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Expression of class I and II MHC receptors in Helicobacter pylori-positive patients with active gastritis and duodenal ulcer*

Aktif gastrit ve duodenal ülseri olan H. pylori-pozitif hastalarda Klas I ve II MHC reseptörleri

Zeygam SULEYMANOV

Azerbaijan Medical Faculty, Baku and Türkiye Yüksek İhtisas Hospital, Ankara

Background/aims: *Helicobacter pylori colonizes on the epithelial surface below the mucous membrane in the gastric antrum, causing chronic active gastritis and duodenal ulcer. However, we know little about the response of the immune system, which is responsible for protecting the organism, to this bacteria and the duodenal tissue damage. The aims of our study were to examine the expression of class I and II MHC receptors in the immune cells in the peripheral venous blood of patients with chronic active gastritis and duodenal ulcer.* ***Methods:*** *The study included a total of 124 cases (Helicobacter pylori-positive patients without prior treatment, 47 of whom had small-sized and 56 medium-sized duodenal ulcer, and 21 healthy individuals). Immune cells carrying receptors CD3, CD4, CD3/4, CD8, CD3/8, CD14, CD45, CD14/45, HLA-DR, CD3/HLA-DR, CD16/56, and CD3/16/56 by applying DAKO Dual Color Reagent, and non-specific indicators of reactivity, were investigated in the peripheral venous blood samples.* ***Results:*** *In both study groups, nonspecific indicators of reactivity in peripheral venous blood were not different from those in the healthy group irrespective of the ulcer size. It was detected that NK cells which express only CD3/16/56 receptor are more aggressive than class I MHC carriers, which were increased in both patient groups more markedly than in the control group ($p<0.05$). The number of CD14 cells in patient groups with small- and medium-sized duodenal ulcers was found to be lower than in the control group. This difference was more marked in the small-sized ulcer group than in the medium-sized ulcer group. While there was a similarity in the number of CD45 cells in all three groups, the number of CD14/45 cells was reduced in the study group. This reduction was found statistically insignificant in the medium-sized ulcer group, but significant in the small-sized ulcer group ($p<0.05$). As compared to the control group, the number of HLA-DR cells was found to be increased in both study groups, and CD3/HLA-DR was found to be increased in the medium-sized ulcer group ($p<0.05$).* ***Conclusions:*** *The reduced number of CD14 cells may have been among the factors predisposing to the development of Helicobacter pylori infection. Such a deficit in cellular defense may be compensated through the upregulation of cells with HLA-DR and CD3/HLA-DR markers by the organism. The increase in the number of cells expressing CD3/16/56 in chronic antral gastritis and duodenal ulcer patients positive for Helicobacter pylori suggests a role for autoimmune events in these diseases.*

