ANALYSIS OF SIGNAL TRANSDUCTION PATHWAY
IN H. PYLORI - MEDIATED GASTRIC CARCINOGENESIS

7.1 INTRODUCTION

H. pylori infection has been recognized as a major risk factor for the development of gastric cancer, but the exact signal transduction mechanism by which it produces the change is yet to be elucidated.

7.1.1 Signal transduction pathways of pathogenesis

Overexpression of several genes implicated in the mitogen-activated protein kinase signaling cascade (mitogen-activated protein kinase, MEK-MAP kinase, Raf-Ras) seems to be most likely responsible for initiated cells acquiring a proliferating phenotype, which facilitates the accumulation of structural changes in additional genes resulting in the generation of autonomously growing preneoplastic and neoplastic lesions (Lewis et al., 1998). The highly conserved family of ras genes in the MAP kinase cascade had been detected as transforming genes in a wide variety of naturally occurring tumors (Kim et al., 2000) and in in vivo and in vitro experimental models after carcinogenic insult (Seger and Krebs, 1995). Although multiple ras effector pathways have been identified, the ras protein kinases which lie downstream of ras were believed to be the primary
mitogenic effectors (Manni et al., 1997) and also the raf proteins were considered as upregulators of the mitogen activated protein kinases (MAPK/ERK) (Salh et al., 1999). The constitutive upregulation of this pathway by oncogenic ras was thought to promote cellular transformation (Shibata et al., 1998) of protein kinase C, a ubiquitous family of eleven related isoforms which are another group of signal transducing molecules deeply implicated in carcinogenesis (Kampfer et al., 1998). The various PKC isoforms are classified into three major sub groups: the classical or conventional PKC isotypes (cPKCs) are Ca\(^{2+}\)-and diacyl glycerol (DAG)-dependent and consist of the isozenzymes cPKC-\(\alpha\), cPKC-\(\beta_1\), cPKC\(\beta_2\) and cPKC-\(\gamma\), novel PKCs (nPKCs) are Ca\(^{2+}\)-independent but DAG-responsive and comprise the isozenzymes nPKC\(\delta\), nPKC-\(\epsilon\), nPKC-\(\eta\) and nPKC-\(\theta\), the PKC isozenzymes aPKC-\(\lambda/1\) and aPKC-\(\zeta\) require neither Ca\(^{2+}\) nor DAG for activation and have been classified as atypical PKC isoforms (aPKCs) (Nishizuka, 1992, 1995; Genot et al., 1995).

The PKC isoforms cPKC-\(\alpha\) (Dean et al., 1996), cPKC-\(\beta_2\) (Sauma and Friedman, 1996; Sauma et al., 1996) and nPKC-\(\epsilon\) (Perletti et al., 1996) have been correlated to Ha-Ras-Mediated signaling transformation. As all of these PKC isoforms are not expressed in all cellular systems, cell type-specific differences are to be expected.

Mitogen activated protein kinases (MAPKs) are serine/threonine kinases activated in response to a variety of internal signals. Three major sub classes of MAPKs, namely, extracellular signal-regulated kinase (ERK),
c-Jun NH$_2$-terminal kinase (JNK) and p38 have been identified (Whitmarsh and Davis 1999; Segar and Krebs, 1995). Various receptor tyrosine kinases, cytokine receptors, G proteins and oncogene products activate MAPKs through phosphorylation by mitogen activated protein kinase or ERK-activated protein kinase (MEK) (Zhang et al., 1999; Lenormand, et al., 1993 Tibbles and Woodgett, 1999). Thus, MAPKs are proposed to be critical integrators of various signal transduction systems and are involved in various cellular processes including cell proliferation, differentiation, apoptosis and transformation. Constitutive activation of these signaling cascades has been noted in the malignant transformation of various cell lines, (Manni, et al., 1997; Mansour et al., 1994) and implicated in carcinogenesis and the metastatic potential of human cancers (Salh et al.,1999; Ito et al.,1998;Leav et al 1996).

