A candidate gene approach of immune mediators effecting the susceptibility to and severity of upper gastrointestinal tract diseases in relation to *Helicobacter pylori* and Epstein–Barr virus infections

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This review focuses on immunogenetic aspects of diseases of the upper gastrointestinal tract in which infectious agents may play a role in the aetiopathogenesis, such as *Helicobacter pylori,* Epstein–Barr virus (EBV) and HIV. Gastric adenocarcinoma is a common cancer all around the world, with declining incidences in Europe and high incidences in Asia and central and south America. Together with gastric atrophy and peptic ulcer disease, gastric adenocarcinoma belongs to the commonest upper gastrointestinal tract diseases. These diseases are multifactorial and factors such as smoking and dietary habits contribute to the pathogenesis. More recently, scientists have turned their eyes on the host. Functional polymorphisms in the genes regulating the host immune system may contribute to the susceptibility to and progression of disease. In multifactorial and polygenetic diseases, candidate gene studies of single nucleotide polymorphisms (SNPs) detect small to moderate relative risks. Unfortunately, only a few functional SNPs have been identified. The candidate gene approach can be seen as a useful first step in exploring causal pathways between genetic determinants and complex diseases such as those mentioned above. To date, little is known about the immunogenetics of upper gastrointestinal tract diseases. We review the literature on *H. pylori*, EBV and gene polymorphisms that affect key immune mediators influencing the pathogenesis of the inflammatory response, such as the genes that code for the IL-1 family, TNF-α, lymphotoxin alpha, and IL-10. IL-1, IL-10, lymphotoxin alpha and TNF-α polymorphisms increase the risk of upper gastrointestinal pathogenesis in *H. pylori*-infected patients, whereas IL-1 and TNF-α polymorphisms confer risk in EBV-infected patients.

European Journal of Gastroenterology & Hepatology 2005, **17**:1213–1224

Keywords: cytokine, Epstein–Barr virus, gastric cancer, *Helicobacter pylori*, immunogenetics, interleukin gene polymorphism, single nucleotide polymorphism

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Received 24 May 2005 **Accepted** 16 August 2005

Introduction

In the traditional point of view, the susceptibility to infection and the severity of ensuing disease depends mainly on environmental and microorganism-based variables. Much attention has been given to the risk of infection and transmission routes, and to the analysis of specific strains of microorganisms with potential differences in virulence, and subsequently the determination of the microorganism genes linked to the more severe phenotypes of disease. However, differences in the course of infection could not be explained by studying only environmental and microorganism-based variables. In recent years we have gained insights into the role of host genetics in infection and disease, and a prominent role for genes encoding immune mediators has been established. The emerging data on the effects of functional polymorphisms in genes encoding cytokines and chemokines on the outcome of infection have provided a wealth of information concerning the susceptibility to and severity of infection and disease. Several studies have demonstrated the effects of functional polymorphisms with different frequencies in diverse ethnic populations on infection and disease. On the basis of these studies, it can be concluded that both the infectious agent and the host immunogenetic background contribute to the determination of the outcome and clinical course of the infection [1–6].

Genetic linkage and positional cloning are appropriate for the identification of relatively high-risk genes, but this approach has not been successful for the identification of genes in complex forms of polygenic diseases such as infectious disease susceptibility and severity [7,8]. Candidate gene studies can detect small to moderate relative risks in the context of aetiological and genetic heterogeneity by studying the relevance of functional

0954-691X

single nucleotide polymorphisms (SNPs) in genes. The field that studies the host genetic background in relation to inflammation, (chronic) infection and (autoimmune) disease susceptibility and severity is called 'immunogenetics'.

Both *Helicobacter pylori* and Epstein–Barr Virus (EBV) have been associated with upper gastrointestinal disease, including gastric adenocarcinoma, the second leading cause of cancer-related deaths in the world [9–11] and other upper gastrointestinal tract diseases [12–19]. *H. pylori* has been associated with peptic ulcer disease and predominantly with the risk of developing gastric and duodenal ulcers. Several *H. pylori* virulence genes have been identified and associated with a risk of developing both gastric and duodenal ulcers [9,14,15,19–22]. The virulence-associated genes include among others the *cag* pathogenicity island. The *cag* genes are involved in the secretion of bacterial proteins and *cag*A is used as a marker for the entire *cag* locus [9]. The *cag*A gene is associated with an increased risk of developing severe gastritis, atrophic gastritis, peptic ulcer disease and distal gastric cancer [9,15,16]. *cag*A has been associated with increased oxidative stress in the gastric mucosa, which is thought to be one of the most important factors in *H. pylori*-mediated gastric mucosal damage [23]. *vac*A, encoding a vacuole formation-inducing protein, has been associated with enhanced gastric epithelial cell injury and distal gastric cancer [9,24,25], while *bab*A, involved in the binding of *H. pylori* to the gastric epithelium, is associated with an increased incidence of gastric adenocarcinoma [9,26,27]. Other well-studied virulence genes include *oip*A [28,29], *ice*A [30–33] and *nap*A [34]. To a lesser extent, associations have been described with EBV [12,17]. The EBV nuclear antigen genes have been implicated in carcinogenesis, mainly via interaction with the tumour (suppressor) genes [13,17,35–37]. The latent membrane proteins may be involved in gastric carcinogenesis [13,17], as may genes controlling the lytic replication of EBV, such as BZLF1 [38]. Abundant transcription of the EBV *Bam*HI-A rightward transcripts was found in a diversity of cancers, including gastric carcinomas. The *Bam*HI-A rightward transcripts and the short EBV RNA are the only two types of transcripts present in all EBV-associated diseases [39]. Despite extensive research, the role of EBV in gastric carcinogenesis is not completely understood.

