THE IMMUNOGENETICS AND PATHOGENESIS OF GASTRIC CANCER

Highlights of the First Sino-European Workshop on the Immunogenetics and Pathogenesis of Gastric Cancer

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Summary

Clinical scientists from eight European countries and China gathered in the ancient Chinese capital of Xi’an on April 26-28, 2001 to discuss collaboration on a modern approach to gastric cancer prevention. Participants at the First Sino-European Workshop on Immunogenetics and Pathogenesis of Gastric Cancer presented their most up-to-date research results on topics ranging from epidemiology and immune mechanisms to Helicobacter pylori and vaccine development. Researchers then formed groups with their Chinese or European counterparts to plan future research endeavors which will benefit Chinese and European populations alike. After 3 years of organization between the Institute of Digestive Diseases of the Fourth Medical University in Xi’an, China and the Laboratory of Immunogenetics, VU University Medical Center in Amsterdam, the first workshop came into being under the joint sponsorship of the Commission of the European Union, National Natural Science Foundation of China and the Institute of Digestive Diseases, Xi’an, China. As gastric cancer is the most prevalent malignant tumor in China, the workshop was of special significance to the Chinese researchers and to the Chinese population in general. During the workshop, presentations on the epidemiology of gastric cancer showed that this disease is in fact common the world over: it is the second most common cancer next to lung cancer and about 1 million new cases were diagnosed in 2000. Three-quarters of the cases of gastric cancer occur in Asia, and approximately 80% of these cases are in China and Japan. Genetic factors and environmental factors such as diet and H. pylori infection play a role in gastric carcinogenesis. As a recognized cause of gastric cancer, H. pylori was the subject of various presentations ranging from immunological studies, molecular analysis of strains and pathogenesis to vaccine development. Specific areas of discussion included bacterial-epithelial interactions in H. pylori infection, epidemiology in China, global distribution of vacA and cagA genotypes, new evidence for host factors, nonsteroidal anti-inflammatory drugs and H. pylori as indepen-
dent risk factor for gastric cancer, new diagnostic techniques for H. pylori using serum levels of pepsinogen I, and autoimmune processes in corpus atrophy. Vaccine development using a variety of strategies against H. pylori was the subject of an entire session of talks. Oral immunization with urease with Escherichia coli heat labile enterotoxin was shown to be safe and immunogenic in humans as a mucosal adjuvant. Results of a study using attenuated Salmonella typhimurium as a vehicle for DNA-mediated immunization in mice were also presented. A final presentation discussed an ongoing trial comparing strain variability in the vacA and cagA gene sequences and disease expression between H. pylori infection in Europe and China. Researchers also discussed the role of IL1 gene family and TNF gene polymorphisms in gastric pathology and various immune mechanisms involved in gastric cancer, such as down-regulation of NFκB, IL-1 and IL-1RA, cyclooxygenase signalling, and identification of MGAg antibodies. An interactive discussion followed each presentation and ideas and suggestions were provided. According to specialty, the presenters were then assigned to groups of four or five to make plans for joint research projects. A number of international and Chinese observers were present, including representatives from the European Commission, the World Health Organization and the Chinese National Center for Biotechnology Development, and offered input on the financial feasibility of such projects.

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OPENING ADDRESS

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May I first of all express my gratitude to the Institute of Digestive Diseases of the Fourth Military Medical University in Xi’an for hosting this workshop. I would also like to express my gratitude to all the participants (see Appendix). Thanks to your enthusiasm and scientific expectations, we are able to work together in the historical city of Xi’an. Of all the experts participating in the workshop, some have come from schools with a long tradition in the area of gastric cancer. I would like to mention two participants from Europe who represent two schools with a long tradition in the area of gastritis and gastric cancer. The first is Dr. Sipponen, who comes from the school of Prof. Siurala in Finland. They were the first to study the natural course of gastritis in the normal population, using gastric biopsies. The second is Dr. Muñoz, who trained with Prof. Pelayo Correa, the first person to describe the sequence of gastritis, focal gastritis, intestinal metaplasia and gastric cancer. Dr. Muñoz also represents the exiting research line of geographic pathology which was created by the World Health Organization in Lyon, France. She is one of the pioneers in the epidemiology of gastric cancer. More than 30 years ago, she showed that the prevalence of gastric cancer in Northern Europe was on the decline.

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Europe                                      China

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**Helicobacter pylori and Gastric Inflammation: Molecular Analysis of Bacterial-Epithelial Interactions**

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There is increasing evidence that *Helicobacter pylori* strains containing the *cag* pathogenicity island (PAI) are associated with more severe gastric pathology and the development of atrophic gastritis. The up-regulation of C-X-C chemokines such as interleukin (IL)-8 and growth-related oncogene-α (GRO-α) is considered to be an important mechanism accounting for more severe clinical outcome. Multiple bacterial genes within the *cag* PAI are essential for this cell signaling response (1, 2).

cDNA array technology has been used to investigate further the changes in epithelial gene expression induced by *cag* PAI-positive and *cag* PAI-negative *H. pylori* strains. In vitro infection of gastric epithelial cells with *H. pylori* induces changes in epithelial gene expression of many cytokines, cytokine receptors and members of the ADAM (a disintegrin and metalloprotease) family, which are important molecules in cytokine and growth factor processing. Marked differences in the epithelial gene expression were observed after infection with *cag* PAI-positive and *cag* PAI-negative *H. pylori* strains (2, 3).

Understanding strain-related differences in gastric epithelial gene expression will improve understanding of the role of the *cag* PAI in disease pathogenesis and identify previously uncharacterized genes of relevance to gastric cancer.

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**Effect of Eating Garlic on the Prevention of Human Gastric Cancer: Inhibition of NFκB Activation Through Up-regulation of Metallothionein Gene in Allitridi-Treated Human Gastric Cancer Cell Lines**


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We reported previously that cells exhibited marked morphological changes, reduced cell proliferation and redifferentiation through the G1 phase arrest on exposure to 25 µg/ml of allitridi and garlic oil. In nude mice, cells showed reduced ability for tumor formation, and apoptosis was confirmed by flow cytometry analysis and DNA-ladder measurement.

In our present data, many changes in gene expression were identified in allitridi-treated tumor cells by using a differential display technique, including up-regulation of more than 20 genes or expressed sequence tags (ESTs) and down-regulation of more than 40 genes or ESTs.

Surprisingly, a high level of expression of metallothionein-IIA (MT-IIA), folate receptor-α (FOLR-α) and calcyclin genes were identified by using Northern blot analysis. We also detected decreased expression of the superoxide dismutases (SOD) gene in the allitridi-treated cells. These results showed that there is a good correlation between up-regulation of MT-IIA gene and inhibition of NFκB activity in garlic-treated human gastric cancer cell lines.

This finding has contributed to our knowledge of the effects of garlic oil and allitridi on suppression of tumor cell growth ability and redifferentiation in human gastric cancer cells. These results suggest that up-regulation of the MT-IIA gene and inhibition of NFκB activation may play a role in the prevention of gastric carcinogenic effects through garlic consumption.

