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Original Article

Increased CD177 expression is associated with *helicobacter pylori*-related gastritis

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Abstract: Objective: *Helicobacter pylori (Hp)* infection is an important cause of gastritis, and neutrophils is involved in the inflammation secondary to HP infection. CD177 expression significantly increases on the circulating neutrophils in response to bacterial infection and is involved in the pathogenesis of some clinical diseases. Currently, the relationship between CD177 and *Hp*-related gastritis is still unclear. This study aimed to investigate the association between CD177 expression and *Hp*-related gastritis. Methods: Paraffin embedded gastric mucosal samples (n=79) were collected by biopsy from patients with gastritis. Of these, Warthin-Starry silver staining showed *Hp* infection in 45 patients, and HP infection was excluded in remaining 34 patients. Their inflammation scores, CD177 expression and correlation between inflammation score and CD177 expression were further analyzed. Results: Patients with *Hp*-related gastritis had higher inflammation scores than in those without HP infection (P=0.001), and that the CD177 expression in HP infection group was significantly higher than in HP-naïve group (P=0.000). Conclusion: Elevated CD177 expression is correlated with *HP*-related gastritis and CD177 may play an important role in the pathogenesis of HP-related gastritis.

Keywords: *Helicobacter pylori,* gastritis, neutrophils, CD177

Introduction

Helicobacter pylori (*Hp*) is a Gram-negative, microaerophilic bacterium that colonizes the stomach of approximately half of the population worldwide. *Hp* infection is associated with chronic gastritis, peptic ulcer, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma. In recent years, the cost for the treatment of *H*p-related gastritis, especially the antibiotic resistant HP infection, is increasing. Therefore, to elucidate the pathogenesis of *Hp*-associated gastritis and to develop strategies for the prevention and control of *Hp* infection has increasingly attracted the attention of clinicians in the department of gastrointestinal diseases. The initial inflammatory response to *Hp* infection in the stomach is characterized by the infiltration of polymorphonuclear cells, which are the key cells in the elimination of microorganisms [1]. Neutrophils are a central participant in the innate immune response, and may act as primary responders by rapidly migrating to the infected site. CD177 is a polymorphic gene involved in the pathogenesis of some clinical diseases, including polycythemia vera, Wegner's granulomatosis, immune-mediated neonatal neutropenia, and severe bacterial infection [2-5].

Recently, studies indicate that CD177, a neutrophil cell surface receptor, has been found to mediate the neutrophil migration across the endothelia through interacting with PECAM1 [6]. CD177 expression is significantly up-regulated on the circulating neutrophils in response to bacterial infection [4]. However, the relationship between CD177 and *Hp*-related gastritis is still unclear. This study aimed to explore the correlation between CD177 expression and *Hp*-related gastritis.

Materials and methods

Ethics statement

This study was approved by the Research and Ethics Committee of the Shanghai Tenth People's Hospital.

Table 1. Distribution of inflammatory scores in *Hp*-related gastritis group and non-*Hp*-related gastritis group

	Inflammation grade			*P*
	Grade 1	Grade 2	Grade 3	
Hp-negative	23 (67.6%)	7 (20.6%)	4 (11.8%)	*P=0.001*
Hp-positive	11 (24.4%)	21 (46.7%)	13 (28.9%)	

Samples

The paraffin embedded gastric mucosal samples (n=79) were collected by biopsy from patients diagnosed with gastritis. Samples were divided into 2 groups according to the status of HP infection: Hp-related gastritis group and non-Hp-related gastritis.

Histological examination

The mucosal tissues were sectioned, followed by hematoxylin-eosin staining. The severity of inflammation was evaluated by the same experienced pathologist in a blind manner. The severity of gastritis was graded based on the degree of mononuclear cell (MNC) and polymorphonuclear leukocyte (PMN) infiltration and atrophy according to the Updated Sydney system [7] on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes.