However, most individuals infected with either *H. pylori* or EBV do not develop severe disease, strongly indicating that host factors are also involved in disease development.

It is known from recent studies that the diffuse type of gastric cancer often has a hereditary component, and *H. pylori* infection may influence carcinogenesis [40,41]. The intestinal type of gastric cancer is related to environmental exposures, such as diet and smoking, and genetic–environmental interactions between *H. pylori* and host polymorphisms have been described in the scientific literature [42–44]. Recent studies have implicated the involvement of the host immune system in the pathogenesis of gastrointestinal tract diseases and gastric cancer [14,15,45,46].

To date, comparatively little is known about the immunogenetics of upper gastrointestinal tract infections. This concerns both the effect of immunogenetics on the susceptibility to or the severity of upper gastrointestinal tract diseases, as well as the incidence and effect on function of gene polymorphisms in different ethnic populations.

So far, only five genes have been associated with upper gastrointestinal tract diseases. First, a short overview of these genes will be given providing an essential background to understand the function of these genes in disease pathogenesis, followed by an overview of the immunogenetic findings in *H. pylori* and EBV infections in upper gastrointestinal disease.

Candidate gene approach

The candidate gene approach of common SNPs can detect small to moderate relative risks in the context of aetiological and genetic heterogeneity. Epidemiological and immunogenetic principles consider the detection of association a crucial step in understanding disease aetiology, rather than the key to determining causality [7]. The first step is to identify potentially relevant genes. The selection of genes is based on a careful consideration of the current knowledge of disease phenotype, expression studies and other infectious disease models. For example, if certain immune mediators are supposed to play a role in disease phenotype, knockout and knockin models in mice could establish the importance of this particular gene. Subsequently, expression profiling of messenger RNA and protein levels in cases and controls could further confirm the role of the gene in disease. The next step would be to identify polymorphisms in this gene, which might influence the function of the gene. Once the polymorphisms are identified, larger scale studies can be performed [47,48].

When a hypothesis on the influence of potential genes on disease is formed, and potentially functional genetic polymorphisms in the genes are identified, the polymorphisms are genotyped in a case–control study. If statistically significant associations are found, then further studies (e.g. expression profiling of mRNA and protein levels) are initiated to identify the exact biological mechanisms through which the genetic polymorphisms influence the disease pathogenesis. Such studies will also provide information on whether this polymorphism is

directly related to the disease pathogenesis or is linked to another polymorphism, which may influence disease. This kind of linkage is referred to as linkage disequilibrium. Linkage disequilibrium (or 'allelic association') describes the tendency of alleles to be inherited together more often than would be expected under random inheritance (e.g. the C allele of *IL-1B*$^{-511}$ is found in 99.5% of cases together with the T allele of *IL-1B*$^{-31}$. Under random segregation, the C allele would have been observed in approximately 50% of the cases with the T allele of *IL-1B*$^{-31}$ and approximately 50% of the cases with the C allele of *IL-1B*$^{-31}$). Associations with a studied polymorphism may thus be caused by the 'linked' polymorphism. This knowledge may one day be used to develop diagnostic tests to identify patients at high risk of disease development or an adverse outcome of disease.

Immune mediators

Several key immune mediators have been studied in relation to gastrointestinal pathogenesis. These include the interleukin (IL)-1 family, IL-10, lymphotoxin alpha (LTA) and tumour necrosis factor (TNF)-α. These cytokines are involved in the regulation of nuclear factor kappa B (NF-κB), which is of paramount importance in the regulation of the inflammatory response, and are described and discussed below.

IL-1 family

Protein

IL-1 consists of four genes coding for IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra) [49] and the IL-1-like protein (IL-1L1) [50,51]. IL-1 is involved in a wide variety of physiological processes, including the regulation of inflammatory, metabolic, haematopoietic and immunological mechanisms. It is produced by macrophages, neutrophils and endothelial cells.

IL-1β initiates the expression of several genes coding for lymphokines. It induces natural killer cells and activates T and B cells [52,53]. IL-1β is a powerful inhibitor of gastric acid secretion and upregulates the expression of cyclo-oxygenase 2 [54].

IL-1ra is the natural inhibitor of IL-1β and is produced by the same cell types as IL-1β. For a 50% inhibition of IL-1, a 10 to 500-fold excess of IL-1ra is required [55].

The binding of IL-1 to the IL-1 receptor induces a signal, via NF-κB-responsive genes in the nucleus, leading to cellular responses such as the stimulation of prostaglandin E_2 synthesis, the upregulation of COX genes [54,56,57], the stimulation of collagenase production and the cytoadherence of leucocytes to endothelial cells [58,59].

The binding of IL-1ra to the IL-1 receptor induces no signal transduction and blocks the receptor for IL-1 binding, thus effectively inhibiting IL-1 function [59]. IL-1 homologues have been described, although their biological roles are as yet unknown [50,51,60].

A graphical representation of IL-1 function is given in Figure 1.

