Flowcytometric Analysis of Helicobacter Pylori on Gastric Mucosa

MİDE MUKOZASINDA HELICOBACTER PYLORI'NİN AKIMSİTOMETRİK ANALİZİ

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Summary

Different Helicobacter pylori (Hp) types could cause different cellular immune responses in Hp infections. Therefore, gastric mucosa of patients with Hp infection was examined by endoscopic, microbiologic, pathologic and flowcytometric methods in order to establish rapid, accurate and special cell typing methods, and relationship between them was investigated. Forty four subjects were included in the study. Female/male ratio was equal to 1 and average age was 44.35 ± 15. Chronic gastritis was diagnosed in 31 subjects (75.6%) and duodenal ulcus in 3 subjects (6.8%) endoscopically. Ten cases were revealed as normal. In 24 cases out of 44 (54.5%) Hp was found as positive. Cellular infiltration simulating chronic lymphoid follicles could not be shown to occur specifically secondary to Hp by pathological findings. However, Warthin-Stariy stain and Hp co-existence was found in these patients. Concentrations of CD2, CD3, CD4, CD8, CD14, CD 19, CD45 and CD56 HLADr were measured by flowcytometric analysis of the tissues. Results of these measurements did not show any difference in the patients who are Hp (+) and Hp (-).

In conclusion, cellular immune response in Hp (+) patients evaluated by flowcytometric analysis could not be attributed specifically to Hp.

Key Words: Helicobacter pylori, flowcytometry, T cell immunity

T Klin J Med Res 1999, 17:96-99

Özet

Helicobacter pylori(Hp) infeksiyonundaki hücresel immün oluşumun farklı Hp suşlarında değişik immün cevap oluşturabilmesi ihtimaline binaen, hızlı doğru ve özel hücre tiplendirme yöntemleri ortaya koyalabilmek amacı ile Hp enfeksiyonlu hastaların mide mukozaları endoskopik, mikrobiyolojik, patolojik ve akımsitometrik incelemeye alındı ve aralarındaki ilişki araştırıldı. Çalışmaya 44 kişi katıldı. Yaş ortalamaları 44.35±15 ve Kadın/ Erkek oranı A'di. Endoskopik olarak olguların 31'inde kronik gastrit (%75.6), 3'ünde duodenal ülser (%6.8) mevcuttu, 10 olgu normaldi, 44 olgunun 24'ünde(%54.5) Hp pozitif bulundu. Patolojik bulgularla kronik lenfoid folükülleri taklit eden hücre inflitrasyonunun spesifik olarak Hp'ye ikincil olduğu gösterilemedi; Ancak bu hastalarda Warthin-Stany boyası ile Hp beraberliği mevcuttu. Dokuların akım sitometrik analizinde CD2, CD3, CD4, CD8, CD14, CD19, CD45, CD56 HLADr konsantrasyonları ölçüldü. Bu değerler Hp pozitif ve negatif hastalarda bir farklılık göstermedi.

Sonuç olarak Hp pozitif hastalarda, akımsitometrik analizle değerlendirilen hücresel immün cevabın spesifik olarak Hp'ye ait olabileceğini söylemek mümkün olmadı.

Anahtar Kelimeler: Helicobacter pylori, Akımsitometri, T hücresel immiinite

T Klin Araştırma 1999, 17:96-99

Received: April 24, 1999

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Helicobacterpylori (Hp) is a gram (-), spiral shaped bacteria and accepted as an important factor in the pathogenesis of gastritis, peptic ulcus, gastric cancer and lymphoma. (1-6) Specific humoral and cellular immunity occurs in Hp infections .(7)

Titration of specific IgG and IgA antibodies showing mucosal humoral immunity has been found to be increased in chronic mucosal infections accompanying Hp (8,9). Mucosal antibodies are more sensitive than serum in Hp infection (7).

The role of gastric T-lymphocytes in Hp infection could not be understood thoroughly. Class II HLA antigens in gastric epithelium stimulate local T-lymphocytes and increase their activities. The concentrations of CD4 and CD8 both in mucosa and peripheral blood have been increased in gastritis. T-lymphocytes can proliferate in response to Hp antigen (7). The mechanism of inflammatory process is still not clear. Inflammatory response in long term occurs as a response of the host to eradicate the bacteria located there by activating local and systemic humoral and cellular immunity (10).

The goal of this study was to demonstrate the cellular migration to the site of inflammation in the described mucosal cellular immune response in Hp infections by flowcytometry and highlight the studies related to cellular immunity.

Materials and Methods

Fourty four subjects, who were accepted to the department of Gastroenterology of Black Sea Medical School and upper gastrointestinal system endoscopy was done, were included in the study. Their ages were between 17 and 75 (median age: 44.35±15) and female/male ratio was equal to 1. The subjects were not taking any H2 receptor blockers, omeprazol or other medication for the treatment of Hp when included in the study. During the endoscopy, four antral biopsies for Hp were obtained from the minor curvature 4 cm proximal to the pylorus. Specimens were stored in sterile glasses for Warthin-Starry staining and Urease medium, in 10% formalin for histological examination and in specifically prepared PBS (Phosphate Buffer Solution) for flowcytometric analysis. Endoscopic devices were cleared with a disinfectant solution Gigasept (Schiilke & Mayr Gmbh) prior to each application. Endoscopic findings were revealed by an endoscopist.