Immunohistochemistry for CD177

Sections were treated with 3% hydrogen peroxide/methanol solution to inhibit endogenous peroxidase activity, followed by antigen retrieval with 10 mM citrate buffer (pH 6.0) in a microwave oven for 10 min at 98°C. Then, sections were incubated with an anti-CD177 antibody (1:100, Abcam). Staining for CD177 was performed using a Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). The CD177 positive cells were scored as follows; score 0 (none, 0-10% of positive cells), score 1 (weak, 10-30%), score 2 (moderate, 30-60%), and score 3 (strong, over 60%), and cases with score 1 or 2 were considered as positive.

Statistical analysis

Data are expressed as mean ± standard deviation, and statistical analysis was performed with SPSS version 17.0 (SPSS Inc, Chicago, USA). Comparisons were done with Chi-square test, and the correlation between CD177 expression score and inflammation grade was evaluated with Spearman correlation analysis. A value of $P<0.05$ was considered statistically significant.

Results

Histological features of Hp-associated gastritis

There were a variety of neutrophils infiltrating in the mucosa of *Hp* infection group. The pathological changes were more serious in *Hp*-related gastritis group as compared to the non-*Hp*-related gastritis group. In non-*Hp*-related gastritis group, the gastric mucosa showed slight histological change, inflammatory cells were mainly found between epithelial cells and faveoli, but were seldom found in the lamina propria. In *Hp*-related gastritis group, the histological change was evident. With increase in the degree of inflammation, increasing inflammatory cells (such as neutrophils, lymphocytes and plasma cells) were observed; inflammatory cells were superficially distributed in the superficial gastric mucosa, but they were diffusely distributed in the lamina propria.

Comparison of inflammatory grade between two groups

There was significant difference in the inflammatory grade between *Hp*-related gastritis group and non-*Hp*-related gastritis group ($P<0.01$) (**Table 1**).

CD177 expression in the gastric tissues of two groups

In non-*Hp*-related gastritis group, the antral mucosa showed a low CD177 expression and the CD177 positive cells were sporadically distributed in the lamina propria (**Figure 1A-C**). However, the antral mucosa showed a significantly increased CD177 expression in *Hp*-related gastritis group. From the superficial mucosa to the submucosa and lamina propria, the CD177 positive cells showed diffuse distribution in *Hp*-related gastritis group (**Figure 1D-F**).

CD177 positive tissues in two groups

In non-HP-related gastritis group, 25 tissues were negative to CD177 expression and 9 positive to CD177 expression. In HP-related gastri-