7.1.2 Signal cascade alterations in H. pylori mediated pathogenesis

Colonisation of the human gastric mucosa by H.pylori induces various diseases, such as atrophic gastritis, peptic ulcer diseases and gastric adenocarcinoma (Maeda et al., 2000; NIH Consensus Developmental Panel 1994). It has been recently demonstrated that H.pylori affects intracellular signal transduction in host cells, leading to the activation of transcriptional factors (Maeda, et al., 2000; Meyer-Ter-Vehn, et al., 2000) and the induction of proinflammatory cytokines (Crabtree et al., 1994; Ogura et al., 1998) H.pylori can activate the three main MAPK signaling pathway (Keates et al., 1999). Thus there is little known about these signal cascade
and its expressions in *H. pylori* mediated gastric carcinogenesis from normal gastric mucosa to malignant carcinoma.

### 7.2 WORKING HYPOTHESIS

In previous chapters, the expression profile of cytokine TNF-α, cell adhesion molecule CD44 isforms and c-H-ras p21 oncogene were examined. To gain further understanding of expression of signal cascades in the pathogenesis induced by *H. pylori* in gastric cancer, in the present study, specific monoclonal antibodies were used to analyse the expression of specific PKC isoform family, MAPK p38, ERK1/2 and protein tyrosine phosphorylation pattern in the sequential evolution from normal mucosa to chronic gastritis, atrophy, intestinal metaplasia and gastric adenocarcinoma. These results will help in identifying drug targets in early stages of the diseases.

### 7.3 STUDY GROUP

The present study was designed to analyse the signal transduction cascade induced by *H. pylori* infection, and to analyse its role in the sequential evolution of gastric cancer by using Immuno blotting. The study population was grouped as follows

- **Group I** - Normal age matched control (4)
- **Group II** - Chronic gastritis (4)
- **Group III** - Atrophic gastritis (4)
- **Group IV** - Intestinal metaplasia (4)
Group V - Adeno carcinoma (4)

Only *H. pylori* positive patients were included for the study. The normal controls were negative for *H. pylori*.

7.4 METHODOLOGY

The histopathological grading was done as mentioned in previous chapters.

7.4.1 Immunoblotting

The tissue lysate (50 μg) was fractionated on a 10% SDS-PAGE and the proteins were electroblotted on to a nitrocellulose membrane. Membranes were probed overnight at 4°C in primary antibody (antibody details given in the Table 7.1) after one hour blocking with 5% skimmed milk in TBS. The membrane was washed thrice in TBST for 5 minutes and incubated with horse radish peroxidase-conjugated secondary antibody for 1 hr. After 3 final washes in TBST, the immune complexes were visualized using DAB substrate. (See Appendix for detailed protocol).]
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Target</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>Anti phosphotyrosine mouse monoclonal antibody</td>
<td>Phosphotyrosine</td>
<td>Cell signaling (Labmate)</td>
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<tr>
<td>Anti PKC rabbit polyclonal antibody</td>
<td>All PKC isoforms</td>
<td>Oncogene Research products USA</td>
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<td>Anti PKC γ mouse</td>
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</table>
7.5 RESULTS

We have analysed the classical PKC family kinases PKC & PKC β and PKC γ P38 MAPK and ERK 1/2 expression along with tyrosine phosphorylation pattern of *H. pylori* infected tissues of different histopathological stages of gastric carcinogenesis.

When the pattern of tyrosine phosphorylation of various histopathological grades analysed in samples infected with *H. pylori* distinct difference in phosphorylation pattern was observed between normal and diseased conditions, and an increase in the number of tyrosine phosphorylated protein bands in infected stages than normal (Plate 7.1) was observed.

Among the 4 normal subjects analysed for PKC α, three were negative and only one was positive. One case of normal showed positive expression for PKC γ and the remaining cases were negative for PKCγ. All the normal cases were not expressing PKC β, whereas the chronic gastritis cases with *H. pylori* infection were found to express PKC β. Only one case showed negativity for PKC α, PKC γ, ERK 1/2 and P38 MAPK and all other cases were expressing all the proteins (plate 7.1a, b, c and e).

