A pro-inflammatory genotype predisposes to Barrett’s esophagus


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Introduction: Severity of mucosal inflammation is shown to be associated with Barrett’s esophagus (BE) development in animals. It has therefore been postulated that a strong pro-inflammatory host response predisposes to BE. Aim: To determine the impact of cytokine gene polymorphisms on the development of BE. Methods: The multiplex SNaPshot™ method was used to determine inter-leukin (IL)-12B (A+C174), IL-10 (C-592A, C-819T, A-1082G), IL-8 (A-251T), IL-6 (G-174C) and IL-2 (G-330T) gene polymorphisms in 255 patients with BE and 247 patients with reflux esophagitis (RE). Results: The presence of the IL-12B C-allele, which is associated with increased IL-12p70 expression, was more frequently observed in BE than in RE patients [odds ratio (OR) 1.8; 95% confidence interval (CI) 1.2–2.7; P = 0.007]. The risk of BE was increased in patients in whom the IL-12B C-allele coincided with a hiatal hernia (OR 2.9; 95% CI 1.32–6.58; P = 0.008). The IL-10-1082 GG genotype, which is associated with higher IL-10 levels, was also associated with a decreased risk of BE when it was associated with the IL-12B C-allele, indicating IL-10-dependent down-regulation of IL-12p70 expression. A combination of the IL-12B AA genotype and the IL-10 AA or AG genotypes was associated with BE (OR 1.4; 95% CI 1.05–1.85; P = 0.011). Conclusion: A genetic profile predisposing to a strong pro-inflammatory host response, mediated by IL-12p70 and partially dependent on IL-10, is associated with BE. This risk further increases when this genotype coincides with a hiatal hernia, suggesting that exposure to gastroesophageal reflux in the presence of a pro-inflammatory genetic background is a driving force in the development of BE.

Introduction
Chronic inflammation predisposes to the development of gastrointestinal malignancies (1,2). This is also true in the esophagus, where the progression from reflux esophagitis (RE) toward esophageal adenocarcinoma follows a sequence of different stages including esophagitis, Barrett’s esophagus (BE), dysplasia and eventual esophageal adenocarcinoma. The presence of BE is associated with a 0.5% annual risk of developing esophageal adenocarcinoma (3) and is therefore the most important risk factor for this disorder.

There is increasing evidence that a more severe esophageal inflammation predisposes to the development of BE. In animal models, the inflammation was more intense in animals developing BE and esophageal adenocarcinoma when compared with animals that did not (4,5). Progression toward BE could be prevented in these animals by the use of cyclooxygenase-2 inhibitors, which reduced the level of mucosal inflammation. BE is associated with a more predominant humoral immune response (Th2) with increased expression of interleukin (IL)-4 and IL-10, whereas RE was associated with a cellular immune response (Th1) expressing high IL-1β, IL-8 and IFN-γ (6,7). In a BE segment, the proximal region was associated with more increased levels of pro-inflammatory cytokines as compared with the distal region (8). The distal region on the other hand was associated with increased expression of regulatory cytokines (8). This suggests that the change in mucosal lining of the esophagus may be paralleled by a shift in mucosal immune response and may be preceded by a strong pro-inflammatory mucosal immune response. Levels of cytokine expression and interaction between key cytokines and inflammatory cells direct mucosal immunity, and thereby the outcome of disease (9). It is therefore conceivable that genetic regulation of cytokine expression levels, resulting in increased or decreased levels of these cytokines in the esophageal mucosal immune response, may alter individual susceptibility to BE.

Single-nucleotide polymorphisms (SNPs) have been reported in the genes encoding for IL-12B, IL-10, IL-8, IL-6 and IL-2, and these SNPs are associated with altered expression levels of these inflammatory mediators. The C-residue at position +1188 in the IL-12B gene encoding for the IL-12p40 subunit is associated with high expression of the pro-inflammatory cytokine IL-12p70 by monocytes (10,11). Expression of the regulatory cytokine IL-10 is partially dependent on polymorphisms in the IL-10 promoter, with the IL-10 C-592C G-1082T (CCG) allele being associated with the highest mucosal IL-10 levels as compared with the IL-10 CCA and ATA haplotypes (12–14). The A-residue at position –251 in the gene encoding for IL-8 is associated with a 2- to 5-fold increased transcription of IL-8 (15–17), the G-residue at position –174 in the promoter of IL-6 with lower IL-6 expression by inflammatory cells (18) and the IL-2 G-residue at position –330 with lower IL-2 expression (19).