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**The Role of IL1β and IL1RN Gene Polymorphisms in the Determination of Gastric Pathology**

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Normal physiological control of the gastric secretory function and the control related to the pathogenesis of *H. pylori*-associated diseases is, to a certain extent, dependent on the genetic control of the inflammatory response (4). The relative risk of developing gastric ulcer, duodenal ulcer or duodenitis in patients with a family history of gastric pathology is higher than in patients without familial occurrence. The prevalence of peptic ulcer is higher in monozygotic twins than in dizygotic twins and the concordance rates for *H. pylori* infection among
pairs of twins reared apart is higher for monozygotic than for dizygotic twins (5). Other host factors, such as the age at which the host acquires the infection, appear to determine the outcome of the interaction between H. pylori and the host. Experimental animal models have provided evidence that the host response is an important determinant in the severity of gastritis (6). T lymphocytes expressing interferon (IFN)-γ and tumor necrosis factor (TNF)-α are generated during the induction of cell-mediated immunity. Macrophages are recruited and become activated. It is now known that IL-12 also facilitates the development of the T helper type 1 (Th1) lymphocytes required for protection against bacteria (7). In principle, the Th1 response is a normal adaptive immune response; however, when this response is too strong it produces severe chronic inflammation. In the stomach, locally produced IL-1β is an important mediator of hypochlorhydria since IL-1β inhibits gastric acid and pepsinogen secretion. The levels of IL-1β in cultured antral biopsy specimens are significantly higher in H. pylori-positive patients than in individuals with negative cultures for H. pylori with normal antral mucosa.

During the inflammatory response a multitude of new genes encoding proinflammatory cytokines such as IL-1α, IL-1β, TNF-α, and lymphotxin (LT)-α as well as other proteins with proinflammatory or antiinflammatory properties are induced. The IL-1A, IL-1B and IL-1RN genes are clustered on the long arm of human chromosome 2. Several functional polymorphisms have been described in these genes. This strongly suggests that individuals who produce high amounts of IL-1β and lower amounts of the IL-1ra have difficulty controlling inflammation. Preliminary results of IL-1 gene polymorphisms in duodenal ulcer patients have shown that the simultaneous carriage of IL-1B+3954 allele 2 and IL-1RN allele 2 is an independent factor associated with reduced risk of bleeding duodenal ulcers (8). In a recent study supporting the role of the IL-1 gene cluster in the progression to disease, El-Omar et al. (9) reported on the association between specific IL-1 gene polymorphisms and increased risk of gastric cancer. According to these authors, carriage of IL-1B-31 allele 2 and IL-1RN*2.2 homozygosity increase both the likelihood of a chronic hypochlorhy- dric response to H. pylori infection and the risk of gastric cancer, presumably by altering IL-1β levels in the stomach. IL-1B+3954 allele 2 homozygotes seemed to play a protective role in gastric cancer, although the effect did not reach statistical significance. More recently, in 102 unrelated Spanish Caucasian patients with duodenal ulcer and 85 ethnically matched healthy individuals we have found that the lack of the “high” IL-1β producer alleles, IL-1B-511 allele 2 and IL-1B+3954 allele 2, in addition to bacterial factors may play a key role in the development of duodenal ulcer disease (10).

The data suggest that the normal physiological control of the gastric secretory function and the control related to the pathogenesis of H. pylori-associated diseases, to a certain extent, are dependent on the genetic control of the inflammatory response.

Until recently the IL-1B family of biallelic gene polymorphisms and the variable number of tandem repeat polymorphism in intron 2 of the IL-1RN gene have been typed with standard polymerase chain reaction (PCR)-based methods, and now the introduction of the TaqMan allelic discrimination test (9, 11) will facilitate the analysis of a large number of samples and comparative population studies. Since different cytokines probably interact in a network in the regulation of the inflammatory response, and their expression depends on a variety of factors, microarray technology such as the one developed by Affymetrix will play an important role in the future. Efforts should be made to acquire this technology to strengthen the collaboration between Chinese and European scientists.

The Role of TNF Gene Polymorphisms in the Determination of Gastric Pathology

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Only 10-20% of H. pylori-infected individuals develop symptoms including gastric and duodenal ulcer disease, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. Twin and family studies have shown that host genetic factors may play a role in the susceptibility to H. pylori infection. Also, the host immune response may help to determine the clinical outcome of disease. It is now known that immunogenetic factors influence the immunological response towards a variety of pathogens, including H. pylori. Polymorphisms in
the genes encoding TNF have been associated with \( \text{H. pylori} \) infection and duodenal ulcer (12). We have previously shown that four biallelic polymorphisms in the TNF and \( \text{LTA} \) genes located in the major histocompatibility complex (MHC) on chromosome 6 occur as only five haplotypes in the Caucasian population (13). In a case-control study of these haplotypes in the TNF and \( \text{LTA} \) genes in Spanish Caucasian duodenal ulcer, gastric ulcer and healthy controls we found evidence for significant differences between gastric and duodenal ulcer. Furthermore, we identified haplotype TNF-I carrier status as an independent risk factor for peptic ulceration in \( \text{H. pylori} \)-infected patients (14).

Recently described polymorphisms in the 5’ flanking region of the TNF gene have been found to influence the transcriptional activity. Genotyping of these polymorphisms, therefore, may reveal the functional polymorphism and will help to refine the TNF haplotypes previously described (15). It is to be expected that the increase in knowledge in the distribution of TNF haplotypes will shed light on the existence of linkage disequilibrium with MHC antigens and may help to explain previously weak associations of human lymphocyte antigen (HLA) genes with peptic ulcer (16, 17).

New technology to study TNF haplotypes in relation to \( \text{H. pylori} \) infection is available. A comparative genetic study in high and low risk regions of gastric cancer in China should be aimed toward studying not only the different strains of \( \text{H. pylori} \) and other environmental factors but also the genetic make-up of the host in these regions. The cytokine gene polymorphisms are interesting candidates to study in relation to peptic ulcer and gastric cancer.

**Identification and Application of Gastric Cancer Associated Antigens MGAGs**

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Gastric cancer is one of the most prevalent malignant tumors in China, causing about 200,000 deaths annually. The efficacy of treatment depends on early detection. Unfortunately, given the lack of early symptoms, most patients seek medical care in the advanced stage when resection of the tumor is rarely possible. General examination using radiological or feasible endoscopic analysis is useful in establishing an early diagnosis, but this is not current practice in China. In order to facilitate the easy and early diagnosis of gastric cancer, 12 murine hybridoma cell lines capable of producing monoclonal antibodies (MABs) (MC series) against gastric cancer were established in our institute. These MABs have been clinically used with encouraging results in immunohistological diagnosis, immunocytochemical diagnosis, radioimmunodiagnosis, and serological diagnosis of gastric cancer. The corresponding antigens defined by the antibodies (MGAGs) have been preliminarily characterized and identified as a group of novel tumor-associated antigens. The epitopes of MGAGs have been recently screened and sequenced by means of a phage display system for random peptide, which are possibly used as candidate molecules for construction of a recombinant gastric cancer vaccine.

**Cyclooxygenase Signaling in Gastric Cancer Prevention**

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Epidemiological studies have shown that chronic aspirin or nonsteroidal antiinflammatory drug (NSAID) users have 50% reduction in incidence of gastric and colon cancer. NSAIDs inhibit the enzymes cyclooxygenase (COX)-1 and -2. Overexpression of COX-2 was demonstrated in a high percentage of both gastric and colon cancer. To understand the mechanisms behind these observations, we have shown that aspirin, indomethacin and specific COX-2 inhibitors inhibited cell proliferation and induced apoptosis in gastric cancer cells. Similar to NSAID-induced apoptosis in colon cancer cells, the induction of apoptosis in gastric cancer is mediated by both COX-dependent and COX-independent pathways.

Firstly, we investigated the role of protein kinase C (PKC) signaling in NSAID-induced apoptosis of gastric cancer. Treatment with indomethacin or a specific COX-2 inhibitor, SC-236, decreased PKC-\( \beta \)-1 expression. Overexpression of PKC-\( \beta \)-1 could both inhibit indomethacin- or SC-236-induced apoptosis and up-regulate p21 expression. Inhibi-
tion of this up-regulation of p21 by its antisense oligonucleotides partially reduced the antipoptotic effect of PKC-β-1. The results suggest that PKC is a novel COX-independent pathway.