Key words: Helicobacter pylori, duodenal ulcer, class I and MHC receptor

Amaç: *Helicobacter Pylori, mide epiteline kolonize olarak kronik aktifgastrit duodenal ülser hastalığına neden olur. Ancak organizlayı koruyan immün sistemin Helicobacter Pylori'ye verdiği yanıt tam olarak bilinmemektedir. Bu çalışmanın amacı Helicobacter Pylori (+) kronik aktifgastritli ve duodenal ülserli hastaların venöz kanındaki immün hücrelerdeki class I ve II MHC reseptör ekspresyonunu araştrmaktır.* ***Yöntem:*** *Çalışmaya daha evvel tedavi görmemiş Helicobacter Pylori (+) 124 olgu alındı (47 küçük çaplı ülser, 56 orta çaplı ülser ve 21 sağlıklı olgu). Olguların periterik venöz kan örneklerinde CD3, CD4, CD3I4, CD8, CD3/8, CD 14, CD45, CD 14/45, HLA-DR, CD3/HCA-DR, CD16/56 ve CD3/16I56 reseptörleri araştırıldı.* ***Bulgular:*** *CD3/16/56 reseptörlerini sunan NK hücreleri hasta grubunda kontrol grubuna göre daha fazla bulunmuştur. CD14 sunan hücrelerin sayısı hasta grubunda kontrol grubuna göre daha düşük bulunmuştur ($p<0.005$). Bu bulgu küçük çaplı ülser grubunda daha belirgin idi. CD45 eksprese eden hücrelerin sayısı 3 grupta da benzer iken, CD 14/45 eksprese eden hücrelerin sayısı çalışma grubunda azalmıştır. Bu bulgu küçük çaplı ülser grubunda belirgindir ($p<0.05$). Kontrol grubu ile karşılaştırıldığında HLA-DR hücrelerinin sayısı çalışma grubunda artmıştır, CD31HLA-DR sayısı ise orta çaplı ülser grubunda artmıştır ($p<0.05$).* ***Sonuç:*** *CD 14 eksprese eden hücrelerin sayısında azalma Helicobakter Pylori infeksiyonu gelişmesine yol açan faktörler arasında olabilir. CD3/16156 eksprese eden hücrelerin sayısınınHeyicobacter Pylori (+) aktif gastritve ülserli hastalarda artmış olması, bu hastalarda otoimmün olayların rolü düşündürmektedir.*

Anahtar kelimeler: Helicobacter pylori, duodenum ülseri, klas I ve II MHC reseptör

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Address for correspondence: Zeygam SULEYMANOV

Azerbaijan Medical Faculty, Baku

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INTRODUCTION

Although gastric mucosa possesses a low number of immune cells (1), it is able to maintain an adequate cell number when needed (2). After taken orally, Helicobacter pylori *(H. pylori)* colonizes on the surface of gastric mucosal epithelium, which predisposes tissue damage (3). However, the question of how they cause this predisposition despite the presence of polymorphonuclear leukocytes, which are extensively encountered in this region, and of subsequently increased T lymphocytes which carry CD4 and CD8 receptors (1,4-6), remains to be answered. The view that both superoxide dismutase and catalase, which are adaptive defense mechanisms developed by the bacterium, prevent the lysis of *H. pylori* in the phagocytic vacuoles of neutrophils is not perfect (7). In gastric biopsies, the number of neutrophils and monocytes, which phagocytose *H. pylori,* is not high. This means that there may be different ways by which these bacteria protect themselves from both nonspecific defense mechanisms and the immune system. On the other hand, it has been proven that individuals infected with *H. pylori* have increased expression of class II MHC antigens on the surface of epithelial cells in the gastric mucosa (6). To clarify this issue, we examined those immune cells expressing MHC class I antigen receptors, and non-specific reactivity cells in the blood, which are the origin of cells migrating as a response to the mucosa, *H. pylori,* and the environment the bacterium produces.

MATERIALS AND METHODS

The study included 103 *H. pylori-positive* patients without any prior treatment, with chronic antral gastritis and duodenal ulcer (DU) who were first diagnosed via endoscopic examination in the Gastroenterology Clinics of the Yüksek İhtisas Hospital Turkey, between October 1994 and February 1997, and 21 healthy individuals. *H. pylori* positivity was confirmed by urease test and histologic examination, while chronic antral gastritis and DU were confirmed by both endoscopic and histologic examinations. While choosing patients, care was given to ensure that they should not have any additional pathological condition, which could affect non-specific reactivity and the immune system. Cases in which the extent of mucosal tissue damage was less than 6 mm were included in the small ulcer (SU) group and those between 6-12 mm in the medium-extent ulcer (MU) group. Absolute number (nx109/L) of neutrophils, lymphocytes and monocytes in the peripheral venous blood and CD3, CD4, CD3/4, CD8, CD3/8, CD16/56, and CD3/16/56 cells which express MHC class I antigen receptors, and CD14, CD45, CD14/45, HLA-DR, CD3/HLA-DR cells, which express class II MHC antigen receptors, were investigated by applying DAKO Dual Color Reagent (Denmark). Statistical procedures were performed using Student's t test in Microsoft Excel 2000. Values were given as means ± standard deviation. P values were considered significant if <0.05.