Mucosal IL-8 and IL-6 levels have been demonstrated to be induced by gastroesophageal reflux and are increasingly expressed in BE (7,20–22). Whether a severe pro-inflammatory immune response also predisposes to the development of BE in humans has not been established yet. This is partially due to the fact that progression from RE toward BE is only infrequently observed in the clinical situation (23), and the majority of patients already have developed BE at the initial endoscopic examination. We postulate that genetic factors controlling the mucosal inflammatory response could affect the individual susceptibility for the development of BE. This study therefore assessed the association between polymorphisms encoding for higher or lower levels of IL-12, IL-10, IL-8, IL-6 and IL-2 and BE. As gastroesophageal reflux is the key mechanism of mucosal inflammation, we included patients with RE as controls and compared them with patients with BE.

Materials and methods

Study design

All eligible Caucasian patients referred to the endoscopy unit of the Erasmus MC—University Medical Center Rotterdam and the IJsselland Hospital, Capelle aan de IJssel, the Netherlands between November 2002 and February 2005 were invited to participate in this study. The study was approved by the institutional ethical review boards of both hospitals. Participants were included when they either had BE or RE without the presence of BE. In total, 1186 gastroscopies were performed in patients referred to the endoscopy unit for the evaluation of reflux-related symptoms, odynophagia or dysphagia, suspected extraesophageal manifestations of gastroesophageal reflux disease or surveillance of BE. Eleven hundred and fifteen patients gave written informed consent (response rate 94%, 96% for RE patients and 89% for BE patients). Most patients were invited to participate in this study after upper endoscopy had been performed and RE or BE was diagnosed. Eighty-five percent of the included BE patients already participated in a surveillance program and were invited to participate in this study prior to endoscopy by a postal letter.
Table I. Primers used for genotyping of the studied polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primers</th>
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<tr>
<td>IL-10 – 592</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
</tr>
<tr>
<td>IL-10 – 819</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
</tr>
<tr>
<td>IL-10 – 1082</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
</tr>
<tr>
<td>IL-12B +1188</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
</tr>
<tr>
<td>IL-8 – 251</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
</tr>
<tr>
<td>IL-6 – 174</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
</tr>
<tr>
<td>IL-2 – 330</td>
<td>Forward: 5′-AGCTGAAAGTTGGAACCAT-3′&lt;br&gt;Reverse: 5′-TACCTCAAAAGTCCCACAAGC-3′&lt;br&gt;SB: 5′-AATCTAATCCAGCTGTCATTTTATTTTACAGACAGCTC-3′&lt;br&gt;Reverse: 5′-TGAACCCCTACGTCCTCTATT-3′</td>
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SBE, single-base extension.
not significantly associated with the presence of BE (Table III) \( (P = 0.078) \). Only the IL-10-1082 GG genotype was more frequently observed in patients with RE \( (74/247; 30\%) \) than in patients with BE \( (54/255; 21\% \) OR \( 1.6; 95\% \ CI 1.06–2.39; \( P = 0.024) \) (Table III).

The association between the IL-12B C-allele and BE remained significant in a multivariate analysis \( (OR 1.8; 95\% \ CI 1.17–2.69; \( P = 0.007) \), but the IL-10-1082 GG genotype and RE did not (Table IV). The association between the IL-12B C-allele and BE was also significant after correction for multiple comparisons by the conservative Bonferroni correction. The IL-12B C-allele was not associated with gender, age, grade of inflammation, length of BE segment or hiatal hernia \( \text{(data not shown)} \).

