

Interleukin-8 (IL-8) Expression Due to *Helicobacter pylori* In A Tissue Culture Model

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Abstract

Helicobacter pylori is the bacterium that causes inflammation and ulcers within the stomach. It is a spiral shaped bacterium found in the gastric mucous layer or adherent to the epithelial lining of the stomach. *H. pylori* has been identified in the past to be the leading cause of chronic gastritis and peptic ulcer disease in humans. Gastritis is the irritation and inflammation of the lining of the stomach. It is hypothesized that cytokine release (interleukin-8) due to *H. pylori* infection causes a release of reactive oxygen species during the inflammatory reaction. Interleukin-8 plays a role in the mucosal inflammation caused by *H. pylori* infection. A gastric epithelial cell line MKN-45 was used to evaluate IL-8 production in response to the *H. pylori* infection. Cells were grown in a 12 well plastic plate in RPMI media. We used 10^6 MKN-45 cells per ml, 10^8 cells per mL of *H. pylori*, and 10ng/10uL solution of TNF- α in the experimental well, while control wells remained uninfected with *H. pylori*. Culture supernatants were collected from each well at 6h, 12h, and 24h. IL-8 was measured in the culture supernatants by Quantikine Human CXCL8/IL-8 Immunoassay kit (R&D Systems). The data show that at the 6h time point there was no significant difference in the production of IL-8 between cells infected with *H.pylori* and cells uninfected with *H. pylori*. However, at the 12 and 24h time points the data did show a significant difference in production of IL-8 between cells infected with *H. pylori* and uninfected cells. We found a 13% increase in IL-8 production at the 12h time point between the infected cells and uninfected cells and a 30% increase at the 24h time point between the infected cells and uninfected cells. It was concluded that the MKN-45 cell line is suitable to study the effects of co-culture with *H. pylori* on IL-8 production.

Introduction

Helicobacter pylori is a bacterium recognized as the cause of chronic gastritis and 90% of all peptic ulcer disease in humans (1). This bacterium resides in the mucous layer of the stomach adherent to the epithelial lining of the stomach (4). It has been estimated that 50% of the world has been infected with the *H. pylori* bacteria (1). This spiral shaped bacterium has been shown to cause inflammation and ulcers within the stomach (3).

Current therapy for those infected with *H. pylori*, is with antibiotics which include omeprazole, amoxicillin, clarithromycin (OAC) for 10 days, bismuth subsalicylate, metronidazole, and tetracycline (BMT) for 14 days, or lansoprazole, amoxicillin, and clarithromycin (LAC) for 10 to 14 days (1). The success rates of these treatments are decreasing due to resistance to antibiotics (2).

It is hypothesized that IL-8 plays a role in the mucosal inflammation caused by *H. pylori* infection. Interleukin is a type of cytokine (6). Cytokines are small secreted proteins which intercede and regulate immunity and inflammation (6). Urease which is produced by *H. pylori* is known to induce epithelial cells to produce inflammatory cytokines, which then stimulate a migration of inflammatory cells including PMNs, lymphocytes, and macrophages (5). Following this the cells release reactive oxygen species (ROSs) which, using its enzyme catalase, *H. pylori* can survive, but damage to the epithelial lining of the stomach may be done by the ROSs (5). Previous studies have concluded that the damage to the stomach is not directly caused by *H. pylori*, but by the inflammatory cells that produce ROSs (5). Antimicrobial therapy for the infection is associated with a decrease in the amount of inflammatory cells. Antioxidant treatment may assist in preventing damage to the epithelial layer by reducing the amount of ROS

present (5). In this report a gastric epithelial cell line MKN-45 was used to examine its production of IL-8 *in vitro* in response to *H. pylori* infection.

Materials and Methods

Bacterial Strain and Cell line

Helicobacter pylori strain ATCC 49503 was obtained from American Type Culture Collection (Rockville, MD). Human gastric adenocarcinoma cells, MKN-45 cell line, obtained from DSMZ (Braunschweig, Germany) were used for this experiment.

RPMI 1640 media obtained from (MP Biomedical, Solon, Ohio) was used as nutrients for the MKN-45 cells. The *H. pylori* was grown on fresh blood agar plates obtained from (BBL, Becton, Dickinson, MD).

IL-8 levels were determined using Quantikine Human CXCL8/IL-8 Immunoassay kit (R&D Systems, Minneapolis, MN).

All other chemical unless otherwise stated, were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and were of analytical grade.