RESULTS

Age, gender, and non-specific reactivity data were similar in the control and patient groups ($p>0.05$). The study included 71 males and 32 females. Although the overall male/female ratio was 2.2/1, this was 1.76/1 in the SU group and 2.73/1 in the MU group. A slight increase in the number of leukocytes, neutrophils, lymphocytes, and monocytes was detected in the peripheral venous blood in the MU group as compared to the other two groups. However, this increase was not statistically significant ($p>0.05$). The number of these cells in the SU group was found similar to that in the control group (Table 1). Table 2 shows the expres-

Table 1. Characteristics of the control and study groups and nonspecific reactivity data (number of cells x 10^9/L)

Parameters	Study groups		
	Control group (n=21)	Small ulcer (n=47)	Medium ulcer (n=56)
Mean age (years)	39.9+7.0	39.6±9.8	38.2±6.9
Age range (years)	26-63	24-62	27-56
Gender (F/M)	9/12	30/17	41/15
Leukocyte	6.88±1.01	6.87±1.09	7.15±1.19
Neutrophil	4.048±0.7	3.895±0.793	4.883+0.747
Lymphocyte	2.103±0.353	2.081±0.05	2.305+0.057
Monocyte	0.514±0.096	0.537±0.115	0.631±0.171

$p > 0.05$ in all comparisons

Table 2. Expression of MHC class I antigen receptors in peripheral venous blood (number of cells x 107L).

Parameters	Study groups		
	Control Group n=21	Small Ulcer n=42	Medium-sized Ulcer n=42
CD3	1.392±0.302	1.619±0.29	1.768±0.361
CD4	0.788±0.134	0.808±0.165	1.060±0.189
CD3/4 ^	0.774±0.137	0.860±0.138	0.944±0.279
CD8	0.828±0.154	0.842±0.199	0.894±0.235
CD3/8	0.590±0.109	0.642±0.18	0.764±0.158
CD16/56	0.547±0.107	0.594±0.149	0.595±0.126
CD3/16/56	0.062±0.01	0.111±0.028*	0.256±0.065**

*p < 0.025, **p < 0.005

sion of class I MHC antigen receptors in peripheral venous blood cells. There is an increase in CD3 cells in the study groups when compared with the control group, and this increase is associated with the extent of ulcer. The number of these cells is higher in the SU group than in the control group, and higher in the MU group than in both control and SU groups. This was similar for CD4, CD3/4, and CD3/8 parameters. CD8 and CD16/56 parameters were very similar in all three groups. There was a statistically significant difference in the number of NK cells distinguished only by CD3/16/56 aggressiveness from those cells expressing class I MHC antigen receptors. The difference between MU and control groups ($p<0.005$) was more marked than that between SU and control groups ($p<0.025$). For this parameter, a significant difference was also detected between the MU and SU groups ($p<0.005$); in other words, the increase in the number of CD3/16/56 cells correlated with the size of the ulcer.

A statistically significant reduction in CD14 cells in the peripheral venous blood was observed in both patient groups as compared to the control group. This reduction was more pronounced in the SU group ($p<0.005$) than in the MU group ($p<0.05$). There was no significant increase in CD45 level in the study groups, but the number of CD14/45 cells was found to be reduced in the SU group when compared with the control group ($p<0.05$), just like CD14 data in the SU group. The reduction in the MU group is statistically insignificant . As compared to the control group, the number of cells carrying HLA-DR receptors was found 1.9 times higher in the SU group and 2.5 times higher in the MU group. The difference was significant in both cases (SU: $p<0.025$; MU: $p<0.005$). While the number of CD3/HLA-DR immune cells was very similar in the control and SU groups, there was an increase in the MU group 2.9 times higher than in the other groups ($p<0.005$) (Table 3).