The presence of a hiatal hernia is associated with BE

It is known that the presence of a hiatal hernia is associated with a more severe reflux-induced mucosal inflammation \( (27–29) \). In the tested population of 255 BE patients, a hiatal hernia was associated with a longer BE segment \( (4.3 \pm 2.5 \text{ cm}; n = 217) \) as compared with patients without this endoscopic finding \( (3.1 \pm 1.1 \text{ cm}; n = 38) \) \( (P = 0.016) \). A hiatal hernia was present in 39/70 \( (56\%) \) of patients with grade A RE, in 142/204 \( (70\%) \) patients with grade B and in 90/98 \( (92\%) \) patients with grade C or D RE \( (P < 0.001) \). The presence of a hiatal hernia was associated with a BE \( (OR 2.6; 95\% \ CI 1.67–4.35, P < 0.001) \).

The combined presence of the IL-12B C-allele and a hiatal hernia is associated with an increased risk of BE

As both the presence of a hiatal hernia and the IL-12B C-allele displayed a positive association with BE, we evaluated whether the IL-12B C-allele in combination with a hiatal hernia was associated with BE. The combined presence of a hiatal hernia and the IL-12B C-allele was more prevalent in patients with BE \( (71/255; 28\%) \) than in patients with RE \( (45/247; 18\%) \) and was associated with BE \( (OR 2.9; 95\% \ CI 1.32–6.58; P = 0.008) \) (Table V) as compared with patients with the IL-12B C-allele without a hiatal hernia. In contrast, absence of the IL-12B C-allele combined with the absence of a hiatal hernia was associated with a significantly decreased risk of BE \( (OR 0.50; 95\% \ CI 0.35–0.72, P < 0.001) \) (Table V).

A pro-inflammatory gene profile is associated with an increased risk of BE

It was tested whether the IL-10-1082 GG genotype would modify the association between the IL-12B C-allele and BE (Table VI). The prevalence of BE or RE was equally distributed between patients with IL-12B_{CA} or CC/IL-10GG and IL-12B_{AA}/IL-10non-GG (Table VI). The combination of IL-12B_{CA} or CC/IL-10non-GG was more frequently observed in BE \( (70/255; 28\%) \) than in patients with RE \( (45/247; 18\%) \) and was associated with BE \( (OR 1.7; 95\% \ CI 1.1–2.7; P = 0.014) \) (Table VI). The combination of IL-12B_{AA}/IL-10GG was more often observed in patients with RE \( (52/247; 21\%) \) than in patients with BE \( (32/255; 12.5\%) \) and was negatively associated with BE \( (OR 0.6; 95\% \ CI 0.34–0.99; P = 0.011) \).
has been shown that both IL-8 and IL-6 expression are induced by increased exposure to the gastroesophageal refluxate (7,8). This introduces the possibility that significantly fewer control patients had similar reflux characteristics as the patients with BE. As it has been shown that both IL-8 and IL-6 expression are induced by reflux components (20,22,33), this raises the possibility that there may be a selection bias based on differences in the composition and extent of gastroesophageal reflux.

We did observe a link between the IL-10-1082 GG genotype and RE (Table III), but that this association was not significant in a multivariate regression analysis (Table IV). This is, however, in contrast to what has been recently reported by Gough et al. (34), who showed that homozygosity for IL-10-1082 allele 2, which is associated with higher IL-10 levels, was linked to male BE patients (OR 1.84; 95% CI 1.04–3.28; P = 0.035). Although not stated by the authors, this allele is likely to be the G-residue at position −1082, as it is associated with an increased risk of BE in a multivariate analysis (Table VI) (37). Patients with an IL-12B AA genotype (low IL-12p70 expression) even had a significantly lower risk of BE when this genotype was present in combination with the IL-10–1082 GG genotype (32). IL-12p70 is therefore believed to be a major link between innate immunity and the recruitment and polarization of adaptive immunity toward a cellular immune response (Th1). The finding of an increased prevalence of the IL-12p70-high genotype (IL-12B C-allele) in BE indicates that a strong host cellular immune response may be involved in the pathogenesis of BE in humans, as was suggested from observations in animal models (4,5). The association between the IL-12B C-allele and BE was modified by the presence of a hiatal hernia, which increased the risk of BE (OR 2.9; 95% CI 1.32–6.58; P = 0.008) (Table V). This supports the hypothesis that an increasing severity of esophageal inflammation induced by increased exposure to the gastroesophageal refluxate and a pro-inflammatory host response predisposes to development of BE.