Secondly, we investigated the role of NFκB during COX-2 inhibition in gastric cancer. We showed for the first time that SC236 could effectively suppress NFκB-mediated gene transcription in gastric cancer cells. The potency of SC236 is more than one magnitude higher than that of the nonspecific COX inhibitor aspirin. Furthermore, it appeared that SC236 acted through different mechanisms. Unlike aspirin, SC236 had no significant effect on phorbol myristate acetate (PMA)-induced IκB-α phosphorylation/degradation. Instead, SC236 worked through suppressing the nuclear translocation of the NFκB subunit, the RelA/p65 protein.

In conclusion, aspirin or COX-2 inhibitors may have a potential chemopreventive effect for gastric cancer by induction of apoptosis through modulation of various signaling pathways and inhibition of NFκB.

**Antitumor Immunity Induced by Tumor Cell Lines**

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T-cell immune response and tumor vaccine are the two major components of immunotherapy for cancer. Induction and expansion of certain numbers of tumor-specific cytotoxic T lymphocytes requires tedious procedure and special techniques such as autologous tumor cell culture. Though recombinant or peptide vaccines have been actively under investigation, tumor cell vaccine remains an important means of tumor vaccine therapy.

By using mixed lymphocyte tumor cell culture, specific cytotoxic T lymphocytes were obtained by stimulation with HLA-A-matched allogeneic gastric cancer cells. These cytotoxic T lymphocytes were CD8+ predominant and proliferated up to 100 times their original number. Moreover, the cytotoxic T lymphocytes could be derived from the peripheral blood of both patients and healthy subjects.

Chemically modified Ehrlich ascites tumor cells were shown to be promising as a tumor vaccine with reliable efficacy in mice. The prolonged survival and decreased tumor burden were associat-

**Epidemiology**

**Epidemiology of Gastric Cancer and Perspectives for Prevention**

N. Muñoz

International Agency for Research on Cancer, Lyon, France

Global burden, geographical distribution and time trends

The most recent estimates indicate that gastric cancer is the second most common cancer in the world after lung cancer with about 1 million new cases diagnosed in 2000. It represents 11% of all cancers in men and 7% of all cancers in women. In developing countries, lung cancer ranks first, representing 13% of all cancers and gastric cancer second (12%). In developed countries gastric cancer ranks fifth, representing 7% of all cancers. Three-quarters of these cases occur in Asia. About 80% of the cases diagnosed in Asia occur in China and Japan.

Steady declines in the rates of gastric cancer have been observed everywhere in the last few decades. However, the total number of new cases diagnosed worldwide is increasing mainly because of increasing and aging of the population. It was estimated that there were 700,000 new cases in 1980, 755,000 in 1985, 900,000 in 1990 and 1,013,000 in 1995 (18-20). This indicates that the impact of gastric cancer in public health is not decreasing.

The exact causes of the decline of the rates of gastric cancer is not well understood, but must include improvements in diet and food storage practices (e.g., refrigeration) and, possibly, a decline in H. pylori infection.
The most recent data on incidence show that the highest rates (over 40 per 100,000) are reported from Japan, Korea, China, Belarus and certain countries in Latin America. The lowest rates (less than 15 per 100,000) are seen among whites in North America, most African countries, India, Thailand, the Philippines and some countries in western Europe. Intermediate rates are seen elsewhere (21).

Risk factors for gastric cancer

Gastric cancer, as all cancers, is the end result of the interplay of many risk factors as well as protective factors. Although epidemiological evidence indicates that environmental factors play a major role in gastric carcinogenesis, a role of genetic factors is suggested by the increased risk of a diffuse type of gastric cancer associated with mutations in the E-cadherin gene. Among the environmental factors, diet and \textit{H. pylori} infection are more amenable to intervention aimed at the prevention of gastric cancer (22).

Diet

Epidemiological evidence derived from dozens of case-control studies conducted all over the world have shown a remarkably consistent protection derived from a high consumption of fruits and fresh vegetables. Salty and preserved foods have also been consistently incriminated as increasing the risk of gastric cancer (22).

\textit{H. pylori}

Epidemiological evidence derived mainly from nested case-control studies indicate that long-standing infection with \textit{H. pylori} is associated with a two- to threefold increase of gastric cancer risk mainly in low-risk countries (23). It has been classified as a class 1 human carcinogen by a multidisciplinary panel of investigators within the International Agency for Research on Cancer monograph program (24). However, a National Institutes of Health consensus panel (25) concluded that the evidence linking \textit{H. pylori} to gastric cancer was not conclusive and that further research was required to clarify its role.

Results of a case-control study on gastric cancer recently conducted in Venezuela showed no association with \textit{H. pylori} (26) and an increased risk with a diet poor in fresh vegetables, fish and meat and rich in starch (27).

Perspectives for prevention

Trials are under way in Colombia, Venezuela, Europe, Japan and China to assess the chemopreventive potential of antioxidant vitamins and of the eradication of \textit{H. pylori} by antibiotics. Endpoints in these trials are reduction of progression or increase in regression of precancerous lesions of the stomach, and reduction in gastric cancer mortality.

Results of the Colombian trial suggest that anti-\textit{H. pylori} treatment and antioxidant vitamins may interfere with the precancerous process, mostly by increasing the rate of regression of precancerous lesions (28).

Conclusions

Gastric cancer, the second most common cancer in the world, represents a very important health problem with about 900,000 new cases diagnosed every year. Diet modifications and possibly vitamin supplements, as well as the control of \textit{H. pylori} infection by the use of antibiotics and safe and effective vaccines, offer great potential for the prevention of this important malignancy.

\textbf{Intervention Study of High-Dose Folic Acid on Gastric Carcinogenesis Induced by N-Ethyl-N-Nitrosoguanidine in Beagles}

S.-D. Xiao\textsuperscript{1}, X.-J. Meng\textsuperscript{1}, Y. Shi\textsuperscript{1}, Y.-B. Hu\textsuperscript{1}, S.-S. Zhu\textsuperscript{1}, Z.-H. Ran\textsuperscript{1} and C.-W. Wang\textsuperscript{2}\textsuperscript{1}

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Epidemiological and nutritional studies have indicated that folate status modulates the risk of developing cancer. A decrease in folic acid and the subsequent DNA hypomethylation or disruption of DNA repair and DNA integrity may be involved in gastric carcinogenesis. We aimed to investigate whether folic acid plays an important role in the chemoprevention of gastric carcinogenesis induced by N-ethyl-N-nitrosoguanidine (ENNG) in beagles.

Sixteen healthy male beagles were randomly divided into two groups: a folic-acid-treated group and a control group. In both groups the beagles were fed ENNG 75 mg/day for 8 months, while in the treated group 20 mg folic acid was given to beagles for 15 months. Gastroscopy and biopsies were performed before and every 2-3 months after the administration of ENNG until the end of the experiment. Histopathological lesions were diag-
nosed according to the criteria for human gastric mucosal biopsies. The serum and gastric mucosal tissue folic acid concentrations were measured by radioimmunoassay or spectrophotometer respectively.

It was found that in the control group, all the beagles developed gastric cancer (8/8), compared with only three (3/8) in the folic-acid-treated group (p <0.05). Moreover, the serum and gastric mucosal tissue folic acid concentrations were markedly elevated 15 months after folic acid administration. The difference was statistically significant between the two groups (p <0.05). Our results indicate that high-dose folic acid plays an important role in the chemoprevention of gastric carcinogenesis induced by the chemical carcinogen ENNG in beagles. However, since all the beagles developed dysplasia, the question of whether supraphysiologic doses of folic acid postpone or prevent the appearance of cancer remains to be elucidated. Further study including a normal group of beagles may be informative.