DISCUSSION

As there is no organized lymphoid tissue in normal gastric mucosa, the immune status of this region is very innocent (1). In addition to this, inadequate recognition of *H. pylori* by defense mechanisms facilitates colonization of bacteria and formation of inflammation in this region. There is a very strong correlation between the number of *H. pylori* in the mucosa and the severity of tissue inflammation (8). Bacteria cause migration of monocytes and neutrophils to the region via chemotactic factor secretion (4,9). In our study, the number of these cells in the peripheral venous blood was found higher in the MU group than in the SU and control groups. While the difference was not statistically significant, this increase may suggest the response of bone marrow

Table 3. MHC class II antigen receptor expression in the peripheral venous blood (n x 107L).

Parameter	Groups		
	Control Group	Small Ulcer	Medium-sized Ulcer
CD14	0.129±0.037	0.021±0.008*	0.048±0.025*
CD45	1.681±0.632	2.502±0.269	2.575±0.686
CD14/45	0.091±0.019	0.044±0.017*	0.056±0.021
HLA-DR	0.582±0.134	1.135±0.241**	1.495±0.247**
CD3/HLA-DR	0.393±0.135	0.388±0.148	1.162±0.301**

*p<0.05, **p<0.05

to chemotactic factor secreted by *H. pylori.*

In healthy adults, distribution of intraepithelial lymphocytes per each 100 epithelial cells of the mucosa is as follows: CD3: 22, CD4: 12, and CD8: 11 cells (10). On the basis of our results, the above ratio of cells in the mucosa, that is 2/1/1, is also seen in the peripheral venous blood (Table 2). As the gastric mucosa lacks organized lymphoid tissue, any change in the number of *H. pylori* will correlate with the peripheral blood cells. CD4 and CD8 cells increase in mucosa under the influence of H. pylori. CD4 is localized predominantly in lamina propria, and CD8 in the epithelium (1). CD3 receptors expressed generally on the surface of T lymphocytes combine with antigens, causing activation of cells and secretion of cytokines. CD4 causes T helper cells to come into contact with MHC II class antigen receptors (11). In this study, an increase in the number of cells expressing CD3, CD4, and CD3/4 was detected in the peripheral venous blood in both study groups as compared to the control group; this increase was dependent on the diameter of ulcer and it was not statistically significant. A similar finding was obtained for CD3/8 — killer/suppressor cells. The number of CD8 - cytotoxic cells was very similar in all three groups. CD8 and CD3/8 cells undertake the function of protecting the organism against foreign invasion. However, such a function could begin to work as a result of acquired immunity after these cells recognize the antigen in advance (11). According to the results we found, there is a problem in recognition of *H. pylori* as foreign by the CD8 - cytotoxic and CD3/8 - killer/suppressor cells which are the basic elements of the immune system. Based on our results, we can say that the same problem also exists for CD16/56 NK cells because in all three groups the number of cells expressing CD16/56 was the same. Although many investigators have correlated this to the liable antigenic structure of *H. pylori,* it is certain that there are unknown factors here. These factors should be explored in the mechanisms underlying the relationship between the cells expressing class I and II MHC antigen receptors.

CD3/16/56 NK cells, distinguished by their greater aggressiveness from the cells expressing class I MHC antigen receptors in the peripheral venous blood from patients with *H. pylori-induced* antral gastritis and duodenal ulcer, were found not to be quiescent in these events. It was seen that the number of these cells in the SU group was 1.7 times as high as that in the control group ($p<0.025$), and 3.9 times as high as that in the MU group ($p<0.005$). This increase was more pronounced in the MU group than in the others. Because CD3/16/56 cells are more aggressive, they may react against their own host, which is then recognized as foreign. This suggests that they may play a role in the epithelial damage in some cases (2,12).