In case of atrophy, intestinal metaplasia and adenocarcinoma, all the cases were positive for all the signal cascade proteins. Fig.7.1.
A. Western blotting analysis of Tyrosine phosphorylation

A. Western blotting analysis of Protein Kinase C α expression

B. Western blotting analysis of Protein Kinase C β expression

C. Western blotting analysis of Protein Kinase C γ expression

D. Western blotting analysis of p38 MAPK expression

E. Western blotting analysis of ERK1/2 expression

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<th>Lane</th>
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<tr>
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<td>2</td>
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<td>Gastric Atrophy</td>
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<td>6</td>
<td>Intestinal Metaplasia and Adenocarcinoma</td>
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<td>Adenocarcinoma</td>
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</table>
1. Normal Stomach  
2. Chronic Gastritis  
3. Atrophic Gastritis  
4. Intestinal Metaplasia  
5. Adeno carcinoma
### Table 7.2 Expression of Signal cascade proteins in *H. pylori* infected gastric tissues of different histopathological stages

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<th>Study Population</th>
<th>PKC-α</th>
<th>PKC-β</th>
<th>PKC-γ</th>
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N = Normal,  
CG = Chronic Gastritis, AG = Atrophic Gastritis, IM = Intestinal Metaplasia, AC = Adeno Carcinoma
Table 7.2 Expression of Signal cascade proteins in *H. pylori* infected gastric tissues of different histopathological stages

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<th>P38 MAPK</th>
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N = Normal, CG = Chronic Gastritis, AG = Atrophic Gastritis, IM = Intestinal Metaplasia, AC = Adeno Carcinoma
7.6 DISCUSSION

Signal cascade involves protein kinase, a key molecule in intracellular signal transducing pathways that transport extracellular stimuli from cell surface to nuclei. MAPK pathway has been revealed to be involved in the physiological proliferation of mammalian cells and also to potentiate them to transform (Sauma and Friedman, 1996; Whitmarsh et al., 1999; Segar and Krebs, 1995). With regard to human gastric carcinogenesis, investigation of alterations in expression and activity, the classical PKC family and MAPK cascade will help to understand the mechanism of carcinogenesis.

It is now known that attachment of *H. pylori* to gastric epithelial cells activate multiple signaling pathways that culminate in IL-8 gene transcription. Previous studies have shown that *H. pylori* is able to induce activation of the transcription factors NF-kB and AP-1, key regulators of many inflammatory genes including IL-8 (Naumann, et al., 1999). Keates et al., (1999) have recently reported that infection of gastric epithelial cell lines with *H. pylori* results in the rapid activation of p38, JNK, and ERK1/2 mitogen-activated protein (MAP) kinases. ERK 1/2 pathway has been linked to cellular proliferation and differentiation (Schlessinger, 2000).
Figure 7.1 Expression of Signal cascade proteins in \textit{H.pylori} infected gastric tissues of different histopathological stages

- PKC-ALPHA
- PKC BETA
- ERK1/2
- P38 MAPK
- PKC-GAMMA

<table>
<thead>
<tr>
<th></th>
<th>Normal n=4</th>
<th>Gastritis n=4</th>
<th>Atrophy n=4</th>
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<td>Gastritis</td>
<td>Atrophy</td>
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<td>Adeno carcinoma</td>
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In the present study, the expression of p38 and ERK 1/2 only in \( H.\text{pylori} \) infected premalignant and malignant stages were observed. Normal tissues did not express these proteins (Plate 7.2). The activity of ERK 1/2 are generally regulated through the activation of cell surface receptors. Numerous stimuli are known to cause activation of ERK 1/2 including \( H.\text{pylori} \) Cag A\(^+\), EGFR transactivation etc., (Hirata et al., 2001 ; Keates et al., 2001). Recently Hirata et al., (2001) reported that \( H.\text{pylori} \) infection induces cell proliferation by stimulating the expression of cyclin D, through MAP Kinase pathway. Liang et al., (2005) have reported the increased expression of ERK1/2 and P38 in the gastric cancer tumorigenesis and metastasis. This result suggests that ERK1/2 and MAPK signal cascades may play a central role in the \( H.\text{pylori} \) mediated gastric carcinogenesis.

