Mucosal IgA & IL-1 β in *Helicobacter pylori* Infection

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Abstract *Helicobacter pylori* infection stimulates strong local inflammatory and specific IgA antibody production. The influence of antibodies on the bacterial colonization is not clear. Here, we have analysed the association between the mucosal IgA level and IL-1 β in various manifestations of the infection seen endoscopically. Antral biopsies of 57 dyspeptic patients were taken for culture, histology and estimation of mucosal levels of anti-*H. pylori* IgA and IL-1 β . Mean mucosal IgA level was higher in patients with normal mucosa compared to all other groups and lower IgA level was associated with higher bacterial density. IL-1 β was higher in ulcer patients and suspicious malignancy group as compared to normal group and higher level of IL-1 β was associated with higher grades of metaplasia. Present study indicates that local immunity seems to have a protective role against *H. pylori* infection and higher level

of IL-1 β induced by the pathogen may be associated with metaplasia and carcinogenesis.

Keywords *H. pylori* · IgA · IL-1 β · Local immunity

Introduction

Helicobacter pylori is a spiral-shaped Gram-negative flagellate bacterium that colonizes the antral region of the human stomach after oral ingestion. The bacterium has infected over half of the world's population, although most infections are asymptomatic. In developing countries, the infection is prevalent among more than 90 % of adults who acquire it mostly in childhood and approximately 30 % of adults are infected in industrialized countries.

Although gastric environment is acidic and hostile to the growth of most bacteria, *H. pylori* can survive within the mucus layer secreting a large amount of the enzyme urease that breaks down urea into carbon dioxide and ammonia, the latter buffering its environment [1]. The infection once acquired generally persists for decades. Infection with the bacterium leads to a chronic active inflammation of the gastric epithelium. Persistent inflammation leads to ulceration and atrophy, a prelude to carcinogenesis. For any microbial infection to persist this long in host effective immune evasion strategies are a must. Following *H. pylori* acquisition, there is rapid host recognition in the form of both innate and acquired immune responses, including generation of specific local and systemic antibodies [2]. Immune activation following *H. pylori* infection is manifested by continuous epithelial cell cytokine signaling and gastric mucosal infiltration by neutrophils, macrophages, and lymphocytes. Gastric mucosa of infected patients has increased levels of pro-inflammatory cytokines such as

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interleukin (IL)-1, tumour necrosis factor alpha, IL-8, and IL-6 [3, 4]. IL-1 β is a potent proinflammatory cytokine which is involved in the host's response to many antigenic challenges and has biological effects that qualify it as arguably the most important cytokine in the gastrointestinal tract. Its proinflammatory properties contribute to the defense against pathogens, its antisecretory and cytoprotective effects contribute to the healing process following challenge to the integrity of the mucosa, and its acid inhibitory effects may have a profound effect on the natural history of *H. pylori* infection.

Both local and systemic antibody responses to the *Helicobacter* infection have been demonstrated that include IgA, IgM, and IgG isotypes [3, 5, 6]. *H. pylori* bacteria reside in the mucus layer covering the gastric epithelium. This ability to survive without tissue invasion in the luminal habitat confers the bacteria ability to evade host immune response. In such scenario the polymeric IgA which are secreted in lumen ought to play a major role in human immunological defense against the pathogen. In fact secretory IgA represents the most efficient and effective immune barrier against a number of pathogens, such as enteric bacterial pathogens which use intestinal mucosal surface to gain access to mammalian host. However, even though *H. pylori* infection stimulates strong local and systemic specific IgA and IgG antibody production, it is still controversial as to what influence antibodies may have on the bacterial colonization [7–9]. While earlier reports claimed eradication as well as protection from the infection by *H. felis* by active oral and passive IgA immunization [8, 10], another study claims that antibodies were not only dispensable for protection, but also impaired elimination of bacteria and the development of gastric inflammation [11].

Herein the present study we have estimated mucosal IgA in the various groups of dyspeptic patients, classified according to their endoscopic findings, in order to evaluate the association between mucosal IgA and *Helicobacter* infection. We also estimated IL-1 β in mucosa of above patients as a marker of inflammation by the infection. These findings were correlated with the histopathological findings of mucosal biopsy specimen. The goal of the study was to establish the relationship of local immunity and inflammation in various stages of this infection in the clinical settings.

Materials and Methods

Study Groups

A total of 57 dyspeptic patients undergoing endoscopy were recruited. Patients were grouped in four categories according to the endoscopic findings. These groups were

peptic ulcer (both gastric and duodenal), gastritis, suspicious cancer and normal mucosa. The number of subjects in each group was 16, 11, 4 and 26 respectively. During endoscopy, biopsies were taken for histology, *H. pylori* culture and measurement of mucosal levels of anti-*H. pylori* IgA and IL-1 β . Proper consent was obtained from each patient. The study was approved by the Ethical Committee of the Institute of Medical Sciences, BHU, India and the funds for the study were provided by the Department of Biochemistry, Institute of Medical Sciences, BHU, India.

