark paper by Broberger and Perlmann,¹⁰ confirmed autoimmunity as a major pathogenetic mechanism in UC. More recent studies provide evidence that hTm5, the dominant hTm5 isoform in colon epithelium, is an important cellular autoantigen that mount humoral and cellular immune responses in UC. Furthermore, surface expression of hTm5 on colon epithelial cells allows the binding of anti-hTm5 autoantibodies present in UC. These autoantibodies are pathogenic to colon epithelium and can cause cell destruction by ADCC and complement mediated lysis, as summarized in Figure 2.⁵⁰ Future studies of mapping the specific peptide(s) of hTm5 involved in the immune responses in UC and extraintestinal manifestations of UC, may provide both diagnostic and therapeutic possibilities in UC.

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