PKC family consists of several isoforms comprising three groups classical, novel and atypical and PKC isoforms are widely distributed in mammalian tissues and have many important physiological functions. PKC subfamily shows significant specificity in tissues distribution. The isoenzymes possess distinct differences in localisation in different cells. PKC isoforms are often overexpressed in disease states such as cancer and play a critical role in regulation of long term cellular events such as proliferation, differentiation and tumorigenesis. An increase in their activities might result in oncogenesis.
An implication of PKC isozymes in the regulation of MAP kinase pathway has been demonstrated in a variety of systems ranging from yeast to higher eukaryotes (Marshall, 1995; Morrison et al., 1996; Liao et al., 1997). In a cell-free system the activation of MAP kinase by a PKC, Raf and MEK-dependent mechanism has been demonstrated (Marquerdt et al., 1994).

In this current study an increase in the expression of cPKC family isoforms especially PKC-β isoform was observed from *H. pylori* infected chronic gastritis to adenocarcinoma. These results suggest that cPKC may be important mediators in *H. pylori* pathogenesis.
EFFECT OF CD44 MONOCLONAL ANTIBODY PRETREATMENT ON *H. PYLORI* ADHESION AND APOPTOSIS IN HEP2 CELLS

8.1 INTRODUCTION

The exposure of epithelial cells to *H. pylori* results in the activation of multiple factors, including NF κB (Munzenmaier, *et al.*, 1997), cytokines, (Sharma *et al.*, 1995), activator protein 1 (AP-1) and mitogen activated protein kinases (Naumann *et al.*, 1999). However, the exact mechanism by which *H. pylori* causes chronic inflammation and gastric pathology remains to be elucidated. In *H. pylori* induced chronic gastritis cells, mucosa increases in size due to epithelial proliferation and this is the striking histological feature (Fan *et al.*, 1996). Epithelial proliferation does not seem to be counter balanced by epithelial necrosis in *H. pylori* induced gastritis (Robert *et al.*, 1993), suggesting that apoptosis may account for the apparent cell loss in chronic gastritis and it can be hypothesized that increased epithelial proliferation may be accompanied by epithelial apoptosis to maintain cellular homeostasis. Recent evidences suggest that the alteration in epithelial cell growth in *Helicobacter pylori*-colonised mucosa is dependent on specific host factors than bacterial factors. Nevertheless, the role of the host factors in the
The pathogenesis of *Helicobacter pylori* associated disease has been largely ignored.

CD44 is one of the highly conserved molecules in mammals (Lesley and Hyman, 1998), mediating adhesion between epithelial cells which was originally described as a glycoprotein surface marker of T lymphocytes. The hematopoietic form of CD44 (70 to 90kDa) in lymphoid cells is the standard unit of CD44 protein. Other isoforms were created by alternative splicing of the mRNA (Underhill, 1992). The reasons for the existence of so many CD44 isoforms are not known. Different isoforms may bind to different ligands (Sunsy et al., 1997). The varied structure and distribution of CD44 isoforms suggest that, the molecule has a variety of functions like cellular adhesion, hyaluronate degradation, lymphocyte activation, lymphnode homing, myelopoiesis and lymphopoiesis, angiogenesis and release of cytokines (Sneath and Manghen, 1998). Although these studies reveal a role for CD44 during inflammatory response, little is known about its *in vivo* role during infections with pathogenic microorganism. In this study we have decided to analyse the effect of CD44 monoclonal antibody on *H.pylori* adherence, cytoskeletal rearrangement and apoptosis. The adherence and apoptosis decreased dramatically when CD44 was blocked by antibody pretreatment.

