Relationship and significance between anti- β_2 -glycoprotein I antibodies and platelet activation state in patients with ulcerative colitis

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Abstract

AIM: To study the relationship between anti- β_2 -glycoprotein I ($\alpha\beta_2$ GP I) antibodies and platelet activation state in patients with ulcerative colitis (UC) and its significance.

METHODS: Peripheral blood samples were collected from 56 UC patients (34 males and 22 females, aged 43.5 years, range 21-66 years), including 36 at active stage and 20 at remission stage, and 25 sex-and age-matched controls. The level of $\alpha\beta_2$ GP I was measured by ELISA. The platelet activation markers, platelet activation complex- I (PAC- I) and P-selectin (CD62P) were detected by flow cytometry.

RESULTS: The A value for IgG $\alpha\beta_2$ GP I in the active UC group was 0.61 ± 0.13 , significantly higher than that in the remittent UC and control groups (0.50 ± 0.13 and 0.22 ± 0.14 , $P < 0.01$). There was a significant difference between the two groups ($P < 0.01$). The A value for IgM $\alpha\beta_2$ GP I in the active and remittent UC groups was 0.43 ± 0.13 and 0.38 ± 0.12 , significantly higher than that in the control group (0.20 ± 0.12 , $P < 0.01$). However, there was no significant difference between the two groups ($P > 0.05$). The PAC- I positive rate for the active and remittent UC groups was $30.6\% \pm 7.6\%$ and $19.6\% \pm 7.8\%$ respectively, significantly higher than that for the control group ($6.3\% \pm 1.7\%$, $P < 0.01$). There was a significant difference between the two groups ($P < 0.01$). The CD62P positive rate for the active and remittent UC groups was $45.0\% \pm 8.8\%$ and $31.9\% \pm 7.8\%$ respectively, significantly higher than that for the

control group ($9.2\% \pm 2.7\%$, $P < 0.01$). There was a significant difference between the two groups ($P < 0.01$). In the active UC group, the more severe the state of illness was, the higher the A value for IgG $\alpha\beta_2$ GP I was, and the positive rate for PAC-I and CD62P was positively correlated with the state of illness ($\text{Fa}\beta_2\text{GP I} = 3.679$, $P < 0.05$; $\text{FPAC-I} (\%) = 5.346$, $P < 0.01$; and $\text{FCD62P} (\%) = 5.418$, $P < 0.01$). Meanwhile, in the same state of illness, the A value for IgG $\alpha\beta_2$ GP I was positively correlated to the positive rates for PAC-I and CD62P.

CONCLUSION: $\alpha\beta_2$ GP I level, platelet activation state and their relationship of them are closely correlated with the pathogenesis and development of UC.

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Key words: β_2 -glycoprotein I; Anti- β_2 -glycoprotein I antibodies; Ulcerative colitis; Platelet activation; Hypercoagulation

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INTRODUCTION

β_2 -glycoprotein I (β_2 GP I) contains 150-200 mg/L glycoprotein in plasma^[1] and is synthesized by hepatocytes^[2]. It participates in fat transfer and metabolism due to its lipotropy^[3], and is also known as apolipoprotein H (apoH)^[4]. It was reported that β_2 GP I is an immunogen in antiphospholipid syndrome (APS)^[5-10]. Antiphospholipid antibody is the only autoantibody that induces blood into hypercoagulability state, just like one bridge between two physiology systems, immunity and coagulation, which may cause formulation of arterial and venous thrombosis with

unknown reasons^[11-15]. β_2 GP I is known as a target antigen of antiphospholipid antibody^[16-19]. Although ulcerative colitis (UC) is an inflammatory bowel disease (IBD) with unknown reasons, it is closely related to immunity, thrombosis formation and blood hypercoagulability state^[20-24]. To research the possible mechanism of blood hypercoagulability state, we measured the $\alpha\beta_2$ GP I level and detected the platelet activation state in serum of UC patients.

