Memory cells in the antral mucosa of children with Helicobacter pylori infection

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Abstract

A total of 106 patients, included in the study, were divided into three groups with regard to Helicobacter pylori infection. Endoscopy and histopathological examination of the stomach, based on the Sydney's System, were performed in all the children. CD45RA and CD45RO cells were identified by means of specific antibodies in the inflammatory infiltrate of the antral mucosa. An increased expression of CD45RO and CD45RA lymphocytes was reported, basing on the results of the study.

Key words: antral mucosa, memory cells, Helicobacter pylori, children.

Introduction

T lymphocytes are activated in response to H. pylori infection. Large amounts of cells with markers: $CD4^+$, CD45RO and $CD69^+$, are usually found in the antral mucosa [2]. The response from $CD4^+$ lymphocytes predominates, whereas $CD8^+$ lymphocytes are not so numerous and an increase in the relation $CD4^+/CD8^+$ takes place [1, 3]. $CD4^+$ lymphocytes, inducing an immune response in combination with the molecules of major histocompatibility complex (MHC) of class II, recognize specific peptides coming from extracellular phagocytized antigens. These cells express an isoform of CD45RO antigen (described as "virgin" or "naive") and CD45RO (described as "memory" and associated with the state of activation). Approximately 85% of $CD4^+$ lymphocytes have CD45RO molecule [3]. CD45RO are more helpful in the production of immunoglobulins by B lymphocytes than the "naive" cells. They produce IL-4, IL-5, IL-10, IFN-gamma. Basing on L-selectin expression on their surface, CD45RO cells are divided into two groups: the group expressing L-selectin and producing mainly IL-4 and IL-5 and the group without expressing L-selectin and producing mainly IFN-gamma. Such a profile of cytokines coincides with the profile of Th1and Th2 lymphocytes [4].

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Material and methods

Group I - 59 children (29 girls and 30 boys; the age range: 2-19 years) with chronic gastritis in the course of H. pylori infection with positive IgG antibody anti- H. pylori. Group II - 29 children (14 girls and 15 boys; the age range: 3-19 years) after past H. pylori infection, without bacterium colonization and gastritis, but with a maintained positive titre of antibodies in IgG class against H. pylori. Group III - 18 children (12 girls and 6 boys; the age range: 5-17 years) with functional disorders of the gastrointestinal tract, without H. pylori infection, with normal IgG concentration against H. pylori (the control group). The samples from the antrum pyloric section were diagnosed, according to the Sydney's System.

All the biopsy specimens from each study group were stained by an immunohistochemical method for the evaluation of CD45RA and CD45RO lymphocytes in the antrum mucosa, performed, using mice monoclonal antibodies against human T lymphocytes (CD45RA and CD45RO) of the DAKO firm. The ABC method was used, according to commercial protocol. The number of CD45RA and CD45RO lymphocytes in the mucosa was counted in discrete areas, measuring 0.785 mm^2 each, by using a light microscope. All the counts were performed, using a magnification of 200x. The numbers of positively stained cells were presented as the mean values per 1 mm^2 of analysed gastric section area. All

Table 1. CD45RA lymphocytes in the antrum mucosa in children

Examined groups	CD45RA lymphocytes in the antrum mucosa /mm								
	Number of patients (N)	Minimum (min)	Maximum. (max)	Arithmetic mean. (x)	Median (M)	Mode	Standard Deviation (SD)	Lower Quartile	Upper Quartile
Group I	17	0	197	46.8	26	3	59.2	4	40.0
Group II	7	0	22	8.0	7	-	7.2	4	10.0
Group III	3	0	5	1.7	0	0	2.9	0	2.5

Table 2. CD45RO lymphocytes in the antrum mucosa in children

Examined groups	CD45RO lymphocytes in the antrum mucosa/mm^2								
	Number of patients (N)	Minimum (min)	Maximum (max)	Arithmetic mean. (x)	Median (M)	Mode	Standard Deviation (SD)	Lower Quartile	Upper Quartile
Group I	20	0	817	262.1	236	0	230.9	132	284
Group II	15	0	270	142.3	147	117	89.5	90	210
Group III	6	67	278	153.5	130	-	85.7	90	212

Figure 1. Correlation between the expression of CD45RA lymphocytes and the severity grade of antral gastritis in children with Helicobacter pylori infection (Group I)

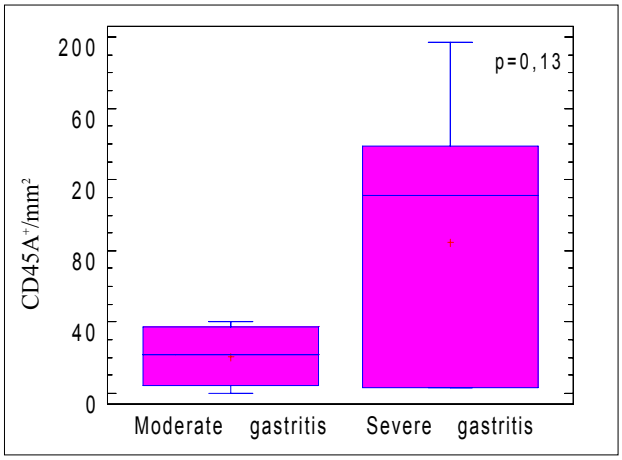
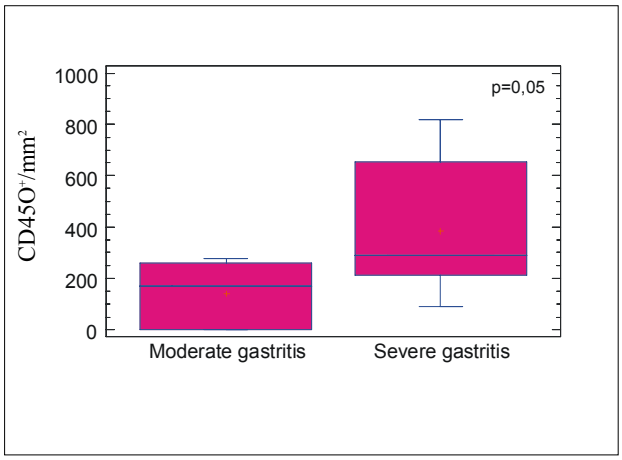


