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• *BRIEF REPORTS* •

Detection of T lymphocyte subsets of children with *Helicobacter pylori* infection

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Abstract

AIM: To study the transformation of T lymphocyte subsets in children with *Heliobacter pylori* (*H pylori*) infection.

METHODS: The *H pylori* infection status were determined by a combination of ELISA and Western blot (immunoblot) technique in 98 children and T lymphocyte subsets in peripheral blood were determined by flow cytometrical analysis.

RESULTS: There were 75 children positive with *H pylori* infection and 23 negative in 98 children. Comparing the proportion of peripheral blood T lymphocytic subsets in children with *H pylori* infection and without *H pylori* infection, it was found that a higher proportion of CD4 T-cells in infected children (39.02±7.71 *vs* 34.25±10.73, $t = 2.246$, $P<0.05$) and higher value of CD4 to CD8 T-cells ratio (1.51±0.52 *vs* 1.25, $t = 2.104$, $P<0.05$) were present, but there were not significant differences in CD3 T-cells and CD8 T-cells (73.11±10.02 *vs* 69.49±17.08, 27.22±6.07 *vs* 28.27±8.67, $P>0.05$).

CONCLUSION: Th1 cell-mediated immune responses may be induced by *H pylori* infection in children.

Key words: Children; *H pylori*; T lymphocyte subsets

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INTRODUCTION

Helicobacter pylori (*H pylori*) infection is one of the causes of many upper digestive diseases, which relates with chronic gastritis and peptic ulcer, in addition it is also closely related to adenocarcinoma of stomach, mucosa-associated lymphoid tissue and lymphoma. The pathogenicity of *H pylori* may differ based on bacteria types, the age of patients and body response. The influence of *H pylori* infection on the immunity in children is unclear and there are few researches on it. We applied ELISA and Western blot (immunoblot) technique in 98 children and T lymphocyte subsets in peripheral blood were determined by flow cytometrical analysis to study the influence of *H pylori* infection on the immunological situation in children that will provide the new information for preventing *H pylori* infection.

MATERIALS AND METHODS

Study population

Ninety-eight Chinese children in Guangxi Luocheng county primary school (9 Han nationality, 26 Zhuang nationality, 31 Mulao nationality and 32 other nationalities) were studied. The age of them is from 6 to 14 years (average 9.40±1.88 years), 56 boys and 42 girls.

Samples collection

As their parents had agreed upon, all of them had no breakfast. Four milliliters of venous blood were taken for the next analysis (2 mL for collecting serum for *H pylori* examination and 2 mL with EDTA for T leukocytes subsets examination).

H pylori antibodies examination

H pylori antibodies were determined by serodiagnosis with ELISA (kit from China Shenzhen Jingmei Biotechnologies Co. Ltd.).The ELISA procedure assay was performed according to the manufacturer's instructions and the results were determined in *A* value with 450 nm wave on BIO-RAD apparatus Model 450 Microplate Reader. All positive serum samples were assayed in duplicate. Because borderline samples tested positive, they were retested to minimize the chance

of false positivity. Negative samples were not retested because they all showed clear negative *A* value.

Determination of VacA and CagA of H pylori

VacA and CagA of *H pylori* were determined by Immunoblotting technique (kit from China Huizhou Allic Biotechnologies, Inc.) and the Immunoblot testing was performed according to manufacturer's instructions. A non-reactive control and reactive control were run with each assay. Reactions that showed a band at CagA(128 116 ku) or any three bands from among VacA(959 187 ku), or the urease subunits (66 ku) were considered positive.

T leukocytes subsets examination

The lymphocyte subset was determined by three color flow cytometry for CD4-FITC/CD8-PE/CD3-Cy5 and the control group with IgG1-FITC/IgG1-PE/Ig1-PE-Cy5. Analyses were performed using a Coulter EPICSXL flow cytometer with 5×10^3 cells per count apparatus from Beckmancoulter. Fluorochrome-tagged (FITC or PE), CyTOROL, and other agents were purchased from Beckmancoulter. The experiments were carried out as follows: (1) standard examination samples: 20 μL of three color monoclonal antibody was added into 100 μL whole peripheral blood (with EDTA antiagglutination) in a 12 mm×75 mm tube, mixed and after room temperature for 20 min, treatment in samples apparatus, added agent for splitting decomposition of red blood cells. Twenty microliters IgG1-FITC/IgG1-PE/Ig1-PE-Cy5 of mice were added into 100 μL whole peripheral blood (with EDTA antiagglutination) for negative control test. (2) T leukocytes subsets were examined by flow-check, all fluorochrome signs were <2. Fluorochrome I is FITC, Fluorochrome II is PE, Fluorochrome IV is PE-Cy5, Chromewave filter were 525, 575 and 675 mm. At the same time, we carried out the negative control tests. The results were obtained and analyzed on flow cytometry apparatus and SYSTMII.

Standard diagnosis of H pylori infection

From the results of ELISA and Immunoblotting, if both the results of experiments were positive, they were recognized as *H pylori* infection.

Statistical analysis

Statistical analysis was performed using the SPSS package (Version 10.0 for Windows). *t*-test and variant analysis were conducted for statistical analyses of all results.

RESULTS

The results of T lymphocyte subsets

T lymphocyte subsets are not significantly different among 98 children from different nationalities (by analysis of variance, $P>0.05$, Table 1).