MATERIALS AND METHODS

Materials

Heparin sepharose CL-6B and sephadex G-100 were from Swedish Pharmacia Company. Flow cytometer (FACSort Calibur Type) was from American Becton Dickinson Company and MB-III enzyme mark instrument was from Beijing New Technology Institute. Fluorescein isothiocyanate labeled against platelet activation GPIIb/IIIa (PAC-I FITC) fibrinogen receptor McAb, phycoerythrin labeled anti-P-selectin (CD62P PE) McAb, peridinin chlorophyll protein labeled platelet glycoprotein III (CD61 per-cp) McAb, negative control RGDS as a PAC-I blocker, phycoerythrin labeled mouse IgG (Mousey1PE), FACS flow TM sheath fluid were all from American Becton Dickinson Company. Horseradish peroxidase labeled goat anti-human IgG was a product from Canadian Anogen, and horseradish peroxidase labeled goat anti-human IgM was from Huamei Company. The rest agents used were of national analytical purity.

Patients

Fifty-six patients attending to No. 1 and 2 Hospitals Affiliated to Jilin University from September 2003 to December 2004 were included in the study as UC group. Of the 56 UC patients (34 males and 22 females at the age of 21-66 years with a mean age of 43.5 years), 36 had active UC, 20 had remittent UC. The average disease course was 18 mo. All the cases were diagnosed based on the diagnostic criteria for inflammatory bowel disease (IBD) revised at the National Inflammatory Bowel Disease Conference held in 2000. Of which, 47 patients were diagnosed based on the findings in clinical, endoscopic and pathologic examinations, 9 patients were diagnosed based on the findings in clinical and endoscopic examinations. None of the patients had hypercoagulability state in the heart, liver, kidney, pulmonary and cerebrovascular diseases. The patients did not take any drugs affecting coagulation and fibrinolytic systems 4 wk before their diagnosis. Twenty-five healthy persons (15 males and 10 females at the age of 22-58 years with a mean age of 42.5 years) served as the control group. There was no significant difference in sex and age between the two groups.

Measurement of β_2 GP I level

Five microlitre of 700 mL/L perchloric acid was added into 200 mL human plasma, stirred for 15 min at 4°C, centrifuged at 12000 r/min for 20 min at 4°C, dialyzed (0.02 mol/L Tris-HCl, pH 8.0, 0.03 mol/L NaCl) and concentrated by heparin sepharose CL-6B affinity chromatography. Linear gradient elution (0.03-0.35 mol/L NaCl) was performed to collect about 0.25 mol/L elution, which was dialyzed (0.02 mol/L Tris-HCl, pH 8.0,

0.03 mol/L NaCl) and concentrated by heparin sepharose CL-6B affinity chromatography. Linear gradient elution was performed (0.15-0.35 mol/L NaCl), and the elution peak containing β_2 GP I was collected and concentrated. The sample was dialyzed (0.02 mol/L Tris-HCl, pH 8.0, 0.03 mol/L NaCl) and concentrated. Sephadex G-100 was separated, elution peak containing β_2 GP I was collected, dialyzed (0.02 mol/L Tris-HCl, pH 8.0, 0.03 mol/L NaCl) and concentrated. Protein was determined with the Lowry method and protein purity was assayed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and ultraviolet scanning.

Detection of $\alpha\beta_2$ GP I by ELISA

IgG $\alpha\beta_2$ GP I and IgM $\alpha\beta_2$ GP I were detected in each serum sample. Multi-wells were set for the two antibodies ($n = 3$). Enzyme labeled 96-well plates were disposed with ⁶⁰Co. Their surface was oxygenized and 100 μ L coating solution containing 4 μ g/mL β_2 GP I was added and stored at 4°C overnight. The coating solution was recycled, the plates were washed three times (3 min each time) with phosphate-buffered solution-Tween 20 (PBS-T) and 6 g/L gelatin-PBS-T was added (100 μ L per well) and sealed at 37°C for 1 h. The plates were washed three times (3 min each time). The diluted serum sample was added (100 μ L per well) and incubated at 37°C for 2 h. The plates were washed three times (3 min each time) with PBS-T. The two antibodies were discarded, the plates were washed three times (3 min each time) with PBS-T. Stained liquid o-phenylene diamine was added (100 μ L per well) and reacted with shade for 5 min, 2 mol/L H₂SO₄ was added (50 μ L per well) and shaken evenly. The reaction was terminated and optical density value at 492 nm was detected with enzyme mark instruments.