Figure 2. Correlation between the expression of CD45RO lymhocytes and the severity grade of gastritis in children with Helicobacter pylori infection (Group I)



the lymphocyte counts were performed by a single observer (I. K.), who was unaware of either the H. pylori status or the subject's clinical group. Descriptive statistics included the arithmetic mean (x), the median (Me), standard deviation (SD), and the minimum (min) and maximum (max) result. The level of the parameters was compared by means of the U Mann-Whitney test for either independent or paired trials. The differences were regarded statistically significant at $p<0.05$. The correlation between the features, measured in the nominal scale, was evaluated by means of an independence test Chi^2 and the results were presented as frequency tables. All the clinical and laboratory tests were performed in the children after a prior consent from their parents and guardians, and of the approval by the Bioethical Board of the Medical University of Białystok.

Results

The quantitative assessment of CD45RA in the antral mucosa by means of an immunohistochemical method showed an increase in their expression in the children with H. pylori infection (46.8 cells/mm^2), in comparison with the children after H. pylori infection (8.0 cells/mm^2) and the controls (1.7 cells/mm^2). Table 1. However, the expression of CD45RA lymphocytes was higher in more severe inflammation. The expression of CD45RA lymphocytes in moderate antral gastritis equalled on the average 20.2 cells/mm^2 in the children with H. pylori infection. In severe antral gastritis, the mean expression of CD45RA lymphocytes was 84.71 cells/mm^2. In mild grade activity gastritis, the mean expression of CD45RA was 11.67 cells/mm^2 in children with Helicobacter pylori (Fig.1).

Moderate activity antral gastritis was characterized by the mean expression of 54.29 cells/mm^2. The quantitative analysis of CD45RO "memory" lymphocytes in the antral mucosa by immunohistochemical method showed a significant increase in the expression of those lymphocytes in children with Helicobacter pylori infection (262.1 cells/mm^2). In children after H. pylori infection, with positive antibodies against H. pylori (142.3 cells/mm^2), the expression of CD45RO lymphocytes was comparable with their expression in the controls (153.5 cells/mm^2) Table 2. When assessing the expression of CD45RO lymphocytes in the antral mucosa in Group I in relation to the severity grade of antral gastritis, the reported differences were on the border of statistical significance (p=0.05). However, the expression of CD45RO lymphocytes was higher in more severe inflammation. In children with H. pylori infection, the mean expression of CD45RO was 139.3 cells/mm^2 in moderate gastritis. Severe gastritis was characterized by the mean expression of CD45RO lymphocytes, which equalled 384.9 cells/mm^2. In case of moderate gastritis, the mean expression of CD45RO lymphocytes was 142.67 cells/mm^2 in Group I (Fig. 2).

Discussion

The quantitative assessment of CD45RA cells in the antral mucosa, using monoclonal antibodies, proved their highest expression in children with H. pylori and a significantly lower expression in children after bacteria eradication, and the lowest one in the controls. The expression of CD45RA correlated positively with the severity and activity grade of antral gastritis in children with H. pylori and was higher in the severe grade inflammation. However, no statistically significant differences were found between the groups. When assessing CD45RO cells in the antral mucosa, their highest expression was established in children with H. pylori infection and approximately, twice lower expression in children after bacteria eradication. The expression of CD45RO was higher in more severe antral gastritis of children with H. pylori infection, and there were statistically significant differences with regard to the severity grade of gastritis ($p<0.05$). The increased expression of CD8$^+$, CD45RA and CD45RO lymphocytes in the antral mucosa may suggest that Th1 and Th2 lymphocytes took part in response to H. pylori infection in the examined children. Hatz et al. [4] obtained results, which were similar to ours, showing an increased expression of CD4$^+$ and CD8$^+$ lymphocytes with predominance of CD4$^+$ in both the lamina propria and the intraepithelium, and an increased relation of CD4$^+$/CD8$^+$. Additionally, they found a doubled expression of CD45RO cells and an activation of TCR α/β^+ receptor on the lymphocytes in the lamina propria of the gastric mucosa. The changes in the expression of cells correlated positively with the severity grade of the inflammatory process. Kikuchi et al. [5] demonstrated an increased secretion of RANTES, a major basic protein (MBP) and an increased expression of CD45RO lymphocytes in an inflammatory infiltrate in the gastric mucosa, in comparison with the respective values in patients after eradication and in healthy people. However, the secretion of RANTES was enhanced up to 2 years after eradication, as well as chronic infiltration with CD45RO cells and eosinophils [5]. Lungren et al. [6], explaining a suppressed immune response to H. pylori infection, showed a suppressed response of T CD4$^+$ memory lymphocytes.

Conclusions

1. An increased expression of memory lymphocytes (CD45RO) was found in the antral mucosa in children infected with Helicobacter pylori, being correlated with the severity grade of inflammation. 2. The quantitative analysis proved the increased expression of CD45RA lymphocytes in children with Helicobacter pylori infection but no correlation was found between their expression and the severity grade of the inflammation.

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