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Expression of integrin alphavbeta6 in the intestinal epithelial cells of patients with inflammatory bowel disease

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Abstract

Background and aims: The prevalence of inflammatory bowel disease (IBD) is about 0.05% in industrialized countries. The pathogenesis of IBD remains to be further understood. The present study aims to elucidate the expression of integrin αvβ6 in the intestinal mucosa of patients with IBD. **Materials and Methods**: Colonic biopsy was obtained from a group of IBD patients. The expression of αvβ6 in the intestinal mucosa was detected by Western blotting. Human colonic epithelial cell line T84 cells were stimulated by microbial antigen flagellin. The expression of αvβ6 in T84 cells was evaluated by quantitative RT-PCR and Western blotting. **Results**: The levels of αvβ6 in the intestinal mucosa were much lower than it in normal control subjects. The serum levels of myeloperoxidase (MPO) were higher in IBD patients that were negatively correlated with the levels of αvβ6 in the intestinal mucosa. The expression of αvβ6 was detectable in T84 cells at naïve status that could be upregulated by exposure to microbial antigen flagellin. Pretreatment with MPO dramatically suppressed the expression of αvβ6 in T84 cells. **Conclusions**: We conclude that the expression of αvβ6 was suppressed in IBD intestinal mucosa, which could be resulted from the high levels of MPO.

Keywords: Inflammatory bowel disease; intestine; mucosa; integrin; oral tolerance.

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Introduction

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn's disease (CD) is a chronic immune inflammation in the intestine. UC mainly affects the colon. CD can distribute to both the ileum and colon or even extends to the whole intestine. The etiology of IBD is not clear [1, 2]. The pathogenesis of IBD needs to be further investigated. Chronic intestinal inflammation causes significant morbidity worldwide. It has a substantial impact on human health and social economy and has emerged as one of the major health problems in the world [3, 4]. Thus, there is an urgent need for fresh ideas on creating effective, targeting therapeutic approaches for IBD. On the other hand, elucidation of the underlying mechanisms of IBD also contributes to the development of novel therapeutic strategies.

A unique feature of the intestinal mucosa is that maintains a relative unresponsiveness (immune tolerance or oral tolerance) to food and commensal flora antigens. Defect in immune tolerance, such as inappropriate immune responses to commensal bacteria, is suggested to be an important factor in the pathogenesis of IBD [5, 6].

Immune tolerant status is believed to be maintained by a complex network of interacting immune cells' activities [7] that include clonal anergy, clonal deletion, and active regulatory processes by T regulatory cells; among those, the local activity of Treg is considered playing a central role in responsible for immune tolerance [8] that has been the study focus in recent years [9]. TGF-β is the key molecule in oral tolerance [10]. However, how the established immune tolerance gets broken-down or fails to develop in the intestine remains largely unknown.

Recent advance of research in chronic inflammation indicates that αvβ6 plays a role in the development of chronic inflammation as well as involving in the immune regulation. Integrins are cellular receptors that have an α and a β subunit; it forms about 24 different dimmers. Integrin mainly mediates cell-cell and cell-ECM interactions [11]. αv integrins family has five members (αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8) that recognize a group of overlapping ligands which generally contain the canonical tripepetide recognition sequence, arginine-glycine-aspartic acid (RGD) [12].

One of the functions of αvβ6 is to convert the latent TGF-β to the active form of TGF-β by dissociating the latency associated peptide from TGF-β molecule. Deficiency of αvβ6 causes low levels of TGF-β in the body that attributes to immune inflammation as reported recently [13]. Yet, what causes the low production of αvβ6 is unknown. Whether the levels of αvβ6 in the intestinal mucosa are suppressed is not clear. Therefore, in the present study, we observed the expression of αvβ6 in intestinal biopsy specimens from a group of patients with IBD. The results indicate that the expression of αvβ6 was still detectable in IBD intestinal mucosa but markedly weaker than in normal control subjects.

Materials and Methods

Diagnosis of IBD and colonic biopsy collection

Diagnosis of IBD was made on the basis of the endoscopic, radiological, histological, and clinical criteria provided by the WHO Council for International Organizations of Medical Sciences and the International Organization for the Study of Inflammatory Bowel Disease [14, 15]. Patients with indeterminate colitis were excluded. The demographic data of IBD patients are presented in Table 1.

Table 1 Demographic and disease features of IBD patients

Group	Crohn's disease	Ulcerative colitis
Sex	Male 4; female 4	Male 4; female 4
Age	38.5 (26 - 59)	32.4 (19 - 64)
Weight (kg)	58.3 (53 - 71)	62 (55 - 80)
Duration (months)	32 (16 - 59)	39 (20 - 66)
Race	Asian	Asian

Written informed consent was obtained from each patient. The collection of colonic biopsy was followed the established procedures in our departments. Another 8 colonic samples were obtained from 8 colonic cancer patients. The marginal "normal" tissue was excised using as normal control. The study using human specimens was approved by the Human Study Ethic Committee at Zhengzhou University.