In this study, the number of CD14 cells in peripheral venous blood of *H. pylori-positive* patients with antral gastritis and duodenal ulcer was found to be lower than in the control group. It is known that CD14 receptors belong to B lymphocytes and a group of phagocytes - neutrophils/monocytes/macrophages - and have a lipopolysaccharide-binding function (5). Furthermore, it has been demonstrated that a big portion of antigenic structures *of H. pylori,* a Gram (-) bacterium, is an endotoxin in the form of lipopolysaccharide (7). These lipopolysaccharides induce a slight IgA immune response in mucosa; on the other hand, as they resemble blood group antigens Lewisx and Lewisy they induce a poor IgG response. However, these antigens react with laminin of basal membrane in the mucosa, causing epithelial cells to break easily (7,8). Many investigators suggest that an immune response to bacteria, whose antigens are in lipopolysacccharide form, is poor (9,10). Thus, the immune response given to *H. pylori* is weak. However, whether this weak response is caused by a specific behavior of the immune system or inadequate number of CD14 receptors that play an important role in recognition of antigens in lipopolysaccharide form is not known. Our study supports the latter since CD14 cells play a major part in the removal of the immune quiescence (10).

On the basis of another finding we obtained, the number of CD45 cells that belong to all leukocytes and that are found on lymphocytes more commonly than on granulocytes was found higher in both patient groups than in the control group, although the difference was not significant (11). The number of CD14/45 cells that have both CD14 and CD45 receptor glycoproteins on their surface was reduced in both patient groups. This reduction was found statistically significant in the SU group, but insignificant in the MU group. It is noted that the number of CD14 and CD14/45 cells was decreased more markedly. It is difficult to explain the reasons why the above-mentioned

cells in the MU group, in which the ulcer size is larger, increased relative to the SU group. Class II MHC antigen receptors (HLA-DR) are strongly expressed on the surface of gastric mucosal epithelial cells in patients infected with *H. pylori* (12). Some investigators correlate this to the intensity of the immune response given to *H. pylori* (13), which is due to the effect of gamma interferon produced by T lymphocytes. However, LA-DR expression begins earlier in gastrointestinal epithelium, and McDonald et al. (14) demonstrated that a 17-week embryo (fetus) had HLA-DR on the surface of villous epithelium in the small intestine. This means that HLA-DR expression is not indicative of local immune response, but is a result of more extensive functions of the organism. HLA-DR cells generally have the ability of phagocytosis, and are located in the organs such as gastrointestinal and respiratory systems, that have the greatest contact with external factors. Therefore, it is normal that the number of HLA-DR-positive cells in the peripheral venous blood from patients we included in the study depended on the ulcer size and increased significantly as compared to the controls. We observed a more marked increase in the MU group than in the control group ($p<0.005$). This difference in SU was not as high as in the MU group ($p<0.025$). That is to say, based on the correlation between the increase in both *H. pylori-positive* patient groups and the ulcer size, this increase can be considered a response given not only to *H. pylori* but to mucosal damage as well. On the other hand, the fact that CD3/HLA-DR parameter, which was an indicator of functionally activated lymphocytes in the MU group, was found higher than that in the control and SU groups further confirms what we have mentioned above.

In conclusion, low number of CD 14 cells is likely to be involved in the predisposition to the development of *H. pylori* infection. Possibly the human organism may have compensated this inadequacy in cellular immune defense by increasing the number of cells which express HLA-DR and CD3/HLA-DR. In addition, there is also a relationship between ulcerative damage in the gastrointestinal mucosa and the number of these cells. Non-specific reactivity indicators in the peripheral venous blood do not respond to *H. pylori* infection, the antral gastritis produced by the bacterium, or the small and medium-sized duodenal ulcers. In this study, NK cells were increased; these cells carry only receptors such as CD3/16/56 and are distinguished by their greater aggressiveness from those cells which express class I MHC antigens. This suggests that autoimmune components are involved in duodenal ulcer as well.

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