T lymphocyte subsets in different situations of H pylori infection

Seventy-five children tested positive for *H pylori* infection and 23 tested negative. There is no significant difference in proportion of $CD3^+$T and $CD8^+$T between children with *H pylori* infection (+) group and *H pylori* infection (-) group (by *t*-test, $P>0.05$). But there is a significant difference in proportion of $CD4^+$T between two groups ($t = 2.246$, $P<0.05$). The value of proportion of $CD4^+$T is comparatively high in *H pylori* infection (+) group; and the ratio of $CD4^+$T /$CD8^+$T is also different ($t = 2.104$, $P<0.05$, Table 2).

Table 1 T lymphocyte subsets

Nationalities	Samples	mean±SD		
		$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)
Zhuang nationality	26	75.542±11.699	39.788±8.668	28.500±5.884
Mulao nationality	31	68.648±12.579	36.816±9.742	25.087±6.559
Han nationality	9	73.867±3.096	36.222±4.118	29.844±5.047
Other nationality	32	72.419±9.193	37.753±7.098	28.119±6.365
Total	98	72.188±10.923	37.856±8.235	27.419±6.327

DISCUSSION

T lymphocyte subsets included T cell population $CD4^+$, antigen restricted by class II major histocompatibility complex and $CD8^+$, which is restricted by class I major histocompatibility complex are called cytotoxic T lymphocytes. T cell population $CD4^+$ are differentiated into three subsets Th0 cells, Th1 cells and Th2 cells that depend on their different cytokines. After accepting the stimulation from antigens, Th0 cells then split up into Th1 or Th2 cells. Th1 cells secrete IL-2, IL-12, IFN-γ, TNF-α, *etc.*, which participate in cell-mediated immune response, that affects the intracellular pathogens (viruses, bacteria, parasites, *etc.*). And Th2 cells secrete IL-4, IL-5, IL-6, IL-10, IL-13, *etc.*, which stimulate the production of antibodies IgA, IgG, IgE, *etc.* which induce humoral immune response. Th1 or Th2 cells are mutually inhibited, Th1 cells secrete IL-2, IFN-γ can restrain the proliferation of Th2 cells, but Th2 cells secrete IL-4, IL-10 which could control the cytokine-production of Th1 cell. $CD8^+$ has an important effect on immunity due to the immunological inhibition of T cell, the activation of $CD8^+$ T needs $CD4^+$Th, IL-2 from Th1 secretion is the factor required for the proliferation of $CD8^+$T. Lymphocytes in the circulating blood come into a select lymphatic tissue, e.g. lymphocytes from lymph nodes

Table 2 T lymphocyte subsets in different situation of *H pylori* infection

H pylori	Samples	mean±SD			
		$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$
+	26	73.107±10.015	39.020±7.714	27.219±6.067	1.5134±0.520[a]
-	31	69.485±17.078	34.250±10.730	28.270±8.670	1.2490±0.409

Positive group compares with negative group: $CD4^+$T $P<0.05$ (39.020±7.714 *vs* 34.250±10.730). $CD4^+$T /$CD8^+$T [a]$P<0.05$ (1.5134±0.520 *vs* 1.2490±0.409).

come into the circulating blood, which usually go back to the lymph nodes, lymphocytes from gastrointestine associated lymphoid tissue (GALT) come into the circulating blood that go back to GALT after circulating in the body. Besides T lymphocytes are major particles that circulate in the blood[1], so we can examine T lymphocyte subsets in peripheral blood to evaluate the immune response of the body.

Some researches showed that there are high concentrations of IL-12, IFN-γ, TNF-α, *etc.* secreted from Th1 cells in children with *H pylori* infection, but IL-4, IL-10, *etc.* from Th2 cell secretions do not vary much[2-5]. The results showed that *H pylori* infection in children principally stimulates Th1 but not Th2 cell response.

There are no reports that relate to the variation of T lymphocytes in peripheral circulation in children with *H pylori* infection in China. Our study shows that there are no significant differences of T lymphocyte subsets in children of different nationalities; there is a significant difference of proportion of $CD4^+$T between *H pylori* infection (+) group and *H pylori* infection (-) group. The ratio of $CD4^+$T /$CD8^+$T also has a significant difference. Our results are the same as those of the reports of Krenska-Wiacek[6], but there is no significant difference of proportion in $CD3^+$T and $CD8^+$T between the two groups, these results also ascertained that *H pylori* infection in children principally stimulates Th1 cell response.

The immunity function of Th1 cells is the effect of the intracellular pathogens, cell-mediated immunity has no effect on *H pylori*, which is negative in normal gastric mucosa. After infection with *H pylori*, the antigens are treated with class II MHC of gastric mucosa, and then transferred to Th0 cell. Antigens of *H pylori* stimulate Th0 cell, which become active immune cells and stimulate Th1 to split up and activate. Th1 cells secrete IL-12, IFN-γ which can stimulate the leukocytes and macrophages continuously and these cells secrete IL-12, IFN-γ, TNF-α furthermore, Th0 cells go a step further splitting into Th1, but IL-12, IFN-γ inhibit Th2 from secreting IL-4, IL-10, that cause the gastric local mucosa to decrease secreting IgA, which is not enough to kill *H pylori*, which may be the reason for the prolonged infection of *H pylori* during a person's lifetime. In the study of Jiang[7], the author discovered that the progress of acute response to *H pylori* infection from children and adults is the same but there are different changes between gastric mucosa of children and adults, which may suggest that the immune response between children and adults are different. Our study shows that the increase in $CD3^+$T and $CD8^+$ T is not significant, the reason is unclear until now. Further studies are required to clarify the potential biological mechanism.

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