Polymorphisms of IL-1

Many SNPs have been reported in the *IL-1B* gene; however, three SNPs, at positions −511 (*IL-1B*$^{-511}$), −31 (*IL-1B*$^{-31}$) and +3954 (*IL-1B*$^{+3954}$) [61–63], are frequently studied. The polymorphisms at positions −511 and −31 are in 99.5% linkage disequilibrium [64–66].

The *IL-1B*$^{-31}$ SNP is located in a TATA box and influences DNA–protein interactions [64,67]. Alleles of *IL-1B*$^{-511}$ (via linkage disequilibrium with *IL-1B*$^{-31}$) and *IL-1B*$^{+3954}$ have been associated with increased IL-1β production.

The *IL-1RN* (receptor antagonist) gene contains a hepta-allelic 86 bp variable number of tandem repeats at position +2951 (in intron 2) [52,55,68]. The most common allele (allele 1) in all populations studied thus far contains four repeats of the 86 bp fragment [64,69]. The second allele of the variable number of tandem repeats is in near perfect linkage disequilibrium with the rare allele of the *IL-1RN* +2018 T > C SNP. This polymorphism has been associated with the increased expression of IL-1RN [68,70,71].

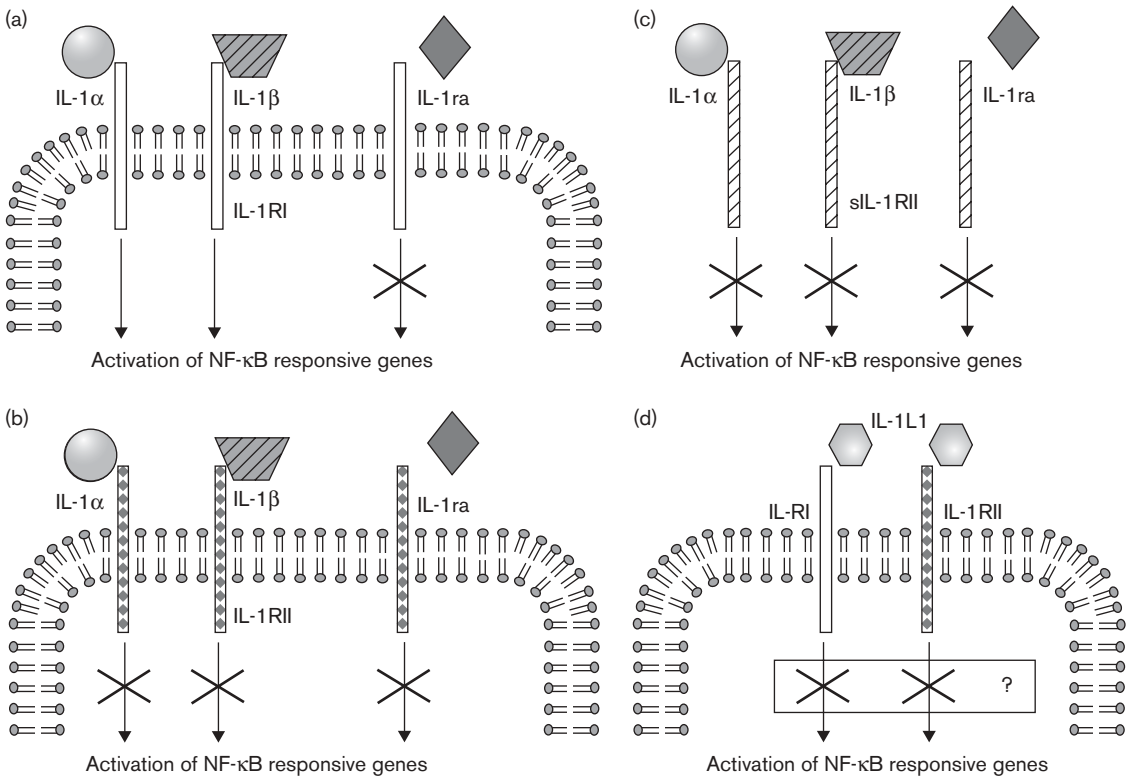
Variations in allele frequencies were found in different ethnic and geographical populations. However, allele 1 is the most common in all populations, and alleles 1 and 2 are present in over 90% of the population [72–74].

Helicobacter pylori

IL-1β is known to be a potent inhibitor of gastric acid [65] and may, through this inhibition, provide a better gastric environment for *H. pylori*. The less frequent T allele of the *IL-1B*$^{-511}$ SNP has been associated with increased mucosal IL-1β levels [75] and decreased acid secretion [76] in Japanese individuals. In a Portuguese study [77], both *IL-1B*$^{-511}$*T and *IL-1RN**2* were associated with an increased risk for gastric carcinoma, especially in patients with high-risk bacterial virulence types (*vac*As1, *vac*Am1 and *cag*A) [24]. A similar association was found in a Chinese population for *IL-1B*$^{-511}$ T/T in *H. pylori*-infected patients [77].

The *H. pylori* virulence genes *oip*A and *cag*A have been associated with gastritis activity, peptic ulcer and gastric adenocarcinoma. Increased frequencies of heterozygous carriers of the *IL-1B*$^{-31}$ and *IL-1RN* SNP were found when compared with gastritis patients, whereas intestinal metaplasia was associated with *cag*A, functional *oip*A and the *IL-1RN*2* allele [29].