Western blotting: Total proteins were extracted from surgical removed nasal mucosa or cultured cells with established procedures [16, 17]. Equal amounts protein extracts of each sample were separated on a precast NuPAGE gel system and blotted onto nitrocellulose membrane. The membranes were then blotted with primary antibodies against target proteins. The proteins were detected using horseradish peroxidase conjugated second antibodies. Horseradish peroxidase enzymatic reaction was detected with enhanced chemiluminescent reagents and recorded with X-ray films.

Detection of myeloperoxidase (MPO)

Blood samples were obtained from IBD patients and normal subjects. The sera were isolated afterwards. A solution of tetramethyl benzidine (1·6 mM) and H_2O_2 (0·1 mM) was added and reacted to an aliquot of the serum and optical density was measured at 650 nm. One unit of MPO activity was defined as degrading 1 μmol H_2O_2 per min at 37 °C. MPO activity was expressed as units per ml of serum (U ml^{-1} serum).

qRT-PCR

Total RNA was extracted from the DCs using an RNeasy Mini kit (Qiagen, Mississauga, ON, Canada). cDNA was synthesized using iScriptTMcDNA Synthesis Kit (Bio-Rad, Mississauga, ON, Canada). The resulting cDNA was subjected to qPCR that was performed with a Light Cycler using a SuperScript III Platinum SYBR Green Two-Step qPCR Kit (Invitrogen, Burlington, ON, Canada). The amplified product was detected by the presence of an SYBR green fluorescent signal. The standard curve was designed with β-actin cDNA. The resulted amplicon was quantified with the standard curve. The primers using in qPCR are available upon request.

Histology

The colonic biopsy specimens were fixed with 4% formaldehyde and processed for paraffin embedding. The paraffin sections were stained with hametoxylin and eosin. Tissue structure and inflammatory cell infiltration were observed under a light microscope.

Statistics

All values were expressed as the means ± SD of at least three independent experiments. The values were analyzed using the two-tailed unpaired Student's t-test when data consisted of two groups or by ANOVA when three or more groups were compared. The correlation between variables was analyzed using Pearson's correlation coefficient. $P<0.05$ was accepted as statistically significant.

Results

Levels of αvβ6 in IBD intestinal mucosa

Integrin αvβ6 is involved in immune regulation as well as

in development of fibrosis [18, 19]. To understand if αvβ6 is also involved in the pathogenesis of IBD, we examined the levels of αvβ6 in intestinal biopsy from a group of IBD patients. As shown by Western blotting (Fig.1), the levels of αvβ6 in IBD intestine were variable from case to case, but much less than those from "normal" controls (samples were obtained from colonic cancer patients; the marginal "normal" mucosa were used as normal controls).

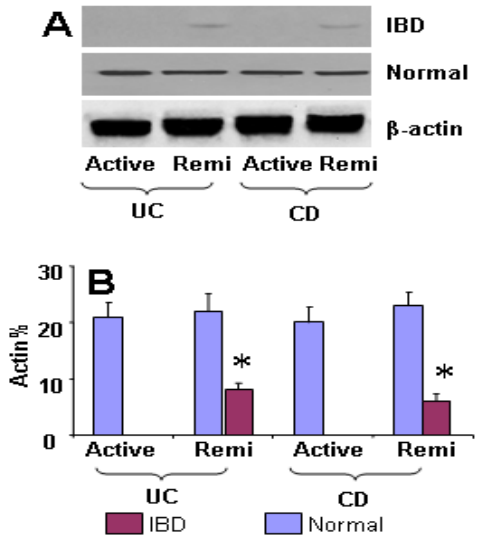

Fig.1 Expression of αvβ6 in IBD intestinal mucosa. Colonic biopsies were obtained from IBD patients. Expression of αvβ6 in the biopsies was examined by Western blotting. Panel A shows αvβ6 immune blots. Panel B shows the densitometry data of band analysis; data were expressed as percentage of β-actin (means ± SD). *: p<0.05, compared with normal control. UC: Ulcerative colitis. CD: Crohn's disease. Active: IBD patients with active inflammation. Remi: IBD patients at remission period.

Serum levels of MPO in patients with IBD

MPO is a lysosomal protein stored in azurophilic granules of the neutrophil, which is used as an indicator of inflammatory activities in IBD study [20]. As shown by Fig.2, low levels of MPO were detected from normal control subjects. The levels of MPO were significantly higher in both UC and CD patients as compared with normal controls.