Although IL-2, IL-6 and IL-8 gene polymorphisms are also associated with increased transcriptional levels of their respective cytokines, we did not observe a link between these polymorphisms and BE. This may be partially explained by a limitation of this study. We selected a control group consisting of patients with endoscopically confirmed RE, since this confirms the presence of gastroesophageal reflux and its ability to induce mucosal erosions. As we have not investigated severity, duration, content and pattern of gastroesophageal reflux, this introduces the possibility that significantly fewer control patients had similar reflux characteristics as the patients with BE. As it has been shown that both IL-8 and IL-6 expression are induced by reflux components (20,22,33), this raises the possibility that there may be a selection bias based on differences in the composition and extent of gastroesophageal reflux.

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In this study, IL-10 genotypes interacted with the IL-12B polymorphism and may have modified the risk of BE associated with the IL-12B C-allele. Homozygosity of the IL-10-1082 G-allele was more prevalent in patients with RE (OR 1.6; 95% CI 1.06–2.39; P = 0.024) (Table III), and when combined with the IL-12B C-allele decreased the risk of the BE (OR 0.2; 95% CI 0.5–1.9; P = 0.10) (Table VI). Patients with an IL-12B AA genotype (low IL-12p70 expression) even had a significantly lower risk of BE when this genotype was present in combination with the IL-10-1082 GG genotype (OR 0.6; 95% CI 0.34–0.99; P = 0.011) (Table VI). The IL-10-1082 GG genotype is associated with higher IL-10 expression (14), and IL-10 inhibits IL-12p70 expression by activated dendritic cells, the IL-10-1082 GG genotype may decrease the IL-12p70 production, and in this way the risk of BE. This is also evidenced by a recent observation that IL-10 genotypes interacted with IL-12B genotypes on the level of IL-12p70 expression (46). IL-10-1082 GG genotype was shown to decrease IL-12p70 expression by lipopolysaccharide-stimulated dendritic cells in spite of the presence of an IL-12p70-high genotype (46). These findings further support the notion that increased expression of IL-12p70 might be important in the pathogenesis of BE.

This interaction between IL-12B and IL-10 polymorphisms in the production of IL-12p70 may also provide an explanation why IL-10 polymorphisms itself were only weakly associated with BE in this study. First, although BE is associated with increased levels of IL-10 (7,8), this was not tested in relation to expression of IL-12. A strong pro-inflammatory response in the esophagus could be mediated by IL-12p70, whereas IL-10 levels may tune the inflammatory response by lowering IL-12p70 expression. Second, BE is the end result of a process leading to its development. Inflammatory characteristics in biopsies from the columnar epithelium of a BE segment may not correlate well with the mucosal characteristics in the squamous...
epithelium just before the change in mucosal lining, as it is believed that the columnar epithelium has a protective effect on reflux-related chemical corrosion. IL-10 was shown to be highly expressed in the distal region of the BE segment, suggesting that progression of BE may be associated with increased IL-10 expression (8). This is further supported by the observation that dysplastic epithelium is able to express IL-10 (8) and that IL-10 polymorphisms are associated with esophageal adenocarcinoma (34). This suggests that IL-10 is mainly involved in neoplastic progression in BE and to a lesser extent in the development of BE itself.

