INTER RELATIONSHIP BETWEEN TNF-α,
CD44 ISOFORMS EXPRESSION AND C- H-RAS P21 ONCOGENE IN H. PYLORI INDUCED GASTRIC CARCINOGENESIS

6.1 INTRODUCTION

The regulation of cell life and death, in vivo, is complex and the relative contributions of various genes may be tissue specific. In H. pylori induced gastric carcinogenesis the interrelated mechanisms which are controlled by different genes of host have not yet been elucidated.

H. pylori is the only one known to consistently tolerate the acidic environment of the stomach. Without treatment, H. pylori infection can persist for many years, causing chronic inflammation. Up to 80% patients with gastric cancer have a current or past H. pylori infection (Peek and Blaser, 2002). How the bacterium contributes to the development of cancer is still not entirely clear, although bacterial proteins, the immune responses and hormonal responses have all been implicated. In addition, current research is beginning to link the chronic inflammation to the formation of tumors.
More recently, CagA has been shown to mark a "pathogenicity island" of genes (Censini et al., 1996) which may encode virulence factors that contribute to the more severe gastric disease associated with *H. pylori* infection. However, the association between CagA and disease or gastric inflammation has not been seen in all studies (Graham, et al., 1996) indicating that other environmental or host factors, including the gastric immune and inflammatory responses, also contribute to the pathogenesis of gastric carcinogenesis associated with *H. pylori* infection.

6.1.1 Interaction between TNF-α, CD44 isoforms and c-H-ras p21

*H. pylori* infection in human is always accompanied by mucosal inflammation with an influx of lymphocytes, plasma cells, and neutrophils. The robust immune response to *H. pylori* generally fails to clear the infection, thus resulting in a chronic inflammatory response thought to be a key element of the carcinogenic activity of the bacterium. Inflammatory cells secrete a large number of cytokines and chemokines that can promote the outgrowth of neoplastic cells. These factors are produced in response to proinflammatory stimuli such as bacterial lipopolysaccharide. Cytokines can also contribute to tumour progression by mechanisms other than direct stimulation of cell growth.
The pro-inflammatory cytokine TNF-α is up-regulated by *H. pylori* infection (Beales and Calem, 1998). Pro-inflammatory cytokines released in the mucosa during infectious diseases upregulate the expression of cell adhesion molecules and induce leukocyte infiltration. TNF-α plays a role as a pleotropic cytokine which is found in increased concentration at sites of inflammation and has shown the ability to regulate neutrophil infiltration and eosinophil recruitment. (Lukaes et al., 1995). CD44 is a surface membrane glycoprotein and is implicated in a number of immunological and oncogenic phenomena such as cell activation and tumor metastasis.

The effect of cytokine on CD44 expression has been investigated in several cell types. Cytokine treatment of different cell types has been reported to have contrasting effects on CD44 expression. For instance, in several human colonic epithelial cell lines, IL-4 upregulated CD44 expression whereas TNF-α had no effect (Trejdosiewicz et al., 1998). However, other studies have shown that IL-4 inhibits CD44 expression, whereas TNF-α induces CD44 expression in monocytes (Levesque and Haynes, 1997). TNF-α increased CD44 binding to HA in monocytes and has been correlated with modified glycosylation of CD44 (Levesque and Haynes, 1999). TNF-α can induce CD44 sulphation in monocytes and in the SR91 myeloid cell line and this correlated with the induction of H.A. binding and SR91 cell adhesion to an endothelial cell line (Maiti et al., 1998; Brown et al., 2001).
6.2 WORKING HYPOTHESIS

According to the hypothesis of Nowell (1976), cancer develops through the stepwise accumulation of genetic events that lead to genetic instability and a progressive loss of growth regulation. This suggests the role of a highly interlinked mechanism underlying molecular pathology and cancer development. As far as the cancer of stomach is concerned, the *H. pylori* infection causes an even more complicated process by interacting with host cell gene regulation system. This bacterial infection may lead to cellular transformation through activation of c-H-ras p21 oncogene expression through bacterial protein induced TNF-α expression (Suganuma *et al.* 1999). Over expression of TNF-α and subsequent immune response is a prerequisite for gastric mucosal transformation (Crabtree *et al.*, 1991). Expression of variant isoform of CD44 may contribute to the tumorigenesis, progression and metastasis (Goodison *et al.*, 1999). All these reports suggest that TNF-α, CD44 isoform and c-H-ras p21 are involved in the process of carcinogenesis, and may be interrelated. Based on this hypothesis, the present study was designed to analyse the interrelationship between the host factors involved in *H. pylori* associated gastric carcinogenesis.