Detection of platelet activation state in UC patients by flow cytometry

Three mL venous blood was taken. The first 2 mL mixed with tissue fluid was discarded, and the remained 1 mL was added into anticoagulative tubes, shaken and mixed evenly. PAC-I FITC, CD62P PE, CD61 per-cp (20 μ L each) were added into experimental tubes for 10 min. PAC-I FITC, IgG1PE, CD61 per-cp (20 μ L each) and 10 μ L RGDS were added into the control tubes, and 5 μ L mixed blood was shaken evenly and added into the tubes separately. After incubated for 20 min at the room temperature, PBS (pH 7.2) was added, shaken evenly for 30 min and stored at a cool dark place.

Statistical analysis

SPSS 11.5 statistical software was used to establish database and analyze the data. Comparison between groups was made by *t* test and comparison between multi-independent samples was carried out by One-way ANOVA, related analysis was performed by Pearson analysis. *P* < 0.05 was considered statistically significant.

RESULTS

Extraction and purity of β_2 GP I

β_2 GP I was detected as one band by three-step

Table 1 Detection results of $\alpha\beta_2\text{GP I}$ in patients among groups (4_{492} , mean $\pm$ SD)

Group	<i>n</i>	IgM	IgG
Active UC group	36	0.43 $\pm$ 0.13 ^b	0.61 $\pm$ 0.13 ^{b,d}
Remittent UC group	20	0.38 $\pm$ 0.12 ^b	0.50 $\pm$ 0.13 ^b
Control group	25	0.20 $\pm$ 0.12	0.22 $\pm$ 0.14

^b $P < 0.01$ vs control group; ^d $P < 0.01$ vs remittent UC group.

Table 2 Positive expression rate for platelet activation marker in different groups of patients (% , mean $\pm$ SD)

Group	<i>n</i>	PAC-I	CD62P
Active UC group	36	30.6 $\pm$ 7.6 ^{b,d}	45.0 $\pm$ 8.8 ^{b,d}
Remittent UC group	20	19.6 $\pm$ 7.8 ^b	31.9 $\pm$ 7.8 ^b
Control group	25	6.3 $\pm$ 1.7	9.2 $\pm$ 2.7

^b $P < 0.01$ vs control group; ^d $P < 0.01$ vs remittent UC group.

chromatography, while molecular weight 50 000 and purity over 90% were determined by SDS-PAGE.

Detection of $\alpha\beta_2\text{GP I}$ in different groups of patients

Comparison of IgM $\alpha\beta_2\text{GP I}$ levels in different groups: there was a statistically significant difference in IgM $\alpha\beta_2\text{GP I}$ level between the active UC and control groups ($t = 7.010$, $P < 0.01$) and between the remittent UC and control groups ($t = 5.000$, $P < 0.01$). No statistically significant difference was found in IgM $\alpha\beta_2\text{GP I}$ level between the active and remittent UC groups ($t = 1.416$, $P > 0.05$).

Comparison of IgG $\alpha\beta_2\text{GP I}$ level in different groups: there was a statistically significant difference in IgG $\alpha\beta_2\text{GP I}$ level between the active UC and control groups ($t = 6.879$, $P < 0.01$) and between the active and remittent UC groups ($t = 3.034$, $P < 0.01$) (Table 1).

Positive expression rate for platelet activation marker in different groups of patients

The positive expression rates of platelet activation markers PAC-I and CD62P were sharply increased in the active UC group. There was a statistically significant difference between the active UC and control groups ($t_{\text{PAC-I}} (\%) = 15.679$, $t_{\text{CD62P}} (\%) = 19.664$, $P < 0.01$) and between the active and remittent UC groups ($t_{\text{PAC-I}} (\%) = 5.142$, $t_{\text{CD62P}} (\%) = 5.551$, $P < 0.01$). The positive expression rate in the remittent UC group was higher than that in the normal control group ($t_{\text{PAC-I}} (\%) = 8.305$, $t_{\text{CD62P}} (\%) = 13.306$, $P < 0.01$) (Table 2).