Fig. 1



IL-1 induced activation of nuclear factor kappa B responsive genes. (a) Both IL-1α and IL-1β induce a proinflammatory response, when bound to IL-1RI. However, binding of IL-1 receptor antagonist (IL-1ra) blocks signal transduction of IL-1RI and inhibits IL-1 signalling. (b) Non-functional, membrane-bound IL-1RII. No signalling occurs because of the non-functionality of the receptor. The same is shown in (c) for the soluble IL-1RII. (d) The IL-1-like protein (IL-1L1) induces no signalling; however, the literature is conflicting on the subject. NF-κB, Nuclear factor kappa B.

Hamajima and colleagues [66] associated *IL-1B*$^{-31}$*T with persistent *H. pylori* infection. In their article Hamajima *et al.* [66] defined the T allele as the mutant allele; however, the T allele represents the normal (wild-type) allele [78]. The associations were made with the correct alleles, although the interpretation is reversed.

In a recent article, Queiroz and colleagues [79] demonstrated associations between *IL-1RN**2, *IL-1B*$^{-31}$*C, *H. pylori cag*A positivity and the degree of corpus gastritis and protection against reflux disease. The likely model is reduced gastric acid production caused by all of these factors. These data corroborate increasing evidence that infection with virulent *H. pylori* may protect against reflux disease [79].

Epstein–Barr virus

The group of zur Hausen *et al.* [80] studied *IL-1B* polymorphisms and EBV in Dutch gastric carcinoma patients, and showed that *IL-1B*$^{-511}$ heterozygotes were at a decreased risk of gastric carcinoma and that this association was not related to the EBV status of the patients. On the basis of these results, the authors postulated that this suggests an early common molecular mechanism for the development of EBV-positive and negative gastric carcinomas.

Tumour necrosis factor alpha

Protein

TNF-α is a multifunctional proinflammatory cytokine, with effects on lipid metabolism, coagulation, insulin resistance, and endothelial function.

It plays a crucial role in host defence against infection, but high concentrations of TNF-α may cause severe pathology, such as epithelial injury.

The TNF-α gene is located within the highly polymorphic class III region of the major histocompatibility complex.

Polymorphisms of TNF-α

TNF-α is partly regulated at the transcriptional level, and studies have implicated polymorphisms in the promoter region as potential determinants of disease susceptibility.

The best-studied mutations are three A to G transitions at positions −376, −308 and −238 in the TNF gene.

The SNPs at positions −308 and −238 have been reported to increase TNF-α production [64,81–83]. However, the effects of the *TNF-A* promoter polymorphisms on TNF-α production remain somewhat controversial [84]. Verweij and colleagues [85,86] reported that several polymorphisms, including $TNF\text{-}A^{-308}$, have no effect on gene transcription, even though other researchers have found positive effects.

Posch *et al.* [87] identified a total of 52 unique *TNF–LTA–HLA* haplotypes. Some *TNF* and *LTA* alleles appear to be associated with *HLA* haplotypes; however, not with specific *HLA* alleles. The majority of the SNPs appear to be randomly associated within and between the loci, except for the *LTA* SNPs at positions −293, +81 and +369. Posch *et al.* [87] suggested that these observations may provide an explanation for the contradictory results of studies associating individual cytokine gene SNPs with expression levels, HLA and disease.

Helicobacter pylori

In a Korean population, Yea and colleagues [81] found that the A allele of $TNF\text{-}A^{-308}$ was associated with CagA-positive *H. pylori* infection in patients with gastric disease, including carcinoma, gastric atrophy, gastric ulcers and duodenal ulcers.

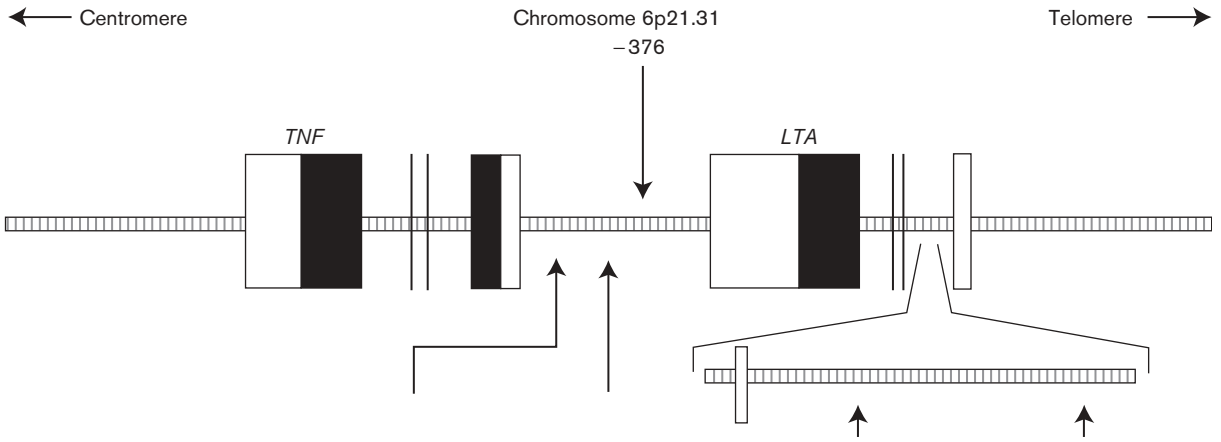
The TNF-I haplotype (Fig. 2) is considered a risk factor for peptic ulceration in *H. pylori*-positive patients, whereas the $TNF\text{-}A^{-308}$*A allele was associated with a decreased risk of gastric ulceration in a Spanish Caucasian population [88,89]. Carriage of the $TNF\text{-}A^{-1031}$*C and $TNF\text{-}A^{-863}$*A alleles is associated with an increased risk of ulcers after *H. pylori* infection and an increased risk of intestinal metaplasia in gastric ulcer patients [90].