Helicobacter pylori Culture

For culture, gastric biopsies were transported in 600 μ l of brain heart infusion agar containing 20 % glycerol. The tissue was homogenized and then plated over the culture media. The media used was Muller Hilton agar supplemented with 7–10 % defibrinated sheep blood, with antibiotics (bacitracin 2,500 IU/100 ml, cycloheximide 5 mg/100 ml, colistin sulphate 1,000 IU/100 ml, cephalosporin sulphate 1.5 mg/100 ml, and novobiocin 0.5 mg/100 ml dissolved in 50 % ethanol in distilled water) (Sigma St. Louis MO, USA). These plates were incubated at 37 °C in a candle jar for incubation. Several tiny colonies could be seen after overnight incubation.

Histology

The specimens for histopathology were fixed in 10 % neutral buffered formalin. Paraffin embedded specimen blocks were prepared and sections of 4 μ m thickness were prepared by conventional methods. These sections were subjected to routine H&E (Haematoxylin and Eosin stain) and Warthin–Starry stain for identification of bacilli. The sections were assessed by independent pathologist for the bacterial density and gastritis was scored as per Sydney criteria (chronic inflammation grading, activity grading and intestinal metaplasia).

Estimation of Mucosal Anti-*H. pylori* IgA and IL-1 β

Each biopsy specimen was weighed and placed in an eppendorf tube containing PBS. The volume of PBS in each tube was separately adjusted to 100 μ l/mg of tissue. The tissue were grounded using a glass tissue grinder over an ice pack. The homogenate was centrifuged at 2,000 $\times$ g for 10 min at 4 °C. The supernatant was removed and stored at –70 °C, until measurement of IL-1 β and IgA. The method used for the estimation of these analytes was ELISA.

The kit for the estimation of IgA was provided by Orion Diagnostica, Finland. The assay was done according to manufacturer's instructions. The A450 was measured using a multiscan plate reader, Power wave XS2 (Biotek, India). Antibody levels were calculated by plotting the absorbencies on the graph obtained by plotting absorbency of calibrator serum against their concentration.

For IL-1 β measurement, the ELISA kit was obtained from Pierce Biotechnology, Inc. The estimation was done as per instructions in the brochure of the kit. The level was calculated from the graph plotted by using serial dilution of calibrator serum and corresponding absorbance.

Statistical Analysis

Chi-square test was used for multiple group comparisons and the student's *t* test was done for two group comparisons. *P* < 0.05 was considered to be significant.

Results

An overall male preponderance (64.9 %) was seen in the study population. Most of patients were in the age group of 31–40 years (22.8 %) followed by age group of 41–50 years (19.3 %). The most common symptom was dyspepsia (40.4 %) followed by pain abdomen (33.3 %). Rapid urease test was positive in 24.6 % of patients while none was positive in control group.

Bacterial Culture

Bacterial culture was positive in overall 63.2 % samples. Peptic ulcer and suspected malignancy was 100 % positive. In cases of gastritis 54.5 % positivity was reported while 38.5 % cultures were positive in normal controls. On analysing the results, the culture positivity was statistically significant in Ulcer (*P* = 0.0001) and suspicious malignancy (*P* = 0.0365) group as compared to the group of patients with normal endoscopic findings.

Histopathological Examination

Bacterial Density Grading

Most of the normal controls had Grade 0 bacterial density while most cases of ulcer had Grade III bacterial density. In gastritis cases all four grades could be seen and but in malignancy mostly Grade I bacterial density was seen. Grade 0 means no characteristic bacteria, Grade 1 refers to occasional spiral bacteria found after searching, Grade 2 means numerous bacteria in most HPF and Grade 3 means scattered bacteria in most HPF/occas groups.

Chronic Inflammation Grading

Severe inflammation could be seen only in ulcer cases and in cases of gastric malignancy. Most cases (35.1 %) had moderate infiltration of plasma cells and lymphocytes. No signs of inflammation were seen in most of the normal controls.

Activity Grading

Activity is denoted by intensity of neutrophilic infiltrations. Grade 0 indicated no infiltration, Grade I is for mild infiltration i.e. involving <1/3 of pits and surface. Grade II denotes moderate infiltration involving 1/3–2/3 of pits and surface and Grade III denotes severe infiltration involving >2/3 of pits and surface. Normal controls had Grade 0 and Grade I while others had Grade I, II and III activity.

Intestinal Metaplasia

This denotes intestinal metaplasia of the foveolar or surface epithelium. This again is graded in four grades, Grade 0, I, II, & III. Grade 0 denotes no metaplastic changes seen, Grade I (mild)— <1/3rd of mucosa involved, Grade II (moderate)—1/3–2/3rd of mucosa involved, Grade III (marked)— >2/3rd of mucosa involved.

Metaplasia could not be seen in most of the cases. When detected, it is generally of mild grade (43.8 % in ulcer and 27.3 % in gastritis group). Severe changes could be seen in cases of suspicious malignancy.

Mucosal IgA

As can be seen from the Table 1, the mean level of the cases is lower than the control group. On statistically analysing the mean values mean IgA in gastritis was significantly lower as compared to endoscopically normal patients with *P* value of 0.029.

