Cytokine Gene Polymorphisms, Cancer Susceptibility, and Prognosis¹-³

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Abstract

IL-10 is a multifunctional cytokine with both immunosuppressive and antiangiogenic functions and may have both tumor-promoting and -inhibiting properties. A large number of polymorphisms (primarily single-nucleotide polymorphisms) have been identified in the IL10 gene promoter. Convincing evidence that certain of these polymorphisms are associated with differential expression of IL-10 in vitro and in some cases in vivo was obtained, and a number of studies investigated associations between IL10 polymorphisms and cancer susceptibility and prognosis. The results from 22 studies in 13 different malignancies are reviewed. In 17 of these studies, positive associations between IL10 genotype or haplotype and disease susceptibility, progression, or both were reported. In some of these cancers genotypes associated with low IL-10 expression were a risk factor for disease or disease progression, whereas in others genotypes associated with high IL-10 expression were a risk factor. Published findings in breast cancer are as yet conflicting. Most but not all of the studies reviewed are based on small sample sizes and a limited number of IL10 polymorphisms. However, the preliminary data indicate that larger studies are required in a number of cancers to confirm initial results, extend studies to include more detailed genotype and haplotype analysis, and combine genotype and gene expression studies in the same subjects. Such studies will contribute significantly to our understanding of the biological role of IL-10 in cancer development. J. Nutr. 137: 194S–199S, 2007.

In recent years there has been an enormous effort by numerous laboratories worldwide to identify genetic mutations that play a major role in genetic predisposition to and disease progression in particular cancers. This approach has met with considerable success, as exemplified by numerous studies in cancers common in the Western world, such as breast cancer. In this malignancy, mutations in the BRCA1 and BRCA2 genes are inherited in an autosomal dominant manner and confer a high disease risk (1,2) but account for only a few percent of breast cancer cases (3). A similar picture has emerged in prostate cancer, now the most common form of male cancer in the Western world (4–9). However, it is highly likely that a number of more prevalent, low-penetrance genes contribute to cancer susceptibility in a larger population of patients and are therefore responsible for a greater proportion of the disease burden (2,3,10,11). Relatively little is known about low-penetrance susceptibility genes, and, continuing with breast cancer as an example, only a few have been identified, including genes involved in carcinogen detoxification and estrogen metabolism (12–14). There is, therefore, considerable interest in identification of more frequent, low- and moderate-penetrance polymorphisms that may be responsible for predisposition to and prognosis in larger numbers of cancer patients worldwide. In cancers in which antitumor immune responses occur, genetic polymorphisms in genes whose products regulate the immune response are obvious candidate polymorphisms for investigation. Associations of human leukocyte antigen with particular cancers was reviewed elsewhere (15).

This short review focuses on evidence for a role for cytokine polymorphisms in cancer predisposition and prognosis, with a special emphasis on studies of the role of the IL10 gene in cancer. Four studies of breast cancer are included.

Cytokine genes and disease

Cytokines are small molecules secreted by cells in response to specific stimuli and alter the behavior of the same or other cells. Cytokines act on target cells generally within the hematopoietic system by binding to specific receptors, initiating signal transduction and second messenger pathways within the target cell. Cytokines function as players in a highly complex and coordinated...
network in which they modulate their own synthesis as well as that of other cytokines and cytokine receptors. Production of numerous cytokines by immune cells in response to both antigen-specific and -nonspecific stimuli is critical to the outcome of inflammatory immune responses.

In recent years, many single-nucleotide polymorphisms (SNPs) and a more limited number of microsatellite polymorphisms were detected within cytokine gene sequences, particularly within the promoter regions of these genes. Several of these polymorphisms may be associated with differential levels of gene transcription, including TNFA-308 and IL10-1082, although cell type and stimulus may also be important. For many well-studied polymorphisms (e.g., TNFA-308), the literature is conflicting. Many genetic studies have tried to correlate these cytokine polymorphisms with immune-mediated diseases. For example, associations were reported between SNPs in the TNFA promoter and rheumatoid arthritis, cerebral malaria, asthma, and cardiac and renal transplant rejection. Similarly, associations between IL10 promoter polymorphisms and systemic lupus erythematosus and asthma were described. However, there is still considerable conflict and uncertainty in the literature with regard to many diseases, genes, and SNPs. Excellent reviews of the literature with regard to cytokine polymorphisms, their relation to gene expression, and disease associations are available, both in print and on line (16,17).