Previously the overexpression of CD44 isoforms was analysed. In this study the human gastric mucosa was analysed to understand the exact role of CD44.
8.2 MATERIAL AND METHODS

8.2.1 Bacterial strains and growth conditions

Two previously characterized clinical isolates of *H. pylori* strains were provided by Dr. Archana Ayyagari, Professor, Department of Microbiology SPGIMS, Lucknow. The isolates were spiral shape, positive for catalase, oxidase, urease and CagA and VagA gene. Stock cultures were maintained in defatted milk at -80°C.

8.2.2 Cells

The Hep-2 cells were maintained in DMEM supplemented with 10% fetal calf serum, 200IU/mL penicillin and streptomycin at 37°C in 5% CO₂-95% air and recultivated once or twice a week.

8.2.3 Immunofluorescence analysis of CD44 expression in Hep-2 cells

Hep-2 cells were cultured on 12mm glass cover slips until semi confluence. Monolayers were analysed for CD44 expression and distribution after being exposed to *H. pylori* at specific time intervals as reported previously (Harry *et al.*, 1998). The cells were fixed for 20 min at room temperature in 4% paraformaldehyde in PBS, then blocked and detergent extracted for 30 mins in isotonic PBS containing 3%(V/V) normal goatserum, 0.02 (W/V) saponin and 0.02% (W/V) NaN3. Primary antibodies were diluted as specified above in the blocking buffer and added to the samples and incubated at 4°C for
4hrs in a moist chamber. After rinsing in PBS, secondary antibody (dilution 1:250) was added and incubated for 1hr. The epifluorescence was observed under Axioscope-II using appropriate filters.

8.2.4 Assay of *H. pylori* adherence to Hep-2 cells and inhibition by CD44 mAb

To assay bacterial adherence, Hep-2 cells were grown to confluence on cover slips in culture flask and the suspension of *H. pylori* (10⁸ cfu/mL) were added to a total volume of 0.5 mL for Hep-2 cultures and allowed to adhere for 4, 8, and 16 hrs at 37°C in 5% CO₂-95% air. The cover slips were washed and stained with modified Grams staining and both the amount of cells adhered by bacteria and bacteria adhering cells were counted among one hundred cells under the light microscope as described previously (Fei et al., 2001). Similarly the assay was repeated for the cells pretreated with 1μg/mL antiCD44 monoclonal antibody (Oncogene Research Products USA). The assay was repeated three times.

8.2.5 Detection of apoptosis in infected cells and antibody pretreated cells.

The apoptosis of the cells was assessed by COMET assay (single cell gel electrophoresis) The *H. pylori* infected and antibody pretreated cells were scraped using glass scrapers after specified time intervals. The COMET assay was performed according to the procedure of Singh et al., (1988) with
few modifications. Briefly, 120 µl of 0.5% normal melting point agarose in 
Ca\(^{+2}\) and Mg\(^{+2}\)-free phosphate buffer at 56°C were quickly layered onto a 
fully frosted slide and immediately covered with a cover-slip. The slides 
were kept at 4°C to allow the agarose to solidify. After gently removing the 
cover-slip, a 50 µl aliquot of cell suspension was mixed with an equal 
volume of 1% low melting point agarose (Sigma, St. Louis, MO) at 37°C 
and quickly pipetted onto the first agarose layer in the same manner. Finally, 
70 µl of 0.5% LMP agarose was added to cover the cell layer. The slide 
sandwiches without cover-slips were immersed in freshly prepared, cold 
lysing buffer [2.5 mol/l NaCl, 100 mmol/l Na\(_2\)EDTA, 10 mmol/l Tris, 1% \(N\)-Lauroyl sarcosine sodium salt, pH 10, with 1% Triton X-100 added just 
before use] and kept at 4°C for 45 min to 1 hour.