A hiatal hernia is associated with a higher total esophageal acid exposure, increased acid clearance time and severity of esophagitis (27,28). Its presence is therefore likely to be associated with increased reflux-related mucosal damage and the induction of a pro-inflammatory tissue response. Patients in whom a high IL-12p70 genotype (IL-12B C-allele) coincided with a hiatal hernia had a higher risk of BE (OR 2.9; 95% CI 1.32–6.58; P = 0.008) (Table V). We could not detect an association between the grade of endoscopic esophageal inflammation and IL-12B C-allele in patients with RE. Nor did we observe a relationship between the IL-12B C-allele and the length of the BE segment (data not shown). A fraction of the population was already diagnosed with BE and used acid suppressant medication for their reflux-related symptoms. Moreover, 46% of the patients with RE used acid suppressant medication as well at the time of upper GI endoscopy. This may have influenced mucosal appearance at the time of endoscopy and could therefore have resulted in an underestimation of the endoscopic grade of inflammation. Second, endoscopic grading of esophagitis by the Los Angeles classification uses macroscopic criteria as marker of the extent of inflammation, but does not give information about the extent of inflammation at the mucosal level. Characteristics of the local immune response may therefore be more important for tissue remodeling than the endoscopic grade of inflammation.

In conclusion, this is the first study providing evidence that the IL-12B C-allele (high IL-12p70 expression) predisposing toward a strong pro-inflammatory host response is involved in the development of BE in humans. This risk increased when the IL-12B C-allele coincided with a hiatal hernia (OR 2.9). This indicates that genetic regulation of cytokine expression levels and their interaction with local conditions, such as a hiatal hernia that is associated with increased gastroesophageal reflux, contributes to a delicate process in which timing and interaction are of crucial importance for changing mucosal homeostasis. Future studies are needed to elucidate and confirm the role of IL-12p70 and the IL-12B polymorphisms in the pathogenesis of BE. Our findings contribute to the growing interest into the role of the inflammatory reaction in the pathogenesis of BE and may also be of relevance to other inflammation-associated diseases of the digestive tract.

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We would like to thank the endoscopy nurses of the Erasmus MC and the Jusselland Hospital for their support with the inclusion of patients for this study. We would also like to thank M.Ouwendijk, J.Francke and A.Heijens for their technical assistance.

Conflict of Interest Statement: None declared.

References


<p>| Table V. The risk of BE in patients with an IL-12B polymorphism and hiatal hernia |</p>
<table>
<thead>
<tr>
<th>Genotype and hiatal hernia</th>
<th>Phenotype</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12B_C- &gt; CC and hiatal hernia</td>
<td>RE (n = 247, n (%)) BE (n = 255, n (%))</td>
<td>2.9 (1.32–6.58)</td>
<td>0.008</td>
</tr>
<tr>
<td>IL-12B_C- &gt; CC without hiatal hernia</td>
<td>20 (9) 15 (3)</td>
<td>0.75 (0.48–1.16)</td>
<td>0.150</td>
</tr>
<tr>
<td>IL-12B_A &gt; and hiatal hernia</td>
<td>104 (47) 125 (54)</td>
<td>1.13 (0.95–1.36)</td>
<td>0.175</td>
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<tr>
<td>IL-12B_A &gt; without hiatal hernia</td>
<td>58 (27) 23 (10)</td>
<td>0.50 (0.35–0.72)</td>
<td>0.000</td>
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<p>| Table VI. Gene profiles consisting of the combination of IL-12B and IL-10 polymorphisms and the risk of developing BE |</p>
<table>
<thead>
<tr>
<th>Genotype combination</th>
<th>RE, n (%)</th>
<th>BE, n (%)</th>
<th>OR (95% CI)</th>
<th>P value</th>
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<tr>
<td>IL-12B_C- &gt; CC and IL-10G</td>
<td>22 (50)</td>
<td>22 (50)</td>
<td>0.98 (0.72–1.34)</td>
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<tr>
<td>IL-12B_C- &gt; CC and IL-10G</td>
<td>45 (39)</td>
<td>70 (61)</td>
<td>1.77 (1.12–2.81)</td>
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<tr>
<td>IL-12B_A &gt; and IL-10G</td>
<td>52 (62)</td>
<td>32 (38)</td>
<td>0.71 (0.54–0.95)</td>
<td>0.011</td>
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<tr>
<td>IL-12B_A &gt; and IL-10G</td>
<td>128 (49)</td>
<td>111 (51)</td>
<td>1.0 (0.83–1.18)</td>
<td>0.920</td>
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