**Helicobacter pylori Infection in the High- and Low-Risk Chinese Population of Gastric Cancer**

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H. pylori is a recognized cause of chronic gastritis and peptic ulcer disease and is strongly suspected of playing an important role in the etiology of gastric cancer. Linqu county in Shandong province is one of the highest risk areas for gastric cancer in China and in the world. The gastric cancer rate in Linqu county is 15 times higher than that of Cangshan county in Shandong province, even though these counties are within 322 km of each other.

In order to evaluate the prevalence of H. pylori infection in Linqu county and Cangshan county, we examined children (aged 3-12 years) and adults (aged 35-64 years). H. pylori infection was determined by 13C-urea breath test in children and by ELISA in adults.

It was found that the prevalence of H. pylori infection were 77.1% and 59.9% in adults, and 69.4% and 28.7% in children in Linqu and Cangshan respectively. The prevalence of H. pylori infection varied greatly with regard to gastric precancerous lesions in the two areas.

The findings of this study support the fact that H. pylori is a risk factor in the development and progression of gastric cancer and precancerous lesions.

**Association of Infection with Gastric Carcinoma: A Meta-analysis of the Chinese Literature**

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In China, the annual average mortality rates for gastric carcinoma are as high as 16 per 100,000, and the H. pylori infection rates are 40-50%. Malignant tumors are the second cause of death in men and the third in women, and among such tumors the gastric variety is the first cause of mortality in China. Since the discovery of H. pylori in 1983, the association between H. pylori and H. pylori-related diseases has become the focus of many gastroenterological studies. Gastric carcinoma is the most important disease among H. pylori-related diseases and it is believed that H. pylori is an etiological factor. However, a definitive conclusion has not been reached and the definite mechanisms of their association remain to be determined.

Many studies in China on H. pylori support the conclusion that there is a relationship between H. pylori and gastric carcinoma, but a few do not. Following the principles of evidence-based medicine, we aimed to determine the combined results of these studies.

More than 100 papers published since 1995 were reviewed on the relationship between H. pylori and gastric carcinoma. Since most of the studies did not have appropriately controlled data and therefore did not meet the requirements for the meta-analysis, 21 papers of case-control studies were selected, including 11 on gastric cancer, seven on gastric precancerous lesions and three on stomach lymphoma. A meta-analysis was conducted to sum up the odds ratios of these studies.

For H. pylori in gastric cancer (intestinal and diffuse type) 11 studies included 820 patients and 11,647 controls. The summary odds ratio from the fixed effect model was 3.0016 (95% CI: 2.4197
In H. pylori and gastric precancerous lesions seven studies included 1,978 patients and 6,076 controls. A random-effect model was used to summarize the odds ratio and was found to be 2.5635 (95% CI: 1.8477–3.5566, \( p < 0.01 \)). For H. pylori and stomach lymphoma three studies included 83 patients and 143 controls. Though there were too few studies for a meta-analysis, the data show that stomach lymphoma is highly associated with H. pylori infection.

It was concluded that most studies on the relationship between H. pylori and gastric carcinoma showed that H. pylori infection is preexisting in gastric carcinoma and precancerous lesions. Therefore, the results of the meta-analysis strongly support the conclusion that H. pylori infection is a risk factor for gastric carcinoma.

There have been many opinions and assumptions regarding the mechanisms of the carcinogenetic process in the gastric mucosa caused by H. pylori, but there is insufficient evidence to support this theory. In China, very few such prospective studies have been carried out. The consensus is that H. pylori infection is one of many factors in the carcinogenesis of the gastric mucosa and that all these factors act together to result in gastric carcinoma. In order to identify all these factors and how they interact in the process of carcinogenesis, more prospective intervention trials must be conducted. A trial of this kind is in progress in Shan Dong and Fu Jian province of China. It will be very useful in revealing the mechanism of H. pylori involved in the process of gastric mucosa carcinogenesis.

**The Global Distribution of Helicobacter pylori Strains**

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H. pylori shows a high degree of genetic heterogeneity due to mutations and frequent recombination. The overall genetic variability can be examined by multiple methods, such as DNA fingerprinting, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and multi-locus sequence typing. In the past, specific genes have been identified which are associated with bacterial virulence. These genes (e.g., vacA, cagA and iceA) have been studied individually, and distinct genotypes have been defined. There is accumulating evidence that the different genotypes of H. pylori show a particular geographic distribution (29–32).

We have developed a PCR-based reverse hybridization tool (called the line probe assay) which permits standardized genotyping of H. pylori strains. This assay discriminates the distinct allelic types of the s- and m-regions of vacA (s1a, s1b, s1c, s2, m1, m2a, and m2b) as well as presence of the cytotoxin-associated gene (cagA, including specific subtypes) (33, 34).

This method was used to assess the vacA and cagA genotypes of a large number of H. pylori strains from more than 25 countries in North and South America, Europe, Asia and Australia. Populations with different gastric cancer incidence rates were compared (35).

In Europe, a distribution gradient of s1 subtypes was observed. In Northern and Eastern Europe, the majority of strains were subtype vacA s1a. In France and Italy, s1a and s1b were equally present, whereas in Spain and Portugal most strains were subtype s1b. Subtypes s1a and s1b were about equally prevalent in North America. In Central and South America virtually all s1 strains were subtype s1b. Subtype s1c was observed in the great majority of the type s1 isolates from East Asia, as well as in European and Canadian patients of East-Asian origin. In most parts of the world vacA m1 and m2a have equal presence, except on the Iberian peninsula and in Central and South America, where m1 (86.2%) is more prevalent than m2a (13.8%). Subtype m2b was observed exclusively among East Asian s1c strains. Analysis of cagA subtypes showed similar results (36).

These data strongly indicate the existence of a nonrandom geographic distribution of H. pylori genotypes which may be associated with migration of human populations since ancient times. These data will aid in understanding H. pylori evolution, population biology and pathophysiology of H. pylori-associated diseases.

**Why is There Significant Variation in the Risk of Gastric Cancer Associated with Helicobacter pylori Infection?**

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H. pylori infection is a risk factor for gastric cancer and most epidemiological studies have estimated a relative risk associated with infection in the order of two- to fourfold. There is interest in the extent to which this risk estimate might vary between different populations. There are a number of population samples that lead to disagreement about the role of H. pylori in gastric cancer:

i) There is a high prevalence of H. pylori infection and low rates of symptomatic disease. More than 80% of the Indian and Thai populations are infected with H. pylori, yet gastric cancer is uncommon.

ii) In certain countries and even in certain areas in the same country, gastric ulcer and gastric cancer are the most usual presentation of H. pylori infection, while in others duodenal ulcer is the norm.

iii) Different ethnic groups living in close proximity may have different patterns of disease. In Singapore and Malaysia, and in the Chinese population, H. pylori infection rates are high and gastric cancer is common. In the Indian community, infection is also very common but gastric cancer is rare. In the Malay population, even in less well-off communities, the level of H. pylori infection is low and gastric cancer is uncommon.

It is believed that gastric carcinogenesis is a multistep, multifactorial process beginning with H. pylori-associated gastritis in most cases. It is therefore likely that environmental and/or genetic cofactors as well as H. pylori strains are important in modifying the risk associated with H. pylori infection, evidence of which is accumulating. Several environmental exposures have already been identified as risk factors. Among them, the association with salt is of particular interest since recent results from both the INTERSALT study and a Japanese study establish a long-suspected correlation between salt intake and gastric cancer risk. As for evidence of a host factor, epidemiological data show that there is high prevalence of gastric cancer in a population with high gastric ulcer/duodenal ulcer ratio, whereas there is a low prevalence of gastric cancer in population with low gastric ulcer/duodenal ulcer ratio.