The slides were placed on a horizontal gel electrophoresis 
platform and covered with cold alkaline buffer [300 mmol/L NaOH, and 1 
mmol/L Na\(_2\)EDTA] for 8 to 20 minutes in the dark at 4°C to allow DNA 
unwinding and the expression of the alkali-labile sites. Electrophoresis was 
conducted at 4°C in the dark for 20 min at 25 V and 300 mA. The slides 
were then rinsed gently twice with neutralizing buffer (0.4 mol/l Tris, pH 
7.5). Each slide was stained with 120 µl of propidium iodide (Sigma) at a 
concentration of 5 µg/ml and covered with a cover slip. DNA fragmentation 
and apoptosis were assessed per 100 cells for each stage and the experiment 
was repeated three times.
8.3 RESULTS

8.3.1 Co-culture of Hep-2 cells with *H.pylori* up regulates CD44 expression.

Uninfected cells showed the sub cellular distribution of CD44 with slight increase at lateral cell-cell junctions and at cell protrusions, but with the reactivity visible over the entire surfaces of most cells. Immunoflourescent staining of infected cells revealed dramatic concentrations of CD44 associated with adherent microcolonies of *H.pylori* (Plate 8.1). CD44 accumulations were observed 4hr after infection and by 8hrs after infection more than 80% of adherent microcolonies of *H.pylori* were associated with CD44 accumulation (Plate 8.1b and c).

8.3.2 CD44mAb pretreatment blocked *H.pylori* adherence to Hep-2 cells

The pattern of fluorescence of cultured Hep-2 cells indicated maximal expression of CD44 at the periphery of the cells, near intercellular junctions between adjacent cells and on the filopodia of nonconfluent cells. This distribution pattern of CD44 as evidenced by immunoflourescence was very similar to the pattern of binding observed by direct microscopy of *H.pylori* bound to Hep-2 cells. To determine whether CD44 was a receptor for *H.pylori*, we used CD44 monoclonal antibody to block the attachment of *H.pylori* to Hep-2 cells. Preincubation of Hep-2 cells with the anti CD44 mAb reduced the attachment of the *H.pylori* to Hep-2 cells by more than
PLATE 8.1

Immunofluorescence analysis of CD44 expression on co-cultured cells

A. Normal Cells without treatment (20 x magnification)

B. Cells infected for 4 hours with *H.pylori* after (40 x magnification)

C. Cells infected for 8 hours with *H.pylori* after 8 hr (40 x magnification)

D. Cells infected for 12 hours with *H.pylori* after 12 hr and (40 x magnification)

E. Cells infected for 16 hours with *H.pylori* after 16 hr (40 x magnification)

F. Anti CD44 antibody pretreated cells infected with *H.pylori* for 16 hr (40 x magnification)
PLATE-8.1
Immunofluorescent Analysis of CD44 in Hep-2 Cells Co-cultured with *Helicobacter pylori*
PLATE 8.2

Adherence analysis of H.pylori with Hep-2 cells

(A) Normal Cells without infection (20 x magnification)

(B) Normal Cells without infection (100 x magnification)

(C) Cells infected with H.pylori for 8 hr (20 x magnification)

(D) Cells infected with H.pylori for 8 hr (100 x magnification)

(E) Cells infected with H.pylori for 16 hr (20 x magnification)

(F) Cells infected with H.pylori for 16 hr (100 x magnification)
PLATE-8.2
Adhesion Analysis of
*Helicobacter pylori* with Hep-2 Cells
Fig. 8.1 Effect of mAb CD44 pretreatment on H. pylori adherence
80% when compared to the attachment of the same *H. pylori* to untreated Hep-2 cells (Plate 8.2).

The rate of adherence were calculated as previously reported (Fei et al., 2001)

The rate of adherence = the amount of cells adhered by bacteria/100X100%

For each cover slip, five fields (one field of about 100 cells) were counted and the results were expressed as mean of all fields. (Table 8.1 and Fig.8.1).