The proinflammatory genotypes of *IL-1B*, *IL-1RN* and *TNF-A* are associated with the risk of non-cardia gastric cancer [45,65,91]. In particular, the combination of the carriership of $IL\text{-}1B^{-511}$*T, *IL-1RN*2* homozygosity and $TNF\text{-}G^{-308}$ defines a high-risk genetic profile for both chronic atrophic gastritis and gastric cancer data [92].

Epstein–Barr virus

Wu and co-workers [93] studied the effects of *TNF-A* and *IL-10* polymorphisms in a Han Chinese population in Taiwan. Both EBV-positive and negative gastric carcinomas were compared with unaffected control subjects.

Fig. 2



Haplotype	− 238	− 308	M.S.	*Asp*HI	*Nco*I
TNF - C	G (1)	G (1)	1	2 (L)	1 (S)
TNF - E	G (1)	A (2)	1	2 (L)	1 (S)
TNF - H	A (2)	G (1)	2	2 (L)	2 (L)
TNF - I	G (1)	G (1)	2	2 (L)	2 (L)
TNF - P	G (1)	G (1)	1	1 (S)	2 (L)

Graphic representation of the *TNF* and *LTA* genes. Open boxes represent untranslated parts of the exons. Closed boxes represent translated parts. Arrows show the positions of the bi-allelic *Asp*HI and *Nco*I restriction fragment-length polymorphisms in the first intron of the *LTA* gene and the single nucleotide polymorphisms at positions −238, −308 and −376 in the promoter region of the *TNF* gene. M.S., Microsatellite. Combinations of the alleles of these sites form the haplotypes: TNF-C, -E, -H, -I and -P. Figure adapted from Lanas *et al.* [88] and Peña *et al.* [89].

Wu *et al.* [93] found that the high-producer allele *TNF-A*$^{-308}$A was significantly associated with EBV-positive gastric carcinoma patients, whereas the high-producer allele *IL-10*$^{-1082}$G was significantly higher in EBV-negative gastric carcinoma patients.

Associations of *TNF-A* could also be caused by linkage with other genes, for example the HLA system. The *TNF-A*$^{-308}$A polymorphism has been shown to be in a strong linkage disequilibrium with HLA haplotypes A1, B8, DR3 in Caucasian patients [93].

Lymphotoxin alpha

Protein

Lymphotoxin alpha, previously known as TNF-β, is a soluble protein secreted by activated lymphocytes and is presumed to act as a modulator of the immune response.

Both lymphotoxin alpha and TNF-α play a role in the formation of lymphoid tissue, and the influences of either lymphotoxin alpha or TNF-α on immune responses may thus be either via direct signalling or via the influence on the organogenesis of lymphoid tissue [94,95].

Polymorphisms of lymphotoxin alpha

Two haplotypes are most often studied in the *LTA* gene, namely in intron 1 at position +368 (*Asp*HI restriction fragment-length polymorphism) and at position +252 (*Nco*I restriction fragment-length polymorphism) (Fig. 2).

Helicobacter pylori

Kunstmann *et al.* [96] reported an association between *LTA Nco*I allele 2 and the prolonged survival of patients with gastric cancer. Lanas *et al.* [88] found an increased frequency of the *LTA Nco*I 2.2 genotype and a lower frequency of carriers of the *TNF-E* haplotype characterized by the *TNF-A*$^{-308}$A in *H. pylori*-positive gastric ulcer patients, when compared with *H. pylori*-positive duodenal ulcer patients. Gastric ulcer patients had a higher frequency of the *TNF-I* haplotype compared with controls, whereas *TNF-E* haplotype carriers were more frequent among duodenal ulcers compared with gastric ulcers. Carriership of the *TNF-I* haplotype was identified as an independent risk factor for peptic ulceration in *H. pylori*-infected patients. These data show that the *TNF* and *LTA* gene polymorphisms and their haplotypes are related to peptic ulceration and may play a role in the pathogenesis of *H. pylori* infection [88].

Interleukin 10

Protein

IL-10 is an important regulator of the function of lymphoid and myeloid cells. It suppresses both cytokine production and antigen-specific proliferation of T helper type 1 cells (Fig. 3). Apart from inhibiting inflammatory mediators, IL-10 suppresses IL-10 production by monocytes and macrophages, suggesting a negative feedback regulation. Furthermore, IL-10 inhibits IL-1β production [97,98]. The ability of IL-10 to suppress inflammatory cytokines, while at the same time upregulating anti-inflammatory IL-1ra, suggests that IL-10 is a potent anti-inflammatory cytokine [97,99,100].

Polymorphisms of IL-10

The polymorphisms at positions −2849 (C > T), −819 (C > T), −592 (C > A), and the IL-10.G (CA repeat microsatellite) in the promoter region of the *IL-10* gene, have been most frequently studied and associations have been found with both high and low IL-10 production [64,101–104]. The influence of the polymorphisms at positions −819 and −592 is yet unclear. Both an increase and decrease in IL-10 production has been reported to be associated with distinct alleles of these polymorphisms [64,103,105].

Remarkable interindividual variations in levels of IL-10 mRNA and serum protein have been described and attributed to genetic control by the polymorphisms in the IL-10 gene promoter [106].