Correlation between $\alpha\beta_2\text{GP I}$ platelet activation marker and disease grades in 36 active UC patients

One-way ANOVA showed that there was a statistically significant difference of $\alpha\beta_2\text{GP I}$ level, PAC-I and CD62P in the disease grades (mild, moderate, severe) of patients with active UC [$F_{\alpha\beta_2\text{GP I}} = 3.679$ ($P < 0.05$), $F_{\text{PAC-I}} (\%) = 5.346$ ($P < 0.01$); $F_{\text{CD62P}} (\%) = 5.418$ ($P < 0.01$, Table 3)]. Pearson correlation analysis displayed that the correlation coefficient of $\alpha\beta_2\text{GP I}$ (IgG) and PAC-I, and $\alpha\beta_2\text{GP I}$

Table 3 Correlation between $\alpha\beta_2\text{GP I}$, platelet activation marker and disease grades in 36 active UC patients

Grade	<i>n</i>	$\alpha\beta_2\text{GP I}$ 492 (IgG)	PAC-I positive rate (%)	CD62P positive rate (%)
Mild	19	0.64	31.8	48.7
Moderate	11	0.80	40.6	60.9
Severe	6	0.92	53.7	71.2
<i>F</i>		3.679	5.346	5.418
<i>P</i>		< 0.05	< 0.01	< 0.01

(IgG) and CD62P PE was $r = 0.472$ and $r = 0.523$ ($P < 0.05$) respectively for mild active UC patients. The correlation coefficient of $\alpha\beta_2\text{GP I}$ (IgG) and PAC-I, and $\alpha\beta_2\text{GP I}$ (IgG) and CD62P was $r = 0.659$ ($P < 0.05$) and $r = 0.714$ ($P < 0.01$) respectively for moderate active UC patients. The correlation coefficient of $\alpha\beta_2\text{GP I}$ (IgG) and CD62P, and $\alpha\beta_2\text{GP I}$ (IgG) and PAC-I was $r = 0.714$ ($P < 0.01$) and $r = 0.878$ ($P < 0.01$) respectively for severe active UC patients. The correlation coefficient of $\alpha\beta_2\text{GP I}$ (IgG) and PAC-I, and $\alpha\beta_2\text{GP I}$ (IgG) and CD62P was $r = 0.878$ ($P < 0.01$) and $r = 0.902$ ($P < 0.01$) respectively for severe active UC patients.

DISCUSSION

$\beta_2\text{GP I}$ is a kind of glycoprotein in normal plasma. It has been shown in a recent study^[19] that $\beta_2\text{GP I}$ plays an important role in the pathophysiological processes of human beings, such as production of APS autoantibody and formation of hypercoagulability state. Its molecule is a single polypeptide chain consisted of 326 amino acids and can be divided into five special functional domains under intermolecular disulfide bond influence, each of which is a circular permutation on the space. There are abundance amino acid sequences with positive charges in the fifth functional domain, the binding sites of which to phospholipids are located on C-terminal end. Cys281- Cys288 is the binding site and can combine with the surfaces of anionic phospholipids and particles with negative charges. One of their important physiological functions is to adjust the distribution of anionic phospholipids expressed on the activated platelets.

It was reported that immune factors play an important role in the pathogenesis of UC^[25-27]. Thromboembolic disease is one of three main causes resulting in death of UC patients. Most scholars believe that hypercoagulability state, micro-thrombosis formation and platelet activation may play an important role in the pathogenesis of UC^[28,29].

The pathogenesis of APS and UC mainly depends on immune factors, presence of hypercoagulability state and thrombosis formulation. $\alpha\beta_2\text{GP I}$ also plays an important role in the pathogenesis of APS^[30].

In the present study, the serum $\alpha\beta_2\text{GP I}$ level in UC patients was measurable. The IgG $\alpha\beta_2\text{GP I}$ level in the active UC group was higher than that in the remittent UC and control groups, indicating that $\alpha\beta_2\text{GP I}$ may have a certain role in the pathogenesis of UC. Antiphospholipid antibody is just like one bridge between the two physiology systems, immunity and coagulation, which may lead to

arterial and venous thrombosis with unknown reason. Moreover, $\alpha\beta_2$ GP I is one of the most important autoantibodies. Whether $\alpha\beta_2$ GP I presence plays an important role in hypercoagulability state and thrombosis formation in ulcerative colitis remains unclear. We have proved, in this study, not only the platelet activation state but also the correlation between platelet activation and disease severity in active UC patients.