**8.3.3 Effect of CD44mAb pretreatment on *H. pylori* infected Hep-2 cells and apoptosis**

In addition to the above experiments, apoptosis was evaluated in hep-2 cell infected with *H. pylori* and mAb CD44 pretreated cells. COMET assay demonstrated apoptosis as the predominant form of cell death in a time dependent fashion. After incubation of cells with *H. pylori*, apoptosis was detected by the appearance of fragmented nuclei and the tail formation in the COMET assay (Plate 8.3). Cells were assessed for apoptosis at specific time intervals and results are depicted in the Table – 8.2 . The cells treated with *H. pylori* alone showed higher apoptosis when compared to CD44 antibody pretreated cells. Control cells (without *H. pylori*) showed only few apoptotic
8.2 Effect of mAbCD44 pretreatment on apoptosis

![Graph showing the effect of mAbCD44 pretreatment on apoptosis across different infection times. The graph includes a bar chart with data points for 4 hours, 8 hours, 12 hours, and 16 hours of infection, comparing control, H. pylori, and mAbCD44 treated conditions.](image-url)
PLATE 8.3

Apoptosis analysis of *H. pylori* infected Hep-2 cells

(A) Normal Cells without DNA fragmentation (40 x magnification)

(B) Early DNA fragmentation of Cells infected with *H. pylori*

(C) Partial DNA fragmentation of Cells infected with *H. pylori*

(D) Complete DNA fragmentation of Cells infected with *H. pylori*
PLATE-8.3

Analysis of Apoptosis in Hep-2 Cells Co-cultured with *Helicobacter pylori* (Comet-Assay)
cells (Fig-8.2). These data suggest that CD44 is not only involved in *H. pylori* adherence but also involved in the *H. pylori* mediated apoptosis.

### 8.4 DISCUSSION

Although a great many ligand–receptor interactions have been suggested to play a role in microbial pathogenesis, there are relatively few for which rigorous experimental evidence supports a significant role *in vivo.* CD44 comprises of a broadly expressed family of variant transmembrane proteins in mammalian cells that are synthesized from differentially spliced mRNA transcripts from a single genetic locus (Sherman et al 1994). CD44 variants are extensively and differently glycosylated and have functions in cell-cell and cell–matrix adhesions, in the presentation of growth factors and chemokines and in the induction of inflammation (Sherman et al 1994). A single antibody that recognizes epitope common to all CD44 variants can detect the localization of entire population of CD44 molecules. CD44 is an adhesion molecule involved in the inflammatory process and is linked to actin cytoskeleton rearrangement and pathogenesis (Moffat, 1996; Jaklien *et al.,* 2003). The present investigation provides evidence that the hyaluronate-binding protein CD44 acts as a receptor for attachment of *H. pylori* to epithelial cells *in vitro.* Several host receptors have been reported before for *H. pylori* attachment on the gastric epithelial cells, however these adhesions failed to mediate effective colonisation and further effects such as apoptosis and oncogene activation. In the current investigation, we explain the
upregulation of CD44 expression and its involvement in actin rearrangement and humming bird formation and apoptosis.

Hasty *et al.*, (1992) have suggested that bacterial adherence to host cells may involve an initial phase of attachment mediated by relatively weak binding between bacterial ligands and their receptors on the host cells, followed by later phase of more avid binding mediated by additional ligand-receptor interactions. We report that *H. pylori* adhere most effectively to cells during initial hours of infection and the adherence and CD44 expression increase up to 16hrs. The causative relationship between *H. pylori*, expression of CD44, and induction of cytoskeletal rearrangement and apoptosis were established *in vitro*. These data suggest that the CD44 mediated attachment may be important not only in the initial interaction but persists and also activates the down stream signaling involved in the pathogenesis.

The recognition of CD44 as a receptor for major microbial pathogens adds a new dimension to the multifaceted role of CD44 in cell-cell communication. The interaction between CD44 and *H. pylori* is a striking example of microbial adaptation to survival within the host through subversion of host intercellular communication pathways. Intervention designed to disrupt this interaction represents a novel potential approach to prevent of *H. pylori* mediated pathogenesis.
SUMMARY

The aim of this research was to analyse the prevalence of *H. pylori* and its significant role in the molecular pathogenesis of gastric carcinogenesis. Considerable importance was given to the understanding of molecular pathology of *H. pylori* infection in gastric carcinogenesis. Thus, the involvement of certain co-factors of host cell and their interaction with *H. pylori* have been investigated in this study.