Considering these data, a hypothesis has been proposed: different gastric acidity causes changes in the distribution of H. pylori-induced gastritis, thus influencing the final disease outcome. Gastric acidity should be related to the size of the parietal cell mass at the time of infection, which should be genetically determined in the individual and population. A recent study from El-Omar et al. (9) examined 100 first-degree relatives of patients with non-cardia gastric cancer. Prevalence of atrophy and hypochlorhydria was increased only in those with evidence of H. pylori infection and was greater in relatives of patients with familial gastric cancer. Further study by El-Omar et al. looked for gene polymorphisms in the same subjects and other groups. They found there were interleukin (IL) gene cluster polymorphisms suspected of enhancing production of IL-1β, a cytokine known to be acid suppressive and linked to H. pylori infection. These gene variations were associated with increased risk of both hypochlorhydria induced by H. pylori infection and gastric cancer. If a person has this mutation and acquires H. pylori infection, the inflammation caused by the bacterium produces more IL-1β and there is therefore more acid suppression than in an infected individual without the polymorphisms and thus normal levels of IL-1β production. It would clearly be important to describe the cofactors that interact with H. pylori infection to increase cancer risk: the better understood the role of cofactors, the better understood will be the role of H. pylori in gastric cancer.

Immunogenetics and pathogenesis of gastric cancer

Telomerase Activity, Telomere Restriction Fragment and a Telomerase Subunit in Different Gastric Mucosal Lesions and the Effect of Antisense HTRT on Biological Behavior of the Gastric Cancer Cell Line SGC-7901

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Gastric cancer is the most common solid tumor in China. This tumor demonstrates significant heterogeneity with respect to pathological, genetic and clinical features. Telomerase activation is regarded as essential for cell immortalization and its inhibition may result in spontaneous regression of neoplasms. Gastric cancer has commonly expressed telomerase activity, and telomerase inhibition is expected to provide a cure for gastric cancer. In this study we examined telomerase activity, telomere restriction fragment (TRF) and a telomerase...
subunit in different lesions of gastric mucosa. We also observed the effect of antisense human telomerase reverse transcriptase (hTRT) on the biological behavior of the gastric cancer cell SGC-7901.

Telomerase activity was detected in 176 different lesions of gastric mucosa with telomeric repeat amplification protocol (TRAP). Telomerase activity was detected in the mucosa of 24.6% (14/57) of chronic atrophic gastritis, 38.9% (7/18) of intestinal metaplasia, 37.5% (3/8) of dysplasia and 92.3% (60/65) of gastric carcinoma lesions. On the contrary, no telomerase activity was detected in normal mucosa. A positive rate of gastric carcinoma was significantly higher than that in chronic atrophic gastritis, intestinal metaplasia and dysplasia (p <0.01). No correlation was found between telomerase activity and clinicopathological parameters in gastric carcinoma.

TRF in 90 different gastric mucosa lesions was determined by Southern blot analysis. The mean TRF length in normal mucosa was decreased with increasing age. The mean TRF length was significantly different to normal age (r=−0.56, p <0.01). Thirty-five mucosae from gastric carcinoma were examined for TRF. Compared with corresponding normal mucosa, 20 (57.1%) showed considerable shortening, 12 (34.3%) showed approximately the same length and three (8.6%) showed elongation. No correlation was found between the mean TRF length and clinicopathological parameters and telomerase activity in gastric carcinoma. We compared the TRF length with microsatellite instability (MSI) and loss of heterozygosity (LOH) of APC, MCC and DCC genes. LOH at the DCC locus was associated with telomere shortening. This tendency was also observed in APC and MCC genes, although there was no statistical significance.

Telomerase subunits were also examined in 60 different lesions of gastric mucosa with RT-PCR and no significant different expression of human telomerase RNA and telomerase-associated protein (TP1) was found among different groups. hTRT was mainly detected in gastric carcinoma and some premalignant lesions. The expression of hTRT paralleled the expression of telomerase.

The sense and antisense hTRT was transfected into SGC-7901 cells with 1,2-dioleoyltrimethyl ammoniumpropane (DOTAP) liposomal transfection. It was found that antisense hTRT could significantly suppress cancer cell growth, and that it down-regulated expression of apoptosis-associated gene bcl-2 and c-myc at their transcription and translation levels, and partially reversed malignant phenotypes of SGC-7901. The mechanism of these phenomena may be mainly involved in inducing differentiation other than apoptosis.

Our results suggest that telomerase activation play an important role in the early stage of gastric carcinogenesis; hTRT may be a good diagnostic biomarker for gastric cancer; telomere erosion is independent of MSI pathway but related to the LOH pathway in gastric carcinoma; and inhibition of hTRT expression by antisense strategy may provide a new approach to gene therapy for human gastric cancer.

Immune Evasion: A Potential Carcinogenic Mechanism of Helicobacter pylori Strains Harboring cag Pathogenicity Island

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H. pylori has been considered an important pathogenic factor in gastritis, duodenal ulcer and MALT lymphoma. Epidemiological studies have strongly suggested a causal association between H. pylori and gastric cancer. This disease manifestation is believed to be partly attributed to the presence of a gene cluster referred to as the cag pathogenicity island (PAI). However, the potential carcinogenic mechanisms have yet to be clearly elucidated. We suggest that immune evasion may contribute to the long-term colonization of cag PAI-positive H. pylori strains and the subsequent inflammatory response in gastric mucosa. This might change the indigenous equilibrium between apoptosis and the growth of gastric epithelium and result in the eventual occurrence of gastric cancer.

When compared with the isogenic cag PAI knock-out H. pylori strain, the cag PAI-positive H. pylori strain was able to stimulate gastric mucosal cells to produce higher amounts of reactive oxygen species (ROS). This would activate stress-associated nuclear factors, such as activator protein-1 (AP-1) and NFκB, and trigger the transcription of some proinflammatory cytokines as well as apoptosis-related genes. Fas ligand expression of gastric T-cells and epithelial cells was up-regulated by ROS with the help of proinflammatory cytokines such as IFN-γ and TNF-α. Subsequently, functional T-cells were depleted through suicide or patricide mecha-
nisms. This immune evasion phenomenon would result in the long-term colonization of the bacterium.

Furthermore, ROS alone and with proinflammatory cytokines was able to induce apoptosis of gastric epithelial cells individually. The proliferation of gastric epithelial cells for the "repair" purpose would be initiated. Some preliminary data have shown that the expression of growth-related genes such as p21, p53, bcl-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in gastric epithelial cells was enhanced as the result of the above process.

Although the findings presented provide new insights into the carcinogenesis of H. pylori, more scientific studies are still required to clarify this complicated mechanism.

PATHOGENESIS AND PATHOLOGY

NSAIDS, Helicobacter pylori and the Risk of Gastric Cancer

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Gastric ulcers, gastritis and complications
H. pylori infection and NSAIDs are the two major causes of gastroduodenal pathology. Both induce inflammation and ulceration of the upper gastrointestinal tract, but their potential interaction in the genesis of mucosal lesions is controversial. The results of six epidemiological studies range from slight enhancement of risk by H. pylori (one study) to no effect (three studies), to protection against NSAID injury by H. pylori (two studies) (37). These findings suggest that NSAIDs and H. pylori act as independent risk factors, without any major effect of H. pylori infection upon NSAID-associated risks. A meta-analysis of endoscopic studies has shown a modest but significant enhancement of ulcer risk in NSAID users infected with H. pylori compared to those who are uninfected (odds ratio 1.8) (38). In patients with no previous ulcer, two studies have shown H. pylori eradication to reduce the risk of gastric ulcer by over 1-2 months (37, 39). Conversely, in patients with a peptic ulcer, H. pylori eradication tended to slow gastric ulcer healing by a proton pump inhibitor (PPI) and there was no discernible effect of H. pylori eradication on duodenal ulcer healing by a PPI (40). H. pylori eradication alone had no effect on recurrence compared to placebo. H. pylori eradication alone was ineffective in preventing recurrent gastric ulcer bleeding compared to maintenance with omeprazole (41).