Flow cytometer and platelet glycoprotein McAb can accurately detect platelet activation. In this study, phycoerythrin labeled CD62P McAb was selected as a molecular marker, CD62P also known as GMP140, a protein on resting platelet α granule membrane. Since granules are released onto the platelet surface when platelets are in an active state, CD62P is expressed on the active platelet membrane instead of on the resting platelet membrane, which is a symbol of active platelets.

GPIIb/IIIa is a glycoprotein on platelet membrane, which can be expressed in resting and active platelets, but only in activated platelets. The binding site of GPIIb/IIIa and fibrinogen can be exposed. Moreover, since PAC-I is a McAb inhibiting the binding site, it is an effective means for detecting activated platelets because they can only combine with active platelets.

In the present study, the positive expression rate for platelet activation markers, PAC-I and CD62P, in active UC patients, was obviously higher than that in the control and remittent UC groups, indicating that platelet activation is one of the main reasons for blood hypercoagulability state in UC patients. At the same time, the positive expression rate for platelet activation markers in remittent UC group was higher than that in the control group ($P < 0.01$), indicating that platelet activation is a difficultly-eliminated factor. Anti-platelet activation therapy for patients at the remission stage can delay the disease progression and further improve prognosis and life quality of UC patients.

Not only high $\alpha\beta_2$ GP I level but also high activation state of platelets in patients with UC was detected in this study and the relationship between $\alpha\beta_2$ GP I level, platelet activation state and disease severity was further studied.

There was a significant difference in the positive expression rates of $\alpha\beta_2$ GP I (IgG), PAC-I and CD62P between the active UC patients, which increased when the disease was aggravated. Moreover, the positive expression rates of $\alpha\beta_2$ GP I (IgG), PAC-I and CD62P were positively correlated with the severity of the disease, suggesting that $\alpha\beta_2$ GP I and platelet activation play an important role in the development of ulcerative colitis and there is a mutual causality between $\alpha\beta_2$ GP I and platelet activation. Excessive inflammatory response may lead to loss of tolerance to some autoantigens. When the autoantibody is induced and damage is aggravated, inflammatory factors may cause platelet activation. Activated platelets and phospholipids with negative charges from the inner layer of cytomembrane are exposed on the surface of cytomembrane, which combines with β_2 GP I easily. After the combination, the concealed antigenic epitopes of β_2 GP I are exposed, thus producing anti- β_2 GP I antibody and other autoantibodies. $\alpha\beta_2$ GP I can not only combine with platelets but also activate platelets and endothelial cells^[31], accelerating adhesion of monocytes

and promoting the development of UC and complications, such as thrombosis.

In summary, $\alpha\beta_2$ GP I is one of the possible causes for blood hypercoagulability state in UC patients. The results of this study provide a new theoretical foundation for the pathogenesis of UC.

COMMENTS

Background

Although UC is an inflammatory bowel disease with unknown reasons, it is closely related to immunity and blood hypercoagulability state, just like APS. Anti- β_2 -glycoprotein antibody plays an important role in the pathogenesis of APS, which is our cut-in point in this study.

Research frontiers

The increased risk of thromboembolic complications in patients with UC is well recognized. It was reported that the incidence of thromboembolic events is 1%-8% in patients with inflammatory bowel disease. In addition, patients with inflammatory bowel disease may experience thromboembolic events at a younger age. Disease activity is a probable risk factor for thromboembolism. The hypercoagulable state in active UC patients is closely related to abnormal platelet function, resulting in a high probability of microvascular thrombosis and microcirculatory dysfunction, but so far, the reasons remain unclear.

Innovations and breakthroughs

Studies on factors such as vWF, XIIa, IX, VIIa, VIIIa, Va, and abnormal platelet function are available. In this study, we explored the cause for hypercoagulable state in UC patients based on the autoimmunity.

Applications

This study provided a new theory for the pathogenesis and development of UC, which is very helpful to estimate illness state and prognosis. Moreover, anti-thrombotic compounds seem to be therapeutically beneficial for UC patients.

Terminology

APS: Anti-phospholipid syndrome, a clinical condition characterized by recurrent thrombotic events and/or pregnancy morbidity associated with the persistence of antiphospholipid antibodies.

Peer review

This paper is very interesting because the authors studied an interesting problem related with the hypercoagulation state in some patients with ulcerative colitis.

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