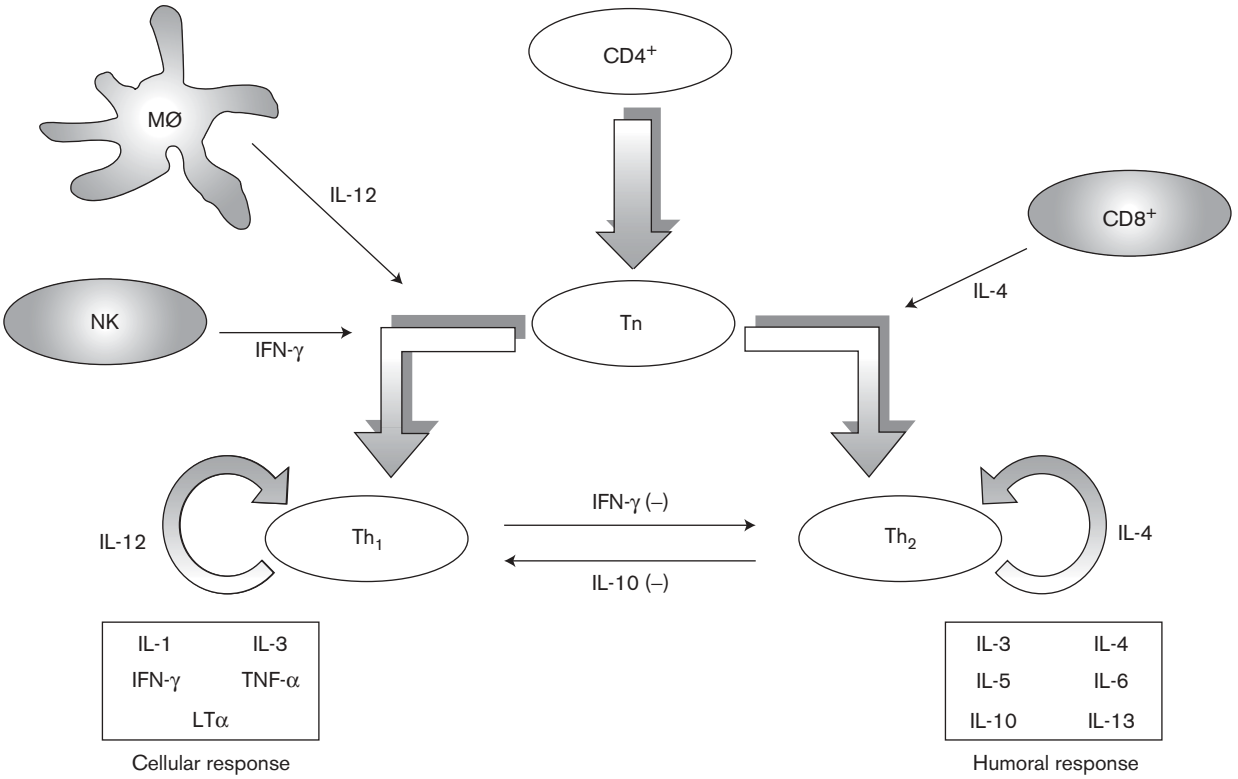
Helicobacter pylori

Wu and colleagues [107] demonstrated that *H. pylori*, smoking and the IL-10 high-producer haplotype (−1082*G/−819*C/−592*C) were independent risk factors for the development of gastric cancer. When these factors were combined increased odds ratios of 2.14–6.00 were found. Rad *et al.* [108] demonstrated elevated levels of mucosal IL-10 mRNA in carriers of the *IL-10 −1082*G/−819*C/−592*C* (GCC) haplotype when compared with ATA haplotype carriers, and showed that carriage of the GCC haplotype was associated with colonization by more virulent *cag*A$^+$, *vac*As1$^+$, and *bab*A2$^+$ *H. pylori* strains. However, Hellmig and colleagues [109] were unable to confirm the results of Rad *et al.* [108] in a similar population. The results of Hellmig *et al.* [109] are comparable with those of Hida *et al.* [110], and further research is necessary to clarify the role of IL-10 in *H. pylori* infection and gastric pathogenesis. High levels of IL-10 may limit an efficient immune response, clearing the way for a more persistent infection. *Helicobacter*-induced tissue damage may thus increase the chances of the development of dysplasia, metaplasia and finally carcinogenesis. In contrast, low levels of IL-10 may not put an adequate stop to the immune response once the infection has been cleared, and may lead to an out-of-control immune reaction, resulting in tissue damage or auto-immune responses.

Chronic inflammation

In the 1970s and 1980s researchers found a more common occurrence of gastric carcinomas in patients with atrophic gastritis with intestinal metaplasia. On the basis of these

Fig. 3



T helper 0–T helper 1–T helper 2 balance. IL-12 and IFN-γ induce T helper type 1 differentiation of naive T cells, whereas IL-4 induces T helper type 2 differentiation. IFN-γ and IL-10 regulate the T helper types 1–2 balance. CD, Cluster of differentiation; LTα, lymphotoxin α; MØ, macrophage; NK, natural killer cell; Th, T helper cell; Tn, naive T cell.