Warthin-Starry staining: Specimens were directly laid on the lam and Warthin-Starry silver impregnation was done. Curled, spiral shaped microorganisms were demonstrated by XI00 immersion microscopy.

Histology: Specimens obtained by antral biopsy were stained with hematoxylen eosin for histological examination. Histological examination was performed by a pathologist. Microorganisms on the mucosal surface of the cells as well as histological changes were evaluated by XI00 immersion microscopy.

Culture: Specimens were inoculated in sterile conditions to bloody medium containing vancomycin, amphotericin and trimetoprim-sulphometoxazol and put into an anaerob jar. Additionally, Campy-BAP kit was added to provide a microaerophillic medium. Then each was incubated at 37°C. In the third and seventh days, each was controlled and proliferation was evaluated.

Flowcytometry : Biopsy specimens kept in the tubes containing PBS solution were divided into smaller parts in a petry container in the flowcytometry laboratory. After being waited in RPIM - 1640 solution (5% fetal calf serum) for some time, they were filtrated and prepared in DNA preparation device and then cells having CD2,CD3, CD4,CD8, CD14,CD19, CD45,CD56, and HLA Dr receptors were analyzed with a flowcytometric analyzer (Coulter Epics Elite Flowcytometer, USA). Concentration of cells was revealed by Multicycle computer software.

Mann Whitney U test was performed for statistical analysis. $P<0,05$ was accepted as significant.

Results

Chronic gastritis was diagnosed in 31 cases (75.6%) out of 44 included in the study. In 22 (70.9%) of the cases having gastritis, Hp was positive. Duodenal ulcus was diagnosed in 3 cases (6.8%) and Hp was positive in two (66.6%) of them. Ten cases were revealed as normal endoscopically, and Hp was negative in all of them (Table 1). Flowcytometric results has been shown in table 2. Hp positive cases (% concentration): [CD2:4.46±1, CD3:0.70±0.08, CD4:0.89±0.19, CD8:0.47±0.18, CD14:4.8±1.5, CD19:0.41±0.5, CD45:0.17±0.02, CD56:1.9±0.12, HLA Dr:1.1l±0.3]. Flowcytometric results of Hp- nega-

Table I. Features of the groups

	HP(+)	HP(-)
Gastritis	22	9
Peptic ulcus	2	1
Normal		10

Table 2. Flowcytometric values of Hp (+) and Hp (-) cases (% concentration ± SEM).

Cells	HP (+)	HP(-)
CD2	4.46 ±1	3.83 ± 2.8
CD3	0.70 ± 0.08	0.48 ± 0.04
CD4	0.89 ±0.19	1.5 ±0.47
CD 8	0.47 ±0.18	0.25 ±0.1
CD14	4.8 ± 1.5	6.75 ±2.1
CD19	0.41 ± 0.5	0.35 ±0.18
CD45	0.17 ±0.02	0.16 ±0.1
CD56	1.9 ±0.12*	0.78 ±0.21
HLA Dr	1.11 ±0.3	2.33 ± 1

**p < 0.05 Difference from Hp (-) cases.*

tive cases (% concentration): [CD2:3.83±2.8, CD3:0.48±0.04, CD4:1.5±0.47, CD8:0.25±0.1, CD14:6.75±2.1, CD19:0.35±0.18, CD45:0.16±0.1, CD56:0.78±0.21, HLA Dr:2.33±l] (Table 2). The increase in concentrations of CD56 demonstrating NK cells was statistically significant. However, there was no significant alteration in the other inflammatory parameters and HLA Dr concentrations.

Discussion

In our study, no significant increase was detected in the rates of local CD4 and CD8 in the patients having Hp-infected gastritis when compared to Hp-negative ones. Significant increase was found only in concentrations of NK cells in the study. This finding was attributed to chronic inflammation. On the other hand, no significant increase was detected in special cell populations in Hp-positive individuals, similar to the results of studies done by microscopic and ELISA methods.

Mucosal mononuclear cell and granulocyte infiltration was found significantly high in Hp-positive patients when compared to Hp-negative ones .(10-11) In invitro studies, Hp was demonstrated to increase the complement-dependent cytotoxic activity of PMNL in peripheral blood (12).

The role of gastric T-lymphocytes in Hp infections could not be understood thoroughly. In various studies, adhesion molecules such as CD44 and CD9 were found to increase in Hp-infected epithelial cells, but there was no change in intraepithelial lymphocytes. HLA II (DR,DP) expression was demonstrated to increase in epithelium and lymphocytes (7,13). Similarly, CD4 and CD8 concentrations were found to increase in urease-positive gastric biopsies in different studies. On the other hand, no significant increase could be found in the concentrations of CD3 and CD71 (14).

In conclusion, the absence of significant increase in cell populations except concentrations of total mononuclear cells with flowcytometric analysis in Hp-positive individuals lead to a question whether the mucosa in Hp-negative individuals has been infected by other infective agents causing inflammation.

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