Gastric cancer
H. pylori infection and NSAIDs have a different effect on the risk of gastric cancer. It is well known that H. pylori infection increases the risk of gastric cancer. However, recent epidemiological studies indicate that NSAID use is associated with a decrease in the risk of gastric cancer. The most recent epidemiological evidence suggests that NSAID use not only reduces the risk of colorectal cancer but the risk of esophageal and gastric cancer as well (42). The reduction of the risk of gastric cancer is dose dependent, suggesting a greater effect with increased numbers of prescriptions of NSAIDs and longer periods of drug exposure. The mechanisms of cancer prevention are unclear but could be related to inhibition of cyclooxygenase (COX)-2 (which is increased in the presence of H. pylori infection), COX-1 and nuclear factors associated with cell cycle regulation (43).

Nonendoscopic Diagnosis of Atrophic Gastritis by Assaying the Serum Levels of Pepsinogen I, Gastrin-17 and Helicobacter pylori Antibodies

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Atrophic gastritis of both H. pylori and autoimmune origin predisposes to gastric neoplasias and impairs gastric physiology. The proper diagnosis of atrophic gastritis requires endoscopy and gastric biopsies. Nonendoscopic diagnosis of antrum-limited, multifocal atrophic gastritis or atrophic gastritis of gastric antrum has not been possible thus far. On the other hand, it is well known that the atrophic gastritis of gastric corpus and fundus can be reliably diagnosed by assaying the serum levels of pepsinogens I (PGI) or the ratio of pepsinogen I to II (PGI/PGI) (44-49).

We developed a new test panel that is targeted to nonendoscopic diagnosis of atrophic gastritis, also including the diagnosis of atrophic gastritis of
the antrum. The test panel is composed of concomitant assays of *H. pylori* antibodies (IgG), serum pepsinogen I (S-PGI) and postprandial serum gastrin-17 (S-G-17prand). The accuracy of the test panel was investigated in a study population of 100 selected dyspeptic patients. The S-G-17prand values were measured 20 min after consuming a protein-rich drink. The S-PGI decreased with an increase in the grade of corpus atrophy. A low S-PGI (<25 µg/l) was found in 32 (84%) of 38 patients with advanced (moderate or severe) corpus atrophy and in three (5%) of 62 patients without this atrophy. The S-G-17prand decreased with increasing grade of antrum atrophy, and low values (<5 pmol/l) were found in 17 (89%) of 19 patients with advanced atrophy limited to the antrum.

In the overall diagnosis of atrophic gastritis (either in antrum or corpus, or in both), the sensitivity and specificity of the test panel (*H. pylori*, PGI, G-17) was 93% (86-100%) and 91% (82-100%), respectively. It was concluded that the test panel is a conceivable tool for the diagnosis of atrophic gastritis from a blood sample, including the diagnosis of atrophic gastritis of the antrum. Correspondingly, the blood test panel provides a comprehensive nonendoscopic possibility for delineation of patients with advanced atrophic gastritis who are at risk for gastric cancer (50-62).

**Molecular Cytogenetics of Gastric Carcinoma, Relationship to Infectious Agents**

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Analysis of DNA copy number gains and losses with reference to normal control by comparative genomic hybridization (CGH) provides a rapid, reproducible study on the entire genome of tumors. A total of 16 gastric carcinomas were studied. Fresh tumor samples were disaggregated and then enriched using immunomagnetic beads. Further study by fluorescence in situ hybridization (FISH) on paraffin-embedded tissue section was performed using α-satellite probes for chromosomes 11 and 17. The results were analyzed in relation to histolytic type, lymph node metastases, presence or absence of Epstein-Barr virus (EBV) and *H. pylori*. It was found that gastric carcinomas showed DNA copy number gains more often than losses. The most common CGH aberrations of the gastric carcinoma were 19+, 17+, 1p+, 22+, 20q+. Gains of chromosome 17 were associated with lymph node metastases (*p* <0.05). Gains of chromosome 19 were associated with *H. pylori* infection. Chromosome 11 gains and 15q losses were associated with EBV (*p* <0.01). There is no significant difference between the tumors with either or both EBV and *H. pylori* compared with the tumors negative for both infectious agents.

It was concluded that molecular cytogenetics provides useful direction for further genetic studies. From the differences observed among the gastric carcinomas, we hypothesize that while EBV and *H. pylori* may act independently as initiators of gastric carcinomas, later development of the tumors may converge to a common path, merging with tumors initiated by factors unrelated to these two infectious agents.

**Gastric Cancer Prevention for a High-Risk Population: A Population Study in China**

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Gastric cancer is one of the most common malignant tumors in China. The latest data provided by the National Tumor Prevention and Treatment Office demonstrate that mortality due to gastric cancer in China is 25.16 per 100,000 inhabitants and it ranks first among all the malignant tumors (23.24%). For many years, basic and clinical research has been carried out on gastric cancer. As a result, the risk factors, geographic distribution and methods of gastric cancer prevention are now becoming better understood. It was therefore worthwhile to carry out a large-scale, comprehensive prevention trial in a population at high risk for gastric cancer.

Zhuang-he county in Liaoning province is made up of 33 towns, 365 villages and has a population of 900,000 people. It is a well-known high-risk area for gastric cancer in northern China. The mortality rate from gastric cancer is 50/100,000 in males and 22/100,000 in females. Previous studies have revealed that 93.97% of the local residents eat salted pork on a daily basis; 81.84% suffer from gas-
H. pylori infection leads to gastritis, gastric and duodenal ulcers, glandular atrophy (i.e., the loss of acid-producing parietal cells and mucus producing cells) which proceed to adenocarcinoma and MALT lymphoma. We will review recent data that suggest that H. pylori-associated corpus atrophy is due to an autoimmune process, and will provide a model for this process.

A substantial number of H. pylori patients develop autoantibodies to gastric parietal cells, in particular the canaliculi. Patients that develop the antiparietal cell autoantibodies have increased corpus atrophy; increased influx of polymorphonuclear leukocytes and B- and T-cells around gastric glands and in the epithelium; increased apoptosis of gastric epithelial cells in the corpus; a decreased occurrence of duodenal ulcers; a decreased pepsinogen I/II ratio; increased gastrin concentrations; and a decreased acid output (63). These pathological characteristics are similar to those of patients suffering from autoimmune gastritis and pernicious anemia (AIG/PA) and it is likely that prolonged H. pylori infection leads to AIG. H. pylori-associated anticanalicular autoantibodies proved to be specific for the α- and β-chain of gastric HK-ATPase, the parietal cell proton pump that is also the target autoantigen in AIG/PA and it is likely that prolonged H. pylori infection leads to AIG. H. pylori develops into a scenario: infection with H. pylori drives an autoimmune process that leads to corpus atrophy; atrophy develops into H. pylori-positive AIG; after loss of the bacteria the process becomes independent of H. pylori and a H. pylori-negative AIG arises. In analogy with experimental AIG we hypothesized that T-cells specific for HK-ATPase are central to this autoimmune process. In agreement with this hypothesis, we were able to clone HK-ATPase specific T-cells from the stomach of H. pylori-negative AIG patients (65). Most of the T-cell clones secreted...
IFN-γ and TNF-α but no IL-4, and hence are of the Th1 class. The T-cells are able to lyse Jurkat target cells by Fas-FasL-mediated apoptosis and HK-ATPase pulsed, EBV-transformed autologous B-cells by perforin. Likewise, HK-ATPase-specific T-cells were isolated from H. pylori-infected AIG patients. The epitope-specificity of the T-cells is under investigation, and to this end 15-mer overlapping peptides of the α and β-chain are being synthesized.