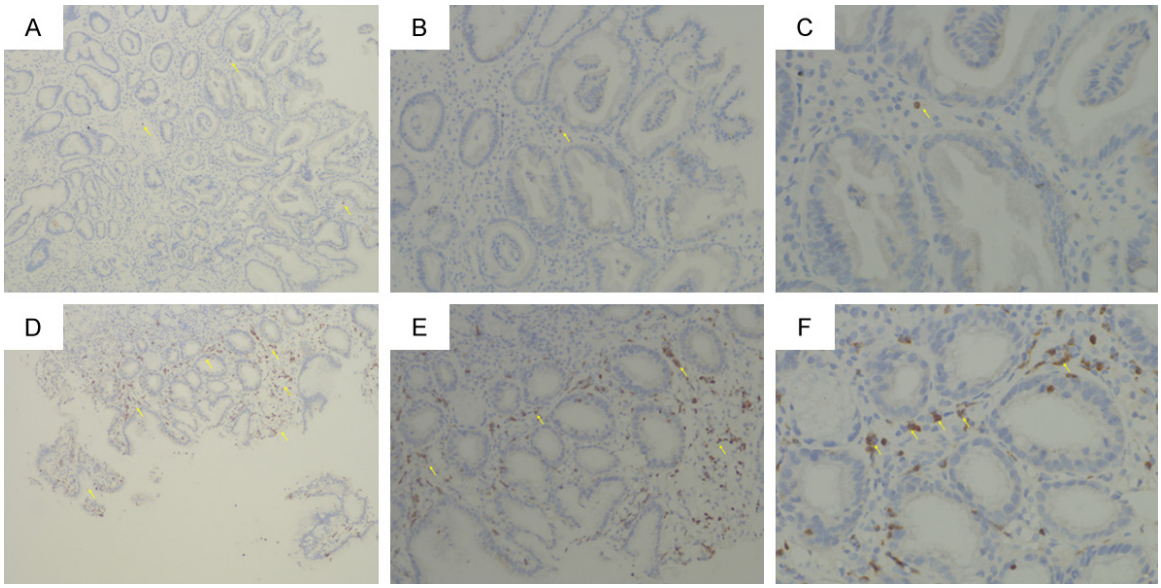

Figure 1. CD177 expression in non-*Hp*-related gastritis group and *Hp*-related gastritis group (inflammation grade 2). In non-*Hp*-related gastritis (A-C), a low CD177 expression was found in the submucosa and lamina propria. In contrast, the CD177 expression in non-*Hp*-related gastritis was significantly lower than in *Hp*-related gastritis (D-F). Magnification ×100 (A and D), ×200 (B and E), and ×400 (C and F).

Table 2. CD177 expression positive tissues in *Hp*-related gastritis group and the non-*Hp*-related gastritis group

	CD177 expression		*P*
	No expression	Expression	
HP negative	25 (73.5%)	9 (26.5%)	*P=0.000*
HP positive	10 (22.2%)	35 (77.8%)	

tis group, 10 tissues were negative to CD177 expression and 35 positive to CD177 expression. There was significant difference in the proportion of CD177 positive tissues between two groups, and the gastric mucosa in HP-related gastritis group was more like to be positive to CD177 expression (**Table 2**).

Relationship between CD177 expression and inflammatory grade

CD177 expression had a positive relationship with inflammatory grade in both groups. CD177 expression increased with the elevation of inflammatory grade in both non-*Hp*-related gastritis group and *Hp*-related gastritis group (**Figure 2**).

In all the patients, inflammation grade was significantly related to CD177 expression score ($P<0.01$). Multiple intercomparisons of the CD-177 expression scores were performed among the three inflammation grade groups, and the differences were significant ($P<0.01$) (**Table 3**).

Discussion

Hp is a Gram-negative, microaerophilic bacterium that infects approximately 50% of the population worldwide [8, 9]. *Hp* infection is closely related to the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric MALT lymphoma. Neutrophils play an indispensable role in the innate immune response to infection. Neutrophils may migrate from the vasculature into the injured site as a response to infection. CD177 expression is significantly up-regulated on the circulating neutrophils in response to bacterial infection [6]. Recently, CD177 is found to be involved in the pathogenesis of many inflammatory disorders, such as polycythemia vera and thrombocythemia, and has been used as a diagnostic marker for various myeloproliferative diseases, such as polycythemia vera, thrombocythemia, and idiopathic myelo-fibrosis [10-13]. However, no studies have been conducted to investigate the relationship between CD177 expression and *Hp*-related gastritis so far. This study was undertaken to investigate the association between CD177 expression and *Hp*-related gastritis.