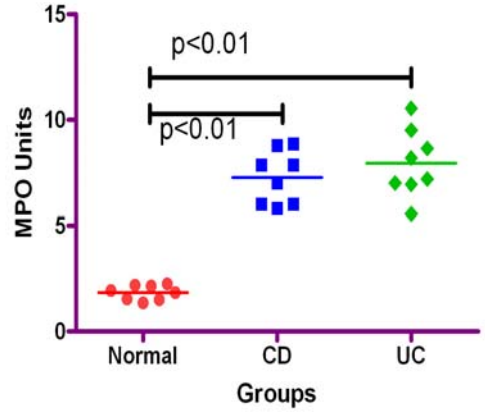

Fig.2 Serum MPO levels of IBD patient. Blood samples were obtained from each patients and control subjects. The sera were isolated and subjected to analysis of MPO levels by ELISA.

Levels of αvβ6 in IBD intestinal mucosa are correlated with serum MPO levels

During data analysis, we noted that the levels of αvβ6 as if correlated with the levels of serum MPO. Thus, a correlation assay was carried out between the data set of αvβ6 (the densitometry data) and the serum MPO levels. As expected, the levels of αvβ6 in IBD intestinal mucosa were significantly negatively correlated with the serum MPO levels (p<0.05).

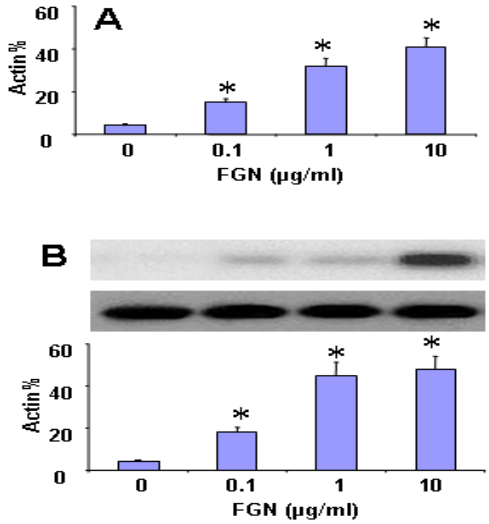

Fig.3 Microbial antigen increases the expression αvβ6 in human colonic epithelial cells. Human colonic epithelial cell line T84 cells were cultured and exposed to FGN for 3 h. A, bars indicate the β6 mRNA levels in T84 cell extracts. B, upper gel shows αvβ6 immune plots of protein extracts from T84 cells. The lower gel shows β-actin's immune plots using as internal control. Bars indicate the densitometry analysis data. Data were presented as mean ± SD. *, p<0.05, compared with group "0".

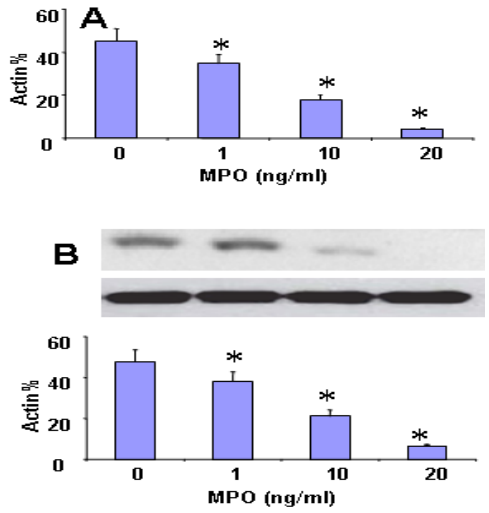

Fig.4 MPO suppresses the expression αvβ6 in human colonic epithelial cells. Human colonic epithelial cell line T84 cells were cultured and exposed to FGN (10 μg/ml) in the presence of MPO at graded doses for 3 h. A, bars indicate the β6 mRNA levels in

T84 cell extracts. B, upper gel shows αvβ6 immune plots of protein extracts from T84 cells. The lower gel shows β-actin's immune plots using as internal control. Bars indicate the densitometry analysis data. Data were presented as mean ± SD. *: $p<0.05$, compared with group "0".

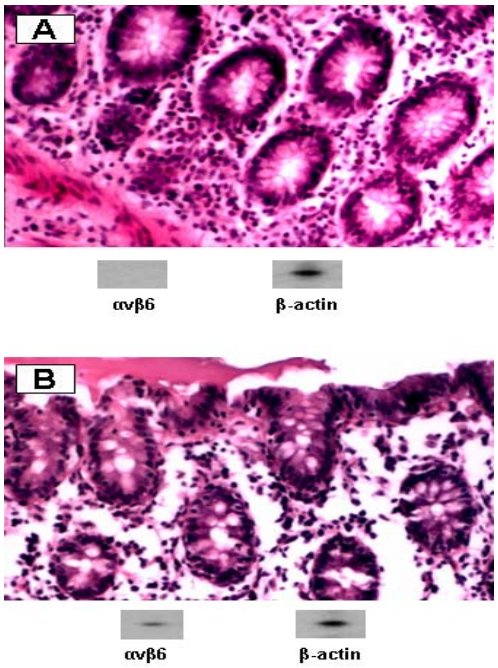

Fig.5 Expression of αvβ6 in colonic mucosa is correlated with inflammatory status. Colonic biopsies were taken from IBD patients in active status (A) and remission period (B). Paraffin sections were stained with hametoxylin and eosin. The Western blotting gels show αvβ6 blots from protein extracts.