study results, Correa [111,112] postulated that chronic infection may result in the development of gastric carcinomas and the 'atrophy–metaplasia–dysplasia–carcinoma' sequence hypothesis was developed. The article by Houghton and colleagues [11] strengthens this hypothesis. This hypothesis is currently a widely used model for gastric carcinogenesis. However, Meining *et al.* [113] reviewed studies showing that diffuse-type carcinomas arise from gastritis that is not associated with intestinal metaplasia and atrophy. Furthermore, Meining *et al.* [113] postulated that intestinal metaplasia appears to be a paracancerous rather than a precancerous condition. The 'gastritis of the carcinoma phenotype' concept has been developed as an alternative to the 'atrophy–metaplasia–dysplasia–carcinoma' sequence hypothesis [113]. The 'atrophy–metaplasia–dysplasia–carcinoma' hypothesis is mainly applicable to gastric adenocarcinomas (mostly non-cardia carcinomas), whereas the 'gastritis of the carcinoma phenotype' concept is applicable to diffuse-type gastric carcinomas. Both hypotheses may thus provide an accurate model for these two types of gastric cancer, and a combination of both hypotheses may provide an accurate model for gastric carcinogenesis in general.

Discussion

The rapid advances in the field of human genetics have offered new opportunities to investigate the role of various immune mediators in disease susceptibility. It is to be hoped that knowledge of the role that host mechanisms play in the pathogenesis of infectious diseases will contribute to the design of new therapeutic strategies.

The *IL-1B*$^{-31}$*C, *IL-1B*$^{-511}$*T and *IL-1RN*2* alleles have been associated with a risk of the development of gastric cancer [24,45,114,115]. The C allele and T allele of *IL-1B*$^{-511}$ and *IL-1B*$^{-31}$, respectively, have been associated with (sero-) positivity for *H. pylori* [66,116], whereas the *IL-1B*$^{-511}$*T and *IL-1RN*2* have been associated with increased mucosal IL-1β levels [29,75,76,117,118]. The *IL-10*$^{-1082}$*G allele may facilitate *H. pylori*-positive gastric cancer by downregulating inflammatory responses. The interplay between the host cytokine genotypes and the *H. pylori* virulence genes probably determines whether *H. pylori* infection leads to mild, severe or carcinogenic disease.

Table 1 Overview of key immunogenetic studies on the influence of *IL-1*, *IL-10*, *TNF-A* and *LTA* polymorphisms on *Helicobacter pylori* and Epstein–Barr virus infection

Gene	*H. pylori*		EBV	
	Author	Results	Author	Results
IL-1	El-Omar *et al.*, 2001 [65]	*IL-1B*$^{-31}$*T and *IL-1RN* 2.2 carriage increases the likelihood of a chronic hypochlorhydric response to *H. pylori* infection and the risk of gastric cancer (OR 2.5 and 3.7)	zur Hausen *et al.*, 2003 [80]	The frequency of *IL-1B*$^{-511}$ heterozygotes was significantly lower in gastric cancer patients. In the patient group men were significantly more frequently EBV positive compared with women
	Machado *et al.*, 2001 [45]	*IL-1B*$^{-511}$*T and *IL-1RN* 2.2 carriers at increased risk of the development of intestinal type gastric cancer (OR 2.7 and 3.1). *IL-1B*$^{-31}$*T + *IL-1RN**2 increases risk even further (OR 9.0)		
IL-10	Wu *et al.*, 2003 [107]	The high producer genotype of *IL-10* (GCC) was significantly associated with gastric cancer	Wu *et al.*, 2002 [93]	*IL-10*$^{-1082}$*G associated with EBV-negative gastric cancer
			Helminen and colleagues, 2001/1999 [102,121]	The *IL-10* ATA haplotype confers protection against primary EBV infection
TNF-A	Lanas *et al.*, 2001 [88]	Gastric ulcer patients had a decreased expression of *TNF-A*$^{-308}$*A compared with controls	Wu *et al.*, 2002 [93]	The high-producer allele of *TNF-A* (*TNF-A*$^{-308}$*A) is associated with gastric cancer in EBV-positive patients
	Lu *et al.*, 2005 [90]	*TNF-A* promoter polymorphisms increased the risk of ulceration in *H. pylori*-infected patients and increase the risk of intestinal metaplasia in gastric ulcer patients		
LTA	Lanas *et al.*, 2001 [88]	Homozygous carriage of the *LTA Nco*I mutation increases the risk for gastric ulceration		
Combined genotypes	El-Omar *et al.*, 2003 [91]	Carriage of single SNP in *IL-1B*, *IL-1RN*, *TNF-A* and *IL-10* had increased risk of gastric cancer (OR 2.8). Combined carriage of multiple SNP increased the OR to 27.3		
	Machado *et al.*, 2003 [92]	Combined carriage of *IL-1B*, *IL-1RN* and *TNF-A* increases risk of chronic atrophic gastritis and gastric cancer		
	Lanas *et al.*, 2001 [88]	Combined carriage of *LTA* and *TNF-A* polymorphisms in the *TNF-I* haplotype increases the risk of gastric ulceration in *H. pylori*-infected patients		

EBV, Epstein–Barr virus; OR, odds ratio; SNP, single nucleotide polymorphism.