Cell culture

Blood agar plates were used to grow *H. pylori*. Bacterial plates were incubated at 37° C in an incubator. A seventy-five ml tissue culture flask was used to grow the MKN-45 cells in. Thirty ml of RPMI media was added to the flask as nutrient for the cells. The flask was then incubated at 37° C, 5% CO₂. Cells were removed by treating the flask with 0.05% trypsin-EDTA. Cells were counted using a haemocytometer and 10⁶ cells were seeded in each well of a 12-well plate.

12 well plates were set up as diagrammed in Figure 1a:

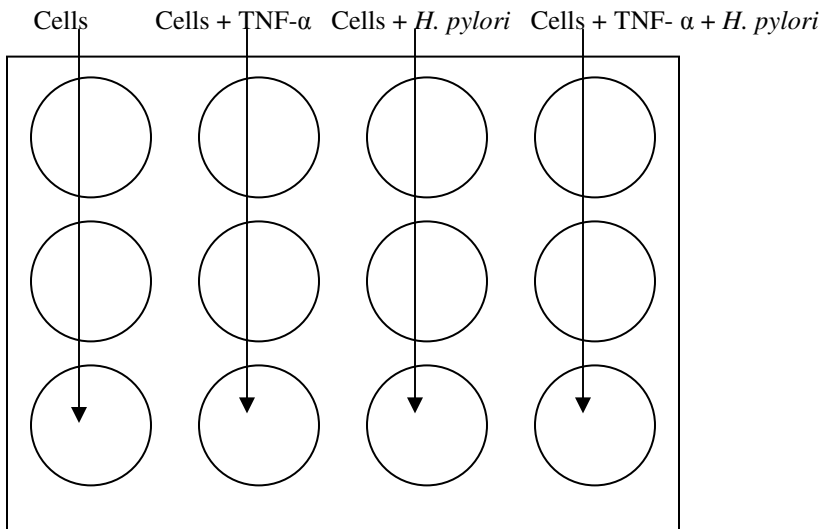


Figure 1a: Preparation of supernatants for IL-8 assays.

Collection of culture supernatants

For the IL-8 assay, 12 well plates were used in which six were control wells with (a) MKN-45 cells alone and (b) MKN-45 cells and TNF- α . Six experimental wells were used containing (c) MKN-45 cells and *H. pylori* and (d) MKN-45 cells, TNF- α and *H. pylori*.

Ten ng of TNF- α solution (to stimulate IL-8 production) was added to wells (b) and (d) and *H.pylori* in the concentration of 10^8 cells per mL were added to wells (c) and (d).

Samples of culture supernatants were collected at 6h, 12h and 24h time points and frozen at -20° C until the assay was performed.

IL-8 Assay

500ml of wash buffer and 100ml of calibrator diluent RD5P was made to perform the IL-8 assay. Standard solutions were taken from the range of 2000pg/mL to 31.2pg/mL. One hundred micro liters of assay diluent RD1-85 (a buffered protein base) was added to each

well. Fifty uL of the standard, control and the samples of culture supernatants collected were then added to the appropriate wells. The wells were washed with 400uL of wash buffer four times and then blocked with 100uL of IL-8 conjugate (polyclonal antibody against IL-8 conjugated to horseradish peroxidase) for one hour at room temperature. The wells were washed again with the wash buffer four times to remove excess unbound IL-8 conjugate. The substrate solution (Color reagents A and B) was made and 200uL was added to each well and then incubated for thirty minutes while protected from light. Fifty uL of stop solution was then added. The plates were placed in an optical density reader to determine the optical density of each well set to 450nm and then 540 nm. A standard curve was plotted and the concentration of IL-8 in the samples was calculated.

Results

This experiment was performed in order to better understand cytokine production (IL-8) from human gastric adenocarcinoma cell line MKN-45 when co-cultured with *H. pylori*. In Figures 2, 3, and 4, results from the IL-8 assay are shown. In the data collected at the 6h time point there was no significant difference in the production of IL-8 between cells infected with *H. pylori* and uninfected cells. However, at 12h and 24h the data showed a significant difference in IL-8 production between cells infected with *H. pylori* and uninfected cells. At the 12h time point we found a 13% increase in IL-8 production in the infected cells compared to the uninfected cells and a 30% increase at 24h in the infected cells compared to uninfected cells.