Mucosal Interleukin 1 β

On comparing the various study groups with the normal as depicted in Table 1, it can be seen that the mean level is

Table 1 Mucosal IL-1 β and IgA levels (mean $\pm$ SD) in various study groups

Study group	Mucosal IL-1 β level (pg/ml)	Mucosal IgA level (U/ml)
Normal	5.0915 $\pm$ 1.02	910.00 $\pm$ 879.745
Gastritis	4.0655 $\pm$ 1.57	358.18 $\pm$ 459.50
Ulcer	9.4581 $\pm$ 19.39	848.81 $\pm$ 840.732
Cancer	35.8025 $\pm$ 62.89	322.50 $\pm$ 290.244

Fig. 1 Mean mucosal IgA $\pm$ SE of mean level in various grades of *H. pylori* density

Fig. 2 Mean mucosal IL-1 β $\pm$ SE of mean level in various grades of *H. pylori* density

significantly higher (P value = 0.0049) in ulcer patients as well as suspicious malignancy group. The level was lower in gastritis group.

Helicobacter pylori Density Versus Mucosal IgA Level

One way analysis of variance shows a P value of 0.0186, which is significant (Fig. 1). Thus we see that as the IgA level falls, the bacterial load gradually increases and this rise is significant.

Metaplasia Grade Versus IL-1 β

On analysing the average interleukin-1 β levels in various grades of metaplasia by applying one way ANOVA, it was seen that mean IL-1 β level was significantly higher in Grade III cases (Fig. 2). ANOVA depicted a P value of 0.0041.

Discussion

The present study was designed with the aim to analyse the relationship of mucosal immunity and inflammation in various *Helicobacter* pathologies. Mucosal tissue IgA was employed to denote the immunity, while IL-1 β was the marker of inflammation in the study.

The mean IgA level of normal group was more than the cases. The difference was statistically significant only with the gastritis group, but the other groups did have a lower value. More over on analysing various grades of bacterial density in the HPE slides and their corresponding IgA level, it was evident that the bacterial density was significantly more in the biopsy specimen with low IgA level. Specific antibody responses against *Helicobacter* antigens after immunization have been documented in serum, saliva, intestinal secretion and in milk [12]. Both these results lead to the conclusion that mucosal IgA level might be protecting the mucosa by lowering the bacterial density. Studies have suggested that locally produced antibodies, particularly IgA [13, 14], might block *H. pylori* infection by preventing colonization and adhesion and allowing killing by neutrophils or monocytes through bacterial opsonisation [15, 16]. *Helicobacter*-specific IgA monoclonal antibodies administered directly into the gastric lumen conferred protection against *H. felis* infection [11, 17] and neutralized the toxic products of *Helicobacter pylori* [18]. The results of present study are in accordance with favourable role indicated in the above mentioned studies. But work of Akhiani et al. is contradictory. In two of their studies involving mouse model they not only refuted the protective role of IgA but went on to prove its role in helping colonisation by bacteria by dampening inflammation. Present study on human subjects with symptomatic pathology does not quiet agree with these results.

On analysis of tissue IL-1 β levels, mean level was higher in both ulcer and malignancy group, though the difference was statistically significant in malignancy group only. On evaluating its relation with metaplasia grading, it was seen that mean tissue IL-1 β was significantly higher in Grade III metaplasia. In general, the inflammation induced by *H. pylori* infection is a chronic active gastritis, which means that both lymphocytes and neutrophils infiltrate the mucosa in a characteristic manner. In addition, apart from the inflammatory infiltrate, foci of intestinal metaplasia with resulting atrophy, lymphatic aggregates and lymphoid follicles occur, and the foveolar epithelium is replaced by regenerative epithelium with correspondingly reduced mucus secretion [19]. Nearly all microbes/microbial products and many non-microbial agents stimulate transcription and synthesis of IL-1 β . The proinflammatory cytokine, IL-1 β is expressed in the gastric mucosa infected with *H. pylori*. It is also a powerful inhibitor of gastric acid

secretion, being 100-fold more potent than proton pump inhibitors and 6,000-fold more potent than H₂ antagonists on a molar basis [20]. It is thought that IL-1 β produced locally in the gastric mucosa is one of the mediators of the inhibitory effects of *H. pylori*-induced inflammation on gastric acid secretion [21]. Increased IL-1 β levels would theoretically result in enhanced suppression of gastric acid secretion allowing more rapid development of gastric atrophy and an increase in the risk of developing gastric cancer. The higher IL-1 β in Grade III metaplasia and in malignancy patients seen in present study supports these facts.

Conclusion

With the help of the above observations, it seems that mucosal IgA has a protective role in *H. pylori* infection and if the bacteria is able to establish itself, it leads to inflammatory changes in the mucosa. The degree of inflammation is proportional to the bacterial load and that decides the clinical presentation of *H. pylori* infection. Increased level of IL-1 β by the *H. pylori* infection may be associated with the metaplastic changes and malignancy.

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Conflict of interest The authors who participated in this study have no potential conflict of interest.

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