The gastric cancers in the Taiwanese EBV-negative group, described by Wu *et al.* [93], may thus be related to *H. pylori* infection or perhaps other carcinogenic factors [119]. Increased levels of IL-10 and IL-12 mRNA were found in *cag*A-positive *H. pylori*-infected patients. The increased IL-12 mRNA levels were associated with peptic ulceration, which may suggest a predominance of T helper type 1 responses [110], a situation that is comparable with that reported for Crohn's disease [59]. The release of TNF-α may lead to the observed cellular responses in EBV-associated malignancies [93]. Three *IL-10* promoter polymorphisms (−1082 G/A, −819 C/T and −592 C/A) were studied by Helminen *et al.* [102]. The ATA haplotype was associated with higher IL-10 levels and with EBV seronegativity [102]. This result is in contradiction with another study by Turner and colleagues [120], which showed decreased IL-10 production in ATA haplotype carriers. Turner *et al.* [120] measured IL-10 production in in-vitro stimulated peripheral blood lymphocytes, whereas Helminen *et al.* [102] measured spontaneous IL-10 plasma levels. Helminen and colleagues [102] stated that EBV-seronegative individuals are more often carriers of the G allele of *IL-10*$^{-1082}$, and that carriage of the A allele increases susceptibility to EBV and is a risk factor for a more severe clinical picture [121]. High IL-10 production has been associated with the *IL-10*$^{-1082}$*A allele and it has been suggested that the high IL-10 levels favour prolonged *H. pylori* infection [98,122,123]. High IL-10 levels may thus increase the risk of gastric carcinogenesis either through *H. pylori* or EBV infection. The conflicting results of Rad *et al.* [108], Hellmig *et al.* [109] and Hida *et al.* [110] indicate the necessity of further studies (see Table 1).

Baas *et al.* [124] reported an increased occurrence of cancer in the intact stomach, associated with *H. pylori* infection. In contrast, EBV infection is more common in gastric stump cancers, which has been reported before in Asian populations and was confirmed by Baas and colleagues [124] in a western population. A recent article by zur Hausen *et al.* [125] provided evidence that EBV plays a late role in gastric carcinogenesis, although the exact role of EBV in gastric carcinogenesis remains unknown. Combining these data, with immunogenetic insights, may provide a better understanding of gastric carcinogenesis and of *H. pylori* or EBV-related gastrointestinal tract diseases.

Future perspectives

Recently discovered immune mediators

Interesting host immunogenetic factors, not or barely studied in relation to upper gastrointestinal tract infections, include the recently described Toll-like receptors (TLRs) [126]. TLR4 could be an important candidate gene. It is a receptor for among others lipopolysaccharide. However, Bäckhed *et al.* [127] reported that gastric mucosal recognition of *H. pylori* is TLR4 independent. The lipopolysaccharide of *H. pylori* is hypoacylated and is therefore not recognized by human TLR4 [128–130]. Both TLR2 and TLR4 are upregulated during intestinal inflammation [131]. Bäckhed and Hornef [128] postulated that a tight regulation of TLR4 responses may prevent excessive immune responses to lipopolysaccharide [128]. These results are corroborated by Smith *et al.* [132] who reported associations between both TLR2 and TLR5 and *H. pylori*-induced NF-κB activation, and the lack of association between TLR4 and *H. pylori*-induced NF-κB activation.

In contrast to these reports, other studies have found TLR4 in gastric cells and have found TLR4-dependent signalling in *H. pylori*-associated gastritis. Further research is necessary to clarify the role of TLR4 in gastric infection.

Other TLR family members may be involved in gastric inflammation. For example, TLR5 recognizes bacterial flagellin and may thus recognize the *Helicobacter* flagellae [133]; however, according to data by Schmausser *et al.* [134] TLR5 is only expressed on the basolateral membrane of the gastric epithelium during *H. pylori* infection. TLR5 could then possibly play a role in the identification of invading bacteria in gastric lesions. Other agonists are recognized by additional TLR family members: bacterial tri-acyl lipopeptides (TLR1), peptidoglycan and human HSP70 (TLR2), bacterial flagellin (TLR5) and bacterial CpG DNA (TLR9) [126].

Multiple polymorphisms and multiple genes

Multiple polymorphisms in one gene could in an increasing manner influence gene expression, protein expression or protein function. For example, several mutations in the TLR4 gene were studied, but no single variant was significantly associated with meningococcal sepsis. However, when multiple rare mutants in the TLR4 gene were combined, an over-representation of these rare mutants was found in systemic meningococcal infected patients [135]. Combinations of multiple polymorphisms across multiple genes may influence pathogenesis. For example, El-Omar *et al.* [91] showed that the carriage of multiple proinflammatory polymorphisms conferred a greater risk of the development of gastric cancer, with increasing odds ratios reaching 27.3 in high-risk genotypes.

Conclusion

Combining the aforementioned studies and the different research methods provides a valuable insight into complex and dynamic host–pathogen interactions. This insight may help to further the understanding of upper gastrointestinal tract pathophysiology and may help identify new research targets and define potential therapeutic targets.

The generated knowledge may benefit the development of medicine and perhaps in future help to establish 'made-to-measure' treatments for patients.

Acknowledgements

The authors would like to acknowledge the PhD-fellowship of AstraZeneca for Sander Ouburg.

Conflict of interest

Sander Ouburg is an AstraZeneca BV (Zoetermeer, the Netherlands) Fellow.

Authors' contributions

S.O. wrote the manuscript. B.C. and S.M. (researchers) critically reviewed the manuscript and especially the technical/immunogenetic aspects. E.K. and C.M. (gastroenterologists) critically reviewed the manuscript and especially the gastroenterological/medical aspects. S.P. (gastroenterologist/researcher) critically reviewed the manuscript with great expertise in both gastroenterology and immunogenetics.

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