6.3 STUDY DESIGN

The analysis of an interrelationship between the oncogenic factors in gastric cancer has been achieved by statistical analysis of the data given in chapters III, IV and V.
The study population is also the same as given in previous chapters. Pearson correlation test was applied for correlation analysis using SPSS 10 software system.

6.4 RESULTS

6.4.1 Interrelationship between proinflammatory cytokine TNF-α and CD44 isoform expression

The previously given data of expression of TNF-α and CD44 were correlated in the same 200 cases inorder to observe whether there is any functional association between the proteins. Statistical analysis showed a positive relation between TNF-α and CD44 expression in relation to histopathological stages and a highly significant association has been noticed between these two proteins. \((p<0.0001)\) \(R=0.686\).

Out of 51 cases with TNF-α negative expression, 36 cases were also negative for CD44 (71%; 36/51). In the 149, TNF-α positive cases, most interestingly 141 cases (95%; 141/149) expressed CD44 and only 8 cases did not express CD44. These results suggest the co-over expression of both the proteins. The same statistical analysis was performed against the CD44v6 expression. This too showed the similar positive association between TNF-α expression and CD44V6 expression. Out of 149 TNF-α positive cases 133 cases (89%; 133 /149 ) expressed CD44v6 isoform. The association was statistically highly significant Fig.6.2.
Figure 6.1 Association between TNF-Alpha and CD44 Isoform expression

[Graph showing the association between TNF-Alpha and CD44 isoform expression. The x-axis represents TNF-alpha negative (n=51) and TNF-Alpha positive (n=149). The y-axis represents CD44 isoform expression. The graph includes bars indicating CD44 negative and CD44 positive.]
Figure 6.2 Association between TNF-Alpha and CD44v6 Isoform expression

- TNF-alpha negative n=51
- TNF-Alpha positive n=149

CD44v6 isoform expression

- CD44v6 negative
- CD44v6 positive
6.4.2 Association between TNF-α expression and proto-oncogene c-H-ras p21 expression

A statistical analysis has been made for the association between TNF-α expression of c-H-ras p21 expression in the same 200 cases. Out of 81 cases with negative TNF-α expression, only 13 (25% ; 13/51) showed positive for ras p21 and all the remaining cases showed negativity for ras p21 expression. In the 149 TNF-α positive cases, 87% of the cases (13/149) showed positivity for ras p21 gene expression.

As shown in Fig.6.3 a highly significant association was observed between TNF-α expression and c-H-ras p21 (p<0.001) (R= 0.596).

6.4.3 Association between CD44 isoform and c-H-ras p21 expression

There was a highly significant positive association between CD44 expression and c-H-ras p21 expression and the results were more or less similar to the relationship between TNF-α and CD44 expression as shown previously . Out of 44 CD44 negative cases, 7 were positive for c-H-ras p21 and remaining 37 (84%) were negative for ras p21 expression. Out of 156 CD44 positive cases, amazingly 87% of the cases (136/156) were positive for c-H-ras p21 and only 20 cases (13%) did not express c-H-ras p21.
Figure 6.3 Association between TNF-Alpha and c-H-ras p21 expression
Figure 6.4 Association between CD44 Isoform Expression and c-H-ras p21 Expression

- c-H-ras P21 Negative
- c-H-ras p21 Positive
Fig 6.5 Association between CD44v6 Isoform Expression and c-H-ras p21 Expression

- CD44v6 Isoform Negative n=59
- CD44v6 Isoform Positive n=141

- c-H-ras p21 Negative
- c-H-ras p21 Positive
A strongly positive association was observed between the expression of CD44 and ras p21 ($p<0.001$) ($R=0.654$) which is shown in Fig.6.4

The same analysis was repeated for CD44v6 expression and c-H-ras p21, which also showed the same kind of association. Out of 141 CD44v6 positive cases 89% (120/141) expressed ras p21. The data are summarised in Table 6.5. As shown in Fig.6.5 a highly significant association was observed between CD44v6 and c-H-ras p21 expression.