In summary, our data suggest that H. pylori infection induces the clonal outgrowth of HK-ATPase-specific T-cells that cause atrophy by parietal-cell cytotoxicity. Precisely how chronic infection causes outgrowth of autoreactive T-cells is under investigation, as is their epitope fine specificity. Finally, we consider it likely that host genetic factors (e.g., gene polymorphisms in cytokine and/or major histocompatibility genes) determine which H. pylori-infected patients develop gastric autoimmunity.

**Vaccine Development**

### Human Experience with Urease-Based Vaccines against Helicobacter pylori

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H. pylori colonizes the gastric mucosa of approximately half the world population, with near universal colonization of adults in developing countries. An estimated 10-20% of the infected population develop significant gastric disease, making H. pylori a worldwide public health problem of considerable magnitude (66). Although eradication therapy is available, its high cost, and emerging drug resistance together with high reinfection rates in developing countries make antibiotic therapy an ineffective approach for global control of infection (67).

The feasibility of producing an effective vaccine against H. pylori has been questioned due to the fact that natural immunity appears to be inadequate for clearing the infection. Vaccination studies in Helicobacter animal models have helped in understanding Helicobacter infection immunobiology and in the development of a human vaccine (69).

The ideal H. pylori vaccine should comprise a well-conserved, immunogenic, H. pylori antigen(s) that will be processed primarily by the MHC II pathway. It should trigger an immune response in the upper digestive tract that is probably biased towards a Th2 type, and is able to prevent initial colonization of the gastric mucosa without any harmful effects to the host.

Initial human trials established the safety of orally administered recombinant H. pylori urease (rUrease) and confirmed the need for a mucosal adjuvant to induce effective immunity (70). Recent studies have shown that oral immunization with urease with Escherichia coli heat labile enterotoxin as a mucosal adjuvant is safe and immunogenic in humans, and is able to decrease gastric bacterial load (71). However, more potent vaccines are still needed to prevent or cure H. pylori infection in humans. Further human studies will be required to define vaccine antigens, adjuvants and delivery systems.

### Development of Oral Salmonella Vaccines against Helicobacter pylori

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H. pylori infection is prevalent worldwide and is associated with gastritis, peptic ulcer, gastric cancer and MALT lymphoma. Treatment of H. pylori infection has been significantly advanced by triple or quadruple therapy combining antibiotics together with proton pump inhibitors. However, the application of this treatment was found to be limited by the drawbacks of pharmacological therapy, such as side effects, poor compliance, and most importantly, the rapid emergence of antibiotic resistance and reinfection after eradication. Prophylactic and/or therapeutic vaccination against H. pylori infection has been expected as an alternative to the current conventional treatment for preventing and controlling H. pylori infection.

DNA vaccine is a novel vaccination strategy. H. pylori DNA vaccine VR1012/UreB was developed previously in our laboratory by using an encoding vector VR1012 and urease subunit B (UreB) gene. It elicited a strong and specific humoral immune re-
spontaneous when administered by intramuscular or subcutaneous injection to mice. However, the plasmid VR1012/UreB should be encapsulated for oral administration to induce a local mucosal immune response. Recent studies have shown that attenuated Salmonella is an effective DNA delivery vehicle for DNA-mediated immunization.

Our study aimed to establish attenuated Salmonella typhimurium producing H. pylori urease subunits B and to determine whether it could be used as an oral vaccine against H. pylori.

H. pylori (SS1 attain) UreB gene fragment was amplified and transfected into attenuated S. typhimurium SL3261 to acquire SL3261/PTc01-UreB. The expression of H. pylori UreB in SL3261 was detected by Western blot. Twelve weeks after oral immunization with SL3261/PTc01-UreB and SL3261 to the control group of mice, antibody responses were evaluated by ELISA assay of serum and intestinal fluid. The supernatants of mouse spleen cell culture were collected for detection of IFN-γ and IL-10 by ELISA. In vitro stability of PTc01-UreB plasmid in S. typhimurium SL3261 was confirmed by growing to 80 generations on Luria broth medium.

It was found that the UreB gene fragment amplified by PCR was consistent with the sequence of the H. pylori UreB upon sequence analysis. Restricted enzyme digestion revealed that the correct PTc01-UreB was obtained. Western-blot showed that a 61 kD protein was expressed in SL3261/PTc01-UreB, which could be recognized by anti-UreB antiserum. Anti-UreB IgA antibodies in mouse intestinal fluid were 0.522 ± 0.16 in serum and 0.11 ± 0.25 (p <0.01) for the control group. IgG antibodies in serum were 0.37 ± 0.10 for the vaccine group and 0.14 ± 0.04 (p<0.01) for the control group. The measurement of cytokine IFN-γ was 167.53 ± 29.93 for the vaccine group and 118.74 ± 16.84 for the controls. IL-10 was 255.18 ± 27.65 for the vaccine group and 56.00 ± 7.15 (p <0.01) for the controls. After 60 generations of continuous culture, the recombinant plasmid PTc01-UreB was stable in SL3261 and showed no obvious toxicity.

This result has shown that oral immunization of mice with attenuated S. typhimurium expressing UreB (SL3261/PTc1-UreB) could induce a specific humoral and cellular immune response. It might be considered as an oral vaccine against H. pylori infection.

Immunogenetics and pathogenesis of gastric cancer

Development of a Vaccine against Helicobacter pylori

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The causative role of H. pylori infection in type B gastritis and peptic ulcer disease is now quite certain. The evidence for this conclusion comes from serological studies, demonstration of disease induction after accidental or voluntary infections of H. pylori in humans and cure of the disease by antibiotic eradication of the infection. Furthermore, substantial epidemiological evidence strongly indicates a pivotal role for the bacteria also in gastric cancer. On account of this, the World Health Organization has declared H. pylori to be a class I carcinogen.

In developed countries, the more severe forms of disease, including peptic ulcer and gastric cancer, are associated with infection by a subset of H. pylori strains (Type I) that produce a potent protein toxin (vacA) and contain a large pathogenicity island in the genome that includes the gene for cagA. In a mouse model, the cytotoxin has been shown to play a key role in H. pylori pathogenesis. Furthermore, in this model, immunization using purified cytotoxin protein or the cagA protein conferred protection from infection by Type I strains. The vacA and cagA proteins are thus strong candidates for inclusion in a vaccine against H. pylori.

As with many microbial pathogens, clinical isolates of H. pylori show a marked strain diversity which may influence virulence and limit the general applicability of vaccines based on single strain types. We are currently involved in an international collaboration to compare strain variability and disease expression between H. pylori infection in Europe and China. H. pylori strains have been isolated from patients in different regions of China and the variation in vacA and cagA gene sequences has been characterized. Chinese variants have been assessed by in vitro and in vivo models for their capacity to induce severe disease. A major vacA variant which accounts for >75% of Chinese isolates has been found to differ in cell specificity from the major variant in Europe. Both variants are however toxic in vivo. Vaccines for use in different geographic areas may need to contain local variants of the protective antigens.