71.5% of the study population including four normal subjects were found to be infected with *H. pylori*. Interestingly, the precancerous lesions like chronic gastritis, atrophy and intestinal metaplasia showed high prevalence of *H. pylori* of about 89%, 85% and 100%, respectively. Out of 86 cases of adenocarcinoma, 55 were (64%) infected with *H. pylori*. Thus, *H. pylori* infection is the key etiologic factor leading to gastric carcinogenesis.

The data from our study suggest that *H. pylori* infection is more strongly associated with gastric cancer with the relative risk (RR) of 13.51. A population based study has also provided epidemiological evidence of the prevalence of *H. pylori*. A thorough knowledge if the prevalence of *H. pylori* may improve the development of novel ways and treatment strategies in the management of *H. pylori* infection and gastric cancer.

*H. pylori* infection has been shown to induce the production of pro-inflammatory cytokine TNF-α, and subsequent chronic inflammatory response, which is a key element for the carcinogenic activity of the
bacterium. Cytokines and chemokines can contribute to tumor progression by mechanisms other than direct stimulation of cell growth.

Over expression of TNF-α was observed in *H. pylori* infected gastric biopsies but not in normal subjects. The expression was highly associated with the histological progression and *H. pylori* status. This cytokine mediated cross talk between the immune system and the resident epithelial cells could play an important role in the development and progression of gastric carcinogenesis.

Cell adhesion molecule CD44 and its isoforms have also been shown to be expressed in the gastric biopsies infected with *H. pylori*. The abnormal CD44 variant expression is highly associated with histological progression and *H. pylori* infection. We have shown the expression of CD44v6 in *H. pylori* infection and histopathological progression.

Expression of the Proto-oncogene c-H-ras p21 was analysed by RT-PCR, western blotting and immunostaining. An overexpression of c-H-ras p21 was observed in *H. pylori* infected precancerous and cancerous lesions. A highly significant association of Ha-ras p21 expression with *H. pylori* infection and with histopathological progression was observed.

This study thus indicates that the cytokine TNF-α, cell adhesion molecule CD44 isoform and proto oncogene c-H-ras p21 are serially modulated in gastric carcinogenesis. A strong, positive correlation between the modulated expression of these cellular oncogenic factors and *H. pylori* infection is indicated. This depicts that the modulation of cellular factors may
Plate-9.1: Proposed Model of H. pylori induced gastric carcinogenesis
be brought about by the *H. pylori* infection. In the current study, the complex interlinked process responsible for gastric carcinogenesis and its progression were also analyzed using statistical association study.

The present study also analysed the role of the modulation of signal transduction cascade in the process of gastric carcinogenesis in limited number of cases and controls. The results suggest that the altered or over-expression of PKC-β and subsequent MAPK pathways are very much interrelated in the process of carcinogenesis induced by *H. pylori*.

Finally, *in vitro* cell culture method was used to identify the precise role of CD44 expression in *H. pylori* pathogenesis using immunofluorescence and antibody blocking methods. The results suggest that *H. pylori*, hyaluronate-binding protein CD44 acts as a receptor for attachment of *H. pylori* to epithelial cells. The CD44 is not only acting as a receptor for *H. pylori* but is also involved as the communicating molecule between the microbe and the host.

In conclusion, a model has been proposed whereby through CD44, *H. pylori* activates the proto-oncogene c-H-ras p21 followed by the abnormal induction of TNF-α. CD44 activates ras p21 through RTK pathway proteins such as erb-2, c-Met and members of Src family. Therefore, this study provides a novel and important insight into the mechanism through which *H. pylori* induces gastric carcinogenesis.
Appendix