In addition, a novel avenue of investigation recently indicated that interactions between cytokine polymorphisms and dietary factors may influence cytokine expression levels. In particular, we and collaborators showed that modulation of tumor necrosis factor-α expression by dietary supplementation with fish oil depends in part on TNFA or LTA genotype (18).

Cytokine polymorphisms and cancer

The literature concerning cytokine polymorphisms and cancer is small but growing rapidly. A number of studies reported associations between TNFA or LTA SNPs and particular cancers, including chronic lymphocytic leukemia (19), non-Hodgkin’s lymphoma (NHL) (20), and breast cancer (21). These associations are, however, refuted by others (22,23). A well-documented association exists between IL-1B and IL-1RN polymorphisms and the development of gastric adenocarcinoma subsequent to Helicobacter pylori infection, as originally reported by El-Omar et al. (24). However, IL10 polymorphisms are of particular interest in relation to cancer because IL-10 has both immunosuppressive (potentially cancer-promoting) and antiangiogenic (potentially cancer inhibiting) properties. A possible role for IL10 polymorphisms in modulating cancer susceptibility and tumor development is therefore considered in more detail.

IL10 gene polymorphisms

The IL10 gene comprises 5 exons, spans ~5.2 kb, and is located on chromosome 1 at 1q31–1q32 (25). A number of groups have pursued intensive studies to identify naturally occurring gene polymorphisms in the IL10 gene and flanking regions. To date, at least 49 IL10–associated polymorphisms have been reported, and an even larger number of polymorphisms are recorded in SNP databases [e.g., Ensembl Genome Browser (26)]. Of these 49 polymorphisms, 46 are SNPs, 2 are microsatellite polymorphisms, and 1 is a small (3 bp) deletion. Twenty-eight polymorphisms occur in the promoter region of the gene, 20 polymorphisms are noncoding intrinsic or synonymous substitutions, and only 1 polymorphism results in a change in amino acid sequence (27). A simplified diagram of the IL10 gene is presented in Figure 1. This shows key SNP and microsatellite promoter polymorphisms and also examples of intronic (+19) and 3’ untranslated region (+117) SNPs to illustrate that polymorphism occurs across the full length of the IL10 gene.

Promoter polymorphisms have been subject to the most scrutiny, particularly with regard to possible influences on gene transcription and protein production. For example, the IL10 –1082 SNP and –1082, –819, and –592 haplotypes were reported to be associated with differential IL-10 expression in vitro, with the –1082 A, –819 T, and –592 A haplotypes associated with decreased IL-10 expression compared with the –1082 G, –819 C, and –592 C haplotypes (28–30). This is thought to reflect, at least in part, differential transcription factor binding associated with –1082 SNP (31). In addition, IL10 R and G microsatellite haplotypes were also shown to be associated with differential levels of IL-10 expression in vitro (31). Figure 1 shows the position of the –1082 and –592 SNPs along with the position of the IL10 R and G microsatellite polymorphisms.

Some workers have suggested that as much as 75% of inter-individual variation in IL-10 expression may be a result of genetic variation (32), although others believe that the contribution of individual SNPs, such as the best-described –1082 SNP, may be much less than this (33). However, the precise role of IL10 promoter polymorphisms, both individually and as part of defined haplotypes and mosaics, in determining IL-10 transcription and expression levels is still a subject under active investigation.