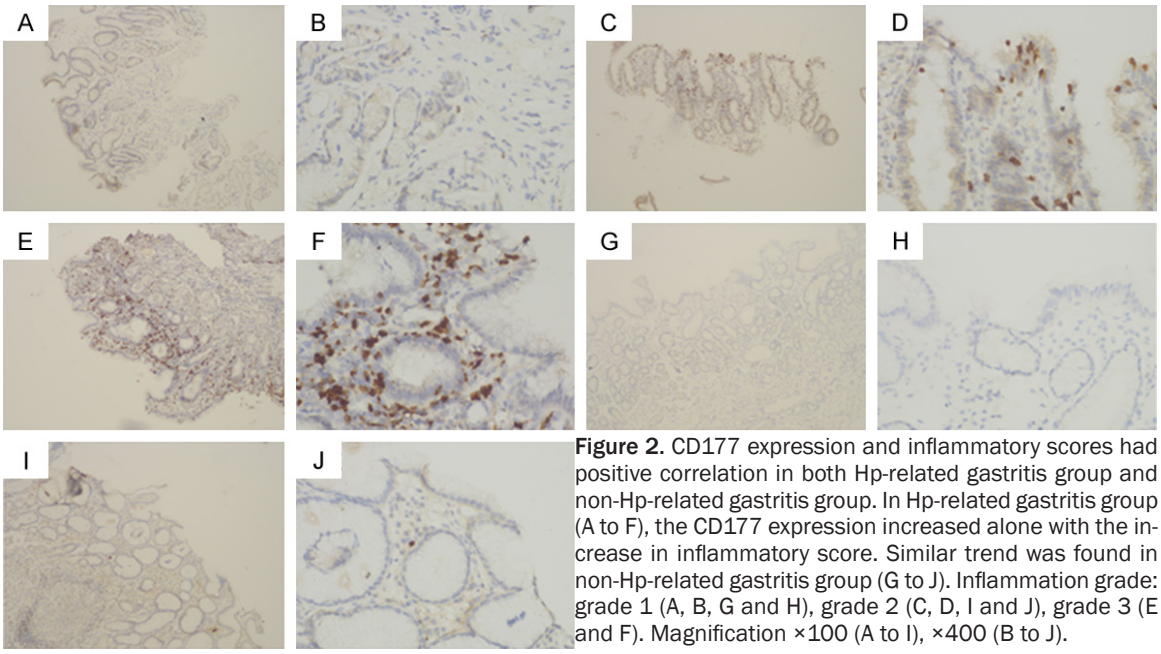

Figure 2. CD177 expression and inflammatory scores had positive correlation in both Hp-related gastritis group and non-Hp-related gastritis group. In Hp-related gastritis group (A to F), the CD177 expression increased alone with the increase in inflammatory score. Similar trend was found in non-Hp-related gastritis group (G to J). Inflammation grade: grade 1 (A, B, G and H), grade 2 (C, D, I and J), grade 3 (E and F). Magnification ×100 (A to I), ×400 (B to J).

Table 3. Correlation between inflammation grade and CD177 expression score in all the patients

Inflammation	CD177 expression score				*P*
	0	1	2	3	
Grade 1	2 (5.9%)	29 (85.3%)	3 (8.8%)	0 (0)	*P=0.000*
Grade 2	0 (0)	6 (21.4%)	18 (64.3%)	4 (14.3%)	
Grade 3	0 (0)	2 (11.8%)	3 (17.6%)	12 (70.6%)	

In our study, results showed that patients with *Hp*-related gastritis had a higher inflammation score than those with non-*Hp*-related gastritis did. In addition, the CD177 expression in *Hp*-positive gastric mucosa was significantly higher than in *Hp*-negative tissues. In both *Hp*-related gastritis group and non-*Hp*-related gastritis group, the increased CD177 expression correlated with the severity of gastric inflammation. Among the specimens with the same histological inflammatory grade and the same inflammatory score, the CD177 expression in *Hp*-related gastritis group was higher than in non-*Hp*-related gastritis group.

CD177 gene is a member of the leukocyte antigen 6 (Ly-6) gene superfamily, and encodes two neutrophil-associated proteins, NB1 and PRV-1 [13, 14]. Gonda et al. reported that CD177 expression in the whole gastric tissue of *H. felis*-infected mice with mucosal dysplasia was reduced after folic acid supplementation [15]. However, it is unclear whether CD177 expression is associated with gastritis or dysplasia. Toyoda et al. proposed that CD177 also acted as a regulator of the adhesion and migration of neoplastic cells in the gastric tumors and reported that the up-regulated CD177 expression was not caused by increased infiltration of neutrophils into the gastric mucosa but by the change in gene expression in tumor cells [16]. However, whether the elevated CD177 expression is dependent on the increased number of neutrophils in *Hp*-related gastritis is unknown, and the association between enhanced CD177 expression and *Hp* infection has never been determined. The mechanism of increased CD177 expression in *Hp*-related gastritis is still unclear. Thus, it is necessary to investigate the mechanism of increased CD177 expression in case of *Hp*-related gastritis. In addition, further studies are also needed to clarify the association between neutrophils and gastric epithelial cells in CD177 expression in *Hp*-related gastritis.

Disclosure of conflict of interest

None.