Human colonic epithelial cells express αvβ6

To take further insight into the event of αvβ6 expression in IBD intestinal mucosa, we carried out a cell culture study. Human colonic epithelial cell line, T84 cells, was cultured with our reported procedures [21]. After cultured with no serum culture media for 2 days, trifle amount of αvβ6 was detected in cellular extracts of T84 cells as shown by qRT-PCR and Western blotting (Fig. 3). However, exposure to microbial antigen flagellin or food antigen ovalbumin for 3 h, the expression of αvβ6 was increased markedly. Pretreatment with MPO efficiently blocked the increase in the expression of αvβ6 (Figs. 4 & 5).

Discussion

The present paper reports a set of novel data that integrin αvβ6 was evaluated in a group of patients with IBD. Only very low levels of αvβ6 were detected in IBD patient's colonic mucosa. The expression of was suppressed by the disease activity. We also detected high serum MPO levels in IBD patients. A significant negative correlation was determined between serum MPO levels and levels of αvβ6 in colonic mucosa. Only trace αvβ6 expression was detected in human colonic epithelial cell line T84 cells at naïve status. Exposure to microbial antigen FGN increased the expression of αvβ6 significantly that could be suppressed by exposure to MPO.

In general, αvβ6 expression is very low or undetectable in healthy adult epithelial cells [12]. However, the expression of αvβ6 is rapidly enhanced upon stimulation such as cell injury, inflammation, cancer, and certain fibrotic disorders. It is also reported that αvβ6 is constitutively expressed in the healthy junctional epithelium linking the gingiva to tooth enamel [13]. Our results are in line with these pioneer studies by showing the expression of αvβ6 is detectable in normal intestinal mucosa and naïve human colonic epithelial cell line T84 cells although the expression is rather weak. A novel finding of in the present study is that the expression of αvβ6 in the intestinal epithelial of IBD patients is not even. The explanation of this event may be the expression of αvβ6 can be modulated by inflammatory molecules since the group of sample we obtained from IBD patients whose inflammatory conditions are from active inflammatory status to remission period.

MPO is derived from neutrophils; its levels are usually high in IBD patients and in parallel with the inflammatory status. Thus, MPO can be an indicator of inflammatory status in IBD as well as in IBD animal model study. In agreement with previous studies, we also detected high levels of MPO in the sera of IBD patients in the present study. A phenomenon caught our attention during the data analysis that the serum levels of MPO showed a negative correlation with the levels of αvβ6 in colonic biopsy tissue. Our further investigation indicates that the correlation between serum MPO and αvβ6 in colonic biopsy tissue is not a coincident event; the high levels of MPO can be a causative factor in the low levels of αvβ6 in the intestine.

Further studies with human colonic epithelial cell line T84 cells allowed us to gain further insight into the mechanism of αvβ6 expression. Similar to previous studies, that αvβ6 can be detected in normal oral mucosa [13], we also found that intestinal epithelial cell line constitutively express αvβ6 as verified by the cell line study. To our knowledge, this is the first report that exposure to microbial antigen FGN can promote the expression of αvβ6 in intestinal epithelial cells.

The fact of levels of αvβ6 in the intestine were negatively correlated with the levels of MPO in IBD patients indicates that MPO might suppress the expression of αvβ6 in the intestine. The present data approved the hypothesis by showing that exposure to MPO inhibits the expression of αvβ6 in T84 cells. Previous reports indicate that αvβ6 plays a critical role in activation of TGF-β1 by cleaving the latent associated peptide [13, 22]. TGF-β is a key molecule in the establishment of oral tolerance by promoting the development of regulatory T cells. Defect of TGF-β is involved in a broad array of immune diseases. IBD is also a chronic immune inflammatory disorder. The low levels of αvβ6 expression in the intestinal mucosa may contribute to the dysfunction of oral tolerance to luminal microflora or other antigens. Since high levels of MPO is a signature phenomenon in IBD, this specific inflammation in the intestine may constantly prevent the production of TGF-β in the intestinal mucosa of patients

with IBD that may consequently prevent the development of regulatory T cells and compromise the oral tolerance.

In conclusion, the present study revealed that the expression of αvβ6 was suppressed in the intestinal mucosa of IBD patients. The levels of αvβ6 were negatively correlated with the serum levels of MPO of patients with IBD. Cell line study discovered that MPO efficiently suppressed the expression of αvβ6 in T84 cells.

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