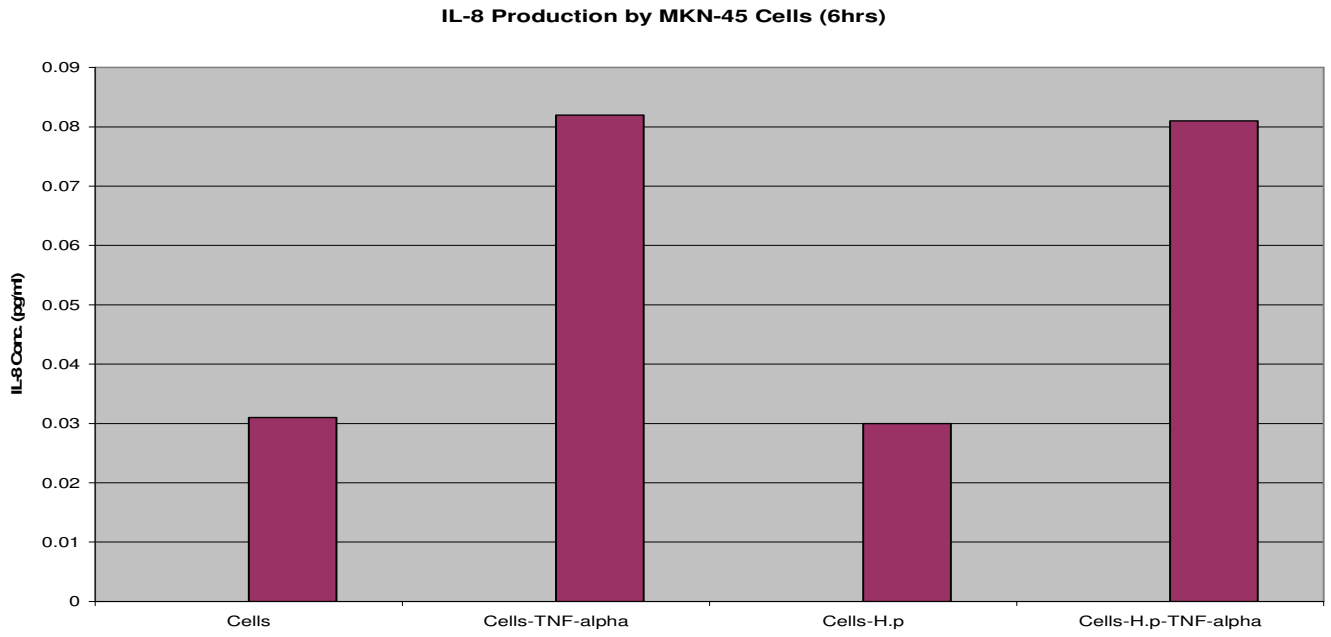


Figure 2: Production of IL-8 when stimulated by *H. pylori* at 6h.

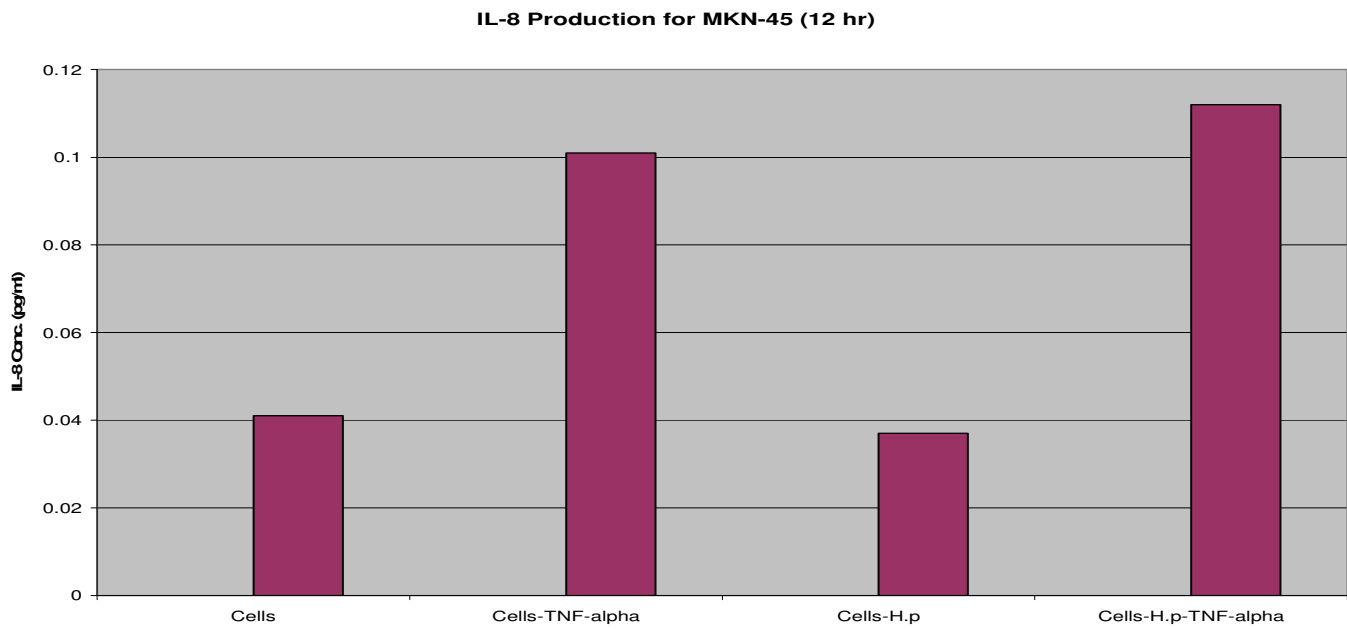


Figure 3: Production of IL-8 when stimulated by *H. pylori* at 12h.