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Monoclonal Antibodies: Development and Production
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Antiserum components: antibodies

In the 1930s, Tiselius and Kabat were the first to distinguish, by liquid phase electrophoresis, the difference between the sera of normal animals and of those that had been immunized. This marked the beginning of a number of studies on the properties of antibodies. Antibodies are used because of their ability to bind to a specific epitope but also because of their ability to attach, by their constant part, to cell receptors or complement.

Monoclonal antibodies

The discovery by Köhler and Milstein in 1975 of a simple technique to obtain MABs completely changed the future of their application. First used for detection of molecules in biological fluids, on the surface or inside the cells, or of pathogens, the MABs have been very helpful in medical diagnoses and fundamental biology.

In therapy, there has been some success, although limited. The technology of rodent MABs, whether mice or rats, remains identical but the development of molecular biology techniques and genetic approaches offer novel possibilities. Using monoclonal antibodies in human therapy is strictly limited by the antigenicity of the rodent antibodies. Various possible approaches for obtaining less antigenic antibodies are discussed below.

Engineering of rodent antibodies

Chimerization

Antibodies have two fragments, a constant part and a variable part. Chimerization consists of replacing the mouse or rat constant part by a human one.

Humanization

A chimerized antibody might be humanized. The binding site of the variable part of an antibody cannot be changed because it will lose its affinity for the recognized epitope. On the contrary, the framework of this part can be replaced by a human one. Humanized antibodies are entirely of human origin, except for the antigen binding sites. These antibodies might have similar avidity to the rodent antibody. Clinical studies are necessary to evaluate the acceptability of chimerized or humanized antibodies.

Direct acquisition of human antibodies

Epstein Barr virus transformation

The method of EBV transformation is easy to use. However, most of the secreted IgGs are of the IgM isotype. Cloning the transformed cells is difficult and the cell lines are generally of poor stability. Some interesting results have been obtained.

Phage library

In 1985, Smith described the use of filamentous phages to express polypeptide sequences. Nucleic acid sequences coding for these polypeptides are inserted into the gene of a protein and few copies are expressed on the surface of the phage and are involved in its surface binding on E. coli, their natural host. Libraries of genes coding for variable parts of antibodies have been formed. Messenger RNA of B lymphocytes are isolated and all the VH and Vκ regions are cloned. Each phage randomly acquires sequence coding for a variable part of the heavy chain and a variable part of the light chain. Formed antigen binding sites are screened on specific antigens.

Transgenic mice

As in other mammals, mouse genome possesses three groups of genes coding for heavy and light (κ and λ) antibody chains. It is possible to knock out these mouse genes and to replace the heavy and the κ groups of genes by human ones.

Reconstituted mice

Mice may have genetic immunodeficiency and can maintain heterografts of human cells. With or without irradiation, these animals can maintain human cells for the time of primary or secondary immune responses.

Immortalization of the production of monoclonal antibodies

Fusion with B-immortalized cells

The first fusions were carried out with lymphoblastoid cell lines or rodent lines. Unfortunately, the results have been of little interest.
Fusion with human myeloma cell line

We have prepared a cell line called Karpas 707H which seems to be excellent for producing human-human hybridomas and human antibodies.

System of production

In most cases, it is necessary to transfer the nucleic acid coding for the interesting human antibody into an expression system. Such a system can be bacteria, baculovirus and insect cells, yeast, eukaryotic cells or even mammals or plants.

Conclusions

Although it was impossible or at least difficult some years ago, the production of human MABs is now possible. A number of technologies exist that have led to these results. At present, there is no direct comparison to evaluate these methods. In the future, it will be possible to choose the best method for a given purpose. In any case, it is very likely that human MABs will be of use in transplantation, infectious diseases, cancer and many other fields, and will provide a wide scope of opportunities in the future.

WORKSHOP SUMMARY

Gastric Cancer: Its Public Health Importance

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Gastric cancer is the second most common cancer in the world and the most common cancer in China. A review of the WHO and Chinese literature on this topic shows that gastric cancer is a leading cause of morbidity and mortality in China and as such constitutes a major public health problem. A collaborative study between the Beijing Cancer Institute and the National Cancer Institute of the US National Institutes of Health has shown that some regions in China have the world's highest prevalence of gastric cancer. It accounts for 42% of deaths from all forms of cancer and shows no sign of decline in mortality since an earlier survey conducted between 1972 and 1975. The Chinese clinicians working in this field believe that gastric cancer is a public health issue of tragic proportions in China for which a range of remedial measures are urgently needed. Sino-European collaboration in this field may make an important contribution to the improvement of the health of the population.

World statistics on stomach cancer mortality and incidence show a distinct geographic variation. In addition to China, the highest rates are found in Japan, Central and South America. In Australia, Canada and the USA the rates have steadily declined and these countries now have the lowest rates of gastric cancer in the world.

The aims of this workshop were: i) to establish the state of the art in several areas of the immunogenetics and pathogenesis of gastric cancer; and ii) to create a framework for long-term collaboration between the Chinese and European experts working in the field of gastric cancer (72-77). We hope that all the investigators gathered in Xi’an, from China and Europe, will participate actively in the further development of this project.

Coordination of Cooperation Activities on the Part of the Vrije Universiteit

H. van der Erve

Vrije Universiteit, Amsterdam, the Netherlands

After several years of preparation, the Fourth Military Medical University in Xi’an and the Laboratory of Gastrointestinal Immunogenetics, Vrije Universiteit in Amsterdam formally started cooperation in the field of gastric cancer in August 1999 when a delegation of the Vrije Universiteit consisting of Prof. S. Shivananda, Dr. J.B.A. Crusius and myself, also on behalf of Prof. A.S. Peña, visited the Fourth Military Medical University. A workshop was then held on the organization of the current workshop and on further planning of future cooperation in the field of research on gastric cancer. The Fourth Military Medical University and the Vrije Universiteit decided to sign a Memorandum of Understanding to create a formal structure for future cooperation. This Memorandum of Understanding was signed by the President of the Fourth Medical University, Prof. Su Bo, and the president of the Vrije Universiteit, Dr. Wim Noomen, on February 21, 2001.

This first Sino-European Workshop on the Immunogenetics and Pathogenesis of Gastric Cancer should lead to future cooperation in this field between Chinese and European partners.
Resulting from this workshop, the most relevant aspects for further research cooperation will be selected. Future activities are to be coordinated by both the Chinese and the European partners. The Vrije Universiteit will coordinate the activities for the European partners. Vrije Universiteit is a fully subsidized private university in the Netherlands with longstanding experience in international cooperation, especially through its International Cooperation Center. This center has since the 1970s coordinated a large number of projects in countries all over the world.

The most important elements of sound coordination are for the project to be “owned” by both sides and therefore designed by both sides on an equal basis. Coordination should be divided into separate responsibilities for content and more managerial matters such as finance, logistics and planning.

Responsibilities and obligations should be clearly formulated. Activities and final outputs should be formulated in such a way that it can be easily assessed whether project objectives are reached or not. For solid and clear project organization a project supervisor and project manager will be appointed to establish a sound division of responsibilities.

The project supervisor will take care of the quality of the contents while the manager will be responsible for budget, logistics, planning of activities and delivering the input mentioned in the project document.

NOTE

A proposal of continuing collaboration is in preparation to present to the Netherlands-China Programme Strategic Scientific Alliances (PSA) with coordinating activities by the Laboratory of Immunogenetics and Department of Gastroenterology (Prof. A.S. Peña), VU University Medical Center; Department of Gastroenterology, Fourth Military Medical University (Prof. Fan Daiming and Prof. Wu Kai-chun); and Department of Gastroenterology of Zhongnan Hospital (Prof. Xia Bing), Medical College of Wuhan, University, Wuhan, China.

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