The interrelationship between H.pylori infection and expression of host factors such as TNF-α, CD44 and c-H-ras p21 are depicted in Fig.6.6.

6.5 DISCUSSION

H.pylori has been recognized as an etiological agent in the development of mucosa associated lymphoid tissue lymphoma and gastric adenocarcinoma (Blaser, 1992; Parsonnet et al., 1994). Several studies indicated that H.pylori induces hyper proliferation of gastric epithelium (Bechi et al., 1996) and an increase in the expression of proto-oncogenes such as c-fos and c-jun and cyclo oxygenase -2 in gastric epithelial cells (Meyer-ter-Vehn, et al., 2000). These reports suggest that the incidence of gastric cancer associated with H.pylori infection might be triggered by an increased expression of the genes related to carcinogenesis.
Figure 6.6 Expression of TNF-alpha, CD44 isoform and c-H-ras p21 in relation to *H. pylori* infection
However, the molecular mechanism of neoplastic transformation by *H. pylori* infection remains unknown. This necessitates a comprehensive investigation of the host cellular response to *H. pylori* in gastric epithelial cells.

Cell-cell interactions play an important and probably central role in a large number of immunological processes in physiological and pathological conditions. These interactions are at least partially mediated by various cell adhesion molecules. In the context of *H. pylori* infection, the production of chemoattractive cytokines and cell adhesion molecules provide a means of recruiting and retaining inflammatory cells within the gastric epithelial layer, contributing to *H. pylori* mediated tissue injury.

Inflammatory mediators such as TNF-α may upregulate the expression of CD44 (Miyake *et al.*, 1990; Sallusto *et al.*, 1994). In the present study, the co-over expression of TNF-α and CD44 isoforms in *H. pylori* induced gastric carcinogenesis is reported. A highly significant association between these two proteins in the *H. pylori* infection was observed. The cytokine mediated cross talk between the immune system and the resident epithelial cells could play an important role in development and progression of gastric carcinogenesis.

During the last few years, oncogene transfection studies revealed the possible implication of genes in carcinogenesis (or) tumour progression exhibiting a phenomenon involving cell membranes and cell surface glycoproteins.

These observations lead to the proposal that oncogenes could control the expression of enzymes involved in the biosynthetic pathways of cell surface glycoproteins.
Very little is known about the relationship between ras genes and CD44 isoforms. Both the proteins activate each other either way. Recently in two different reports, H-ras oncogene was found to be involved in the activation and redistribution of CD44 in NIH3T3 cell lines (Teramato et al., 2004; Kawano et al., 2000). Turely et al., 1993 demonstrated the expression of CD44 in ras transformed cells. Fitzgerald et al., (2000) have reported the activation of ras expression by CD44 in T024 colonic cell lines via aPKCC.

In the current investigation, we observed a highly significant association between CD44 isoform expression and c-H-ras p21 expression in the sequential evaluation of gastric carcinogenesis. The co-over expression of CD44 and c-H-ras p21 suggests that there is a cooperation between these two factors.

In conclusion, we propose that, through CD44, *H.pylori* infection leads to the activation of proto-oncogene c-H-ras p21 followed by the induction of excess TNF-α. However, the mechanism by which ras is recruited into the signaling pathways following interaction between CD44 and *H.pylori* is unclear. It has been demonstrated that tyrosine kinase activation is a key to early post receptor signaling event in CD44-mediated signaling pathways and the role of ras proteins as down stream effectors of tyrosine kinase activation pathways are well established. (Pawson, 1994). CD44 has been shown to interact with receptor tyrosine kinases such as Erb-2, c-Met and members of the Src nonreceptor tyrosine kinase family (Vander Voort, et al., 1999; Ilangumaran et al., 1998). Therefore, this study provides a novel and important insight into the mechanism involving synergistic action of various host factors through which *H.pylori* induces gastric carcinogenesis.
Chapter-VII