IL-8 Production for MKN-45 (24hr)

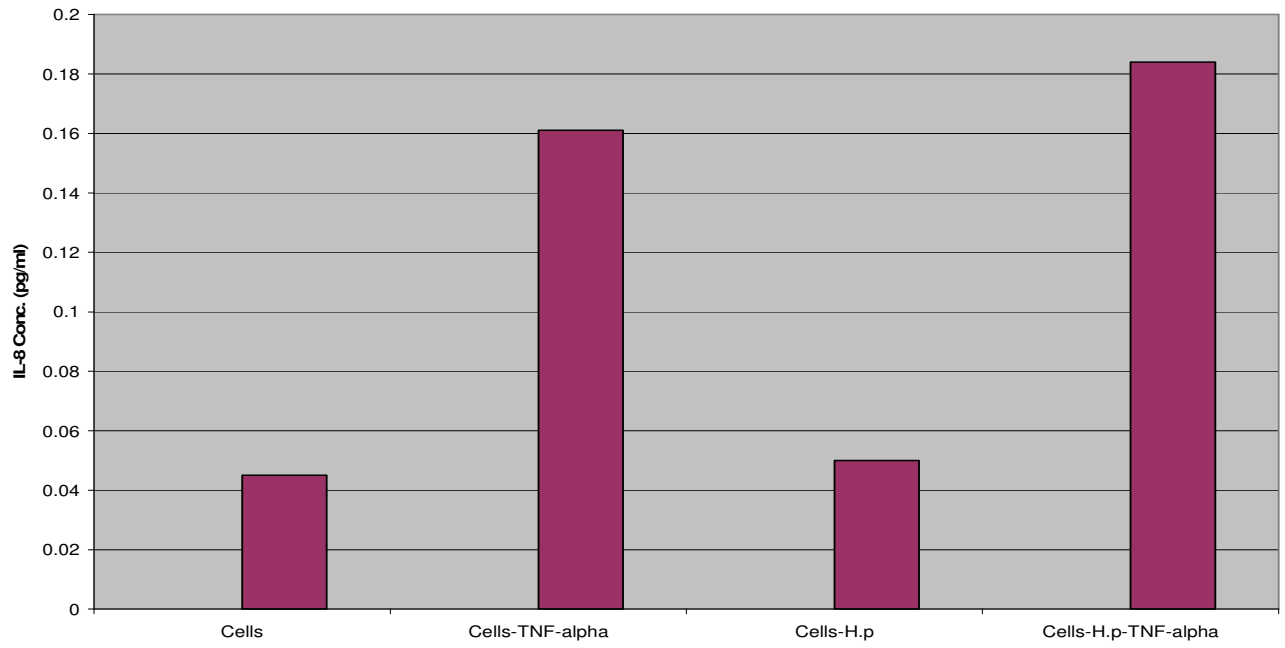


Figure 4: Production of IL-8 when stimulated by *H. pylori* at 24h.

Discussion

Our results indicate that MKN-45 cell line is appropriate to study the effects of co-culture with *H. pylori* on IL-8 production. The production of IL-8 by the MKN-45 cells is significantly greater in *H. pylori* exposed cells compared to non-exposed cells at 12 h and 24 h post-infection.

In previous reports, multiple human cell lines have been demonstrated to produce an increased amount of IL-8 when co-cultured with *H. pylori* (5). Among them, KATO III, AGS, NCI-87N, SNU-5, HAC 1739, MKN28, HEp-2, and Int407 have been shown to have increased production of IL-8 (5). In addition, research has shown that IL-8 secretion in human cell lines is influenced by factors other than the *H. pylori* cytotoxin (5). In contrast, previous research has shown that IL-8 production is not influenced by some products of *H. pylori*, such as catalase (5). In this study, we found the MKN-45 cells to confirm the results of previous studies in their increased production of IL-8 in response to *H. pylori* infection.

Limitations to this study include its *in vitro* nature. In the future, animal and human studies should be performed to confirm or deny results obtained from this study.

Conclusion

Due to the information obtained from the study, it is concluded that the MKN-45 cell line is suitable to study the effects of co-culture with *H. pylori* on IL-8 production.

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