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References

[1] Hofman V, Ricci V, Galmiche A, Brest P, Auberger P, Rossi B, Boquet P and Hofman P. Effect of helicobacter pylori on polymorphonuclear leukocyte migration across polarized T84 epithelial cell monolayers: role of vacuolating toxin VacA and cag pathogenicity island. Infect Immun 2000; 68: 5225-5233.

[2] Bettinotti MP, Olsen A and Stroncek D. The use of bioinformatics to identify the genomic structure of the gene that encodes neutrophil antigen NB1, CD177. Clin Immunol 2002; 102: 138-144.

[3] Caruccio L, Bettinotti M, Director-Myska AE, Arthur DC and Stroncek D. The gene overexpressed in polycythemia rubra vera, PRV-1, and the gene encoding a neutrophil alloantigen, NB1, are alleles of a single gene, CD177, in chromosome band 19q13.31. Transfusion 2006; 46: 441-447.

[4] Gohring K, Wolff J, Doppl W, Schmidt KL, Fenchel K, Pralle H, Sibelius U and Bux J. Neutrophil CD177 (NB1 gp, HNA-2a) expression is increased in severe bacterial infections and polycythaemia vera. Br J Haematol 2004; 126: 252-254.

[5] Lalezari P, Murphy GB and Allen FH Jr. NB1, a new neutrophil-specific antigen involved in the pathogenesis of neonatal neutropenia. J Clin Invest 1971; 50: 1108-1115.

[6] Xie Q, Klesney-Tait J, Keck K, Parlet C, Borcherding N, Kolb R, Li W, Tygrett L, Waldschmidt T, Olivier A, Chen S, Liu GH, Li X and Zhang W. Characterization of a novel mouse model with genetic deletion of CD177. Protein Cell 2015; 6: 117-126.

[7] Dixon MF, Genta RM, Yardley JH and Correa P. Classification and grading of gastritis. The updated sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol 1996; 20: 1161-1181.

[8] Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev 2000; 22: 283-297.

[9] Go MF. Review article: natural history and epidemiology of Helicobacter pylori infection. Aliment Pharmacol Ther 2002; 16 Suppl 1: 3-15.

[10] Martini M, Teofili L and Larocca LM. Overexpression of PRV-1 gene in polycythemia rubra vera and essential thrombocythemia. Methods Mol Med 2006; 125: 265-273.

[11] Michiels JJ, Bernema Z, Van Bockstaele D, De Raeve H and Schroyens W. Current diagnostic criteria for the chronic myeloproliferative disorders (MPD) essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF). Pathol Biol (Paris) 2007; 55: 92-104.

[12] Sirhan S, Lasho TL, Elliott MA and Tefferi A. Neutrophil polycythemia rubra vera-1 expression in classic and atypical myeloproliferative disorders and laboratory correlates. Haematologica 2005; 90: 406-408.

[13] Stroncek DF, Caruccio L and Bettinotti M. CD177: a member of the Ly-6 gene superfamily involved with neutrophil proliferation and polycythemia vera. J Transl Med 2004; 2: 8.

[14] Kissel K, Santoso S, Hofmann C, Stroncek D and Bux J. Molecular basis of the neutrophil glycoprotein NB1 (CD177) involved in the pathogenesis of immune neutropenias and transfusion reactions. Eur J Immunol 2001; 31: 1301-1309.

[15] Gonda TA, Kim YI, Salas MC, Gamble MV, Shibata W, Muthupalani S, Sohn KJ, Abrams JA, Fox JG, Wang TC and Tycko B. Folic acid increases global DNA methylation and reduces inflammation to prevent helicobacter-associated gastric cancer in mice. Gastroenterology 2012; 142: 824-833, e827.

[16] Toyoda T, Tsukamoto T, Yamamoto M, Ban H, Saito N, Takasu S, Shi L, Saito A, Ito S, Yamamura Y, Nishikawa A, Ogawa K, Tanaka T and Tatematsu M. Gene expression analysis of a Helicobacter pylori-infected and high-salt diet-treated mouse gastric tumor model: identification of CD177 as a novel prognostic factor in patients with gastric cancer. BMC Gastroenterol 2013; 13: 122.