Although a large number of investigations of possible associations between IL10 genotypes and immune-mediated disease were performed (17,18), the literature with regard to IL10 polymorphisms and cancer is as yet small but is growing: ~23 studies have been published considering 13 different malignancies. Results are summarized in Table 1. These published studies include both solid tumors and hematological malignancies and common and less-common diseases. Most of these studies comprise small, single-center case-control investigations, which may be prone to sampling bias and type I errors. In addition, in most of the 13 malignancies, only 1–3 studies were performed in each disease in a range of human populations and ethnic groups. Although more than 1 study has been performed for a single cancer, results are not always in agreement. In addition, relative risks for disease, or odds ratios, are modest, ranging from 1.5 to 4, and probability values are modest (P = 0.01–0.05). Relative risk for developing a disease indicates the increased (or decreased) likelihood of developing a disease if an individual carries a particular genotype; values >1 indicate an increased risk, whereas values <1 indicate a reduced risk or a protective effect. A relative risk of 1.5 indicates that an individual carrying the genotype in question has a 50% increased risk of developing disease compared with a noncarrier. True relative risks are difficult to calculate and for a rare condition approximate the odds ratio, which can be calculated by a comparison of the frequency of the genotype in question in a patient group and an appropriately matched healthy control group in a case-control study design.

**Figure 1** The IL10 gene showing key SNPs and microsatellite polymorphisms. Black boxes: exon sequences.
Despite the above caveats, it is striking that in 17 of the 23 studies summarized in Table 1, positive associations between IL10 genotype or haplotype and disease susceptibility and/or progression were detected. In some of these cancers (e.g., cutaneous malignant melanoma, noncardia gastric cancer, and renal cell carcinoma), genotypes associated with low IL-10 expression were a risk factor for disease development or disease progression, whereas in others (e.g., cervical cancer, cardia gastric cancer, hepatocellular carcinoma after hepatitis B virus infection, posttransplant squamous cell carcinoma of the skin, and multiple myeloma), genotypes or haplotypes believed by the authors to be associated with high IL-10 expression were a risk factor.

Results with respect to breast and prostate cancer and NHL are conflicting. In breast cancer, 4 studies were performed. One small study reported that the −1082 AA low-expression genotype was associated with susceptibility to the disease. Conversely, a larger case-control study (500 cases, 500 controls) demonstrated an association between the −592 AA genotype and reduced breast cancer risk. In the same study no associations were found with respect to IL10 −592 genotype and tumor size, histological grading, estrogen or progesterone receptor status, or age at diagnosis. In this study the −592 SNP was studied as a marker of the −3575, −2763, −1082, −819, and −592 haplotypes with the −592 A allele marking the TCATA low-expression haplotype. Accordingly, these results are interpreted to suggest that genetically programmed low IL-10 expression is protective in susceptibility to breast cancer but not in further development of the disease (40). However, 2 independent studies from our laboratory failed to confirm these findings, most notably in a larger series of over 2000 breast cancer cases and 2000 controls (41).

### TABLE 1: IL-10 polymorphisms and cancer

<table>
<thead>
<tr>
<th>Disease</th>
<th>IL10 polymorphism</th>
<th>Cases</th>
<th>Controls</th>
<th>Association</th>
<th>Genotype, allele, or haplotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous malignant melanoma</td>
<td>−1082, −819, −592</td>
<td>153</td>
<td>158</td>
<td>Susceptibility, advanced stage of disease, greater tumour thickness</td>
<td>−1082 AA</td>
<td>Howell et al. (34)</td>
</tr>
<tr>
<td>Cutaneous malignant melanoma</td>
<td>−1082, −819, −592</td>
<td>42</td>
<td>48</td>
<td>Survival (shorter)</td>
<td>ACC/ATA</td>
<td>Martinez-Escricano et al. (35)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>−1082</td>
<td>247</td>
<td>263</td>
<td>Susceptibility</td>
<td>−1082 AA</td>
<td>McCarron et al. (36)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>−1082, −819, −592</td>
<td>1320</td>
<td>1255</td>
<td>No</td>
<td>—</td>
<td>Michaud et al. (37)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>−1082</td>
<td>144</td>
<td>263</td>
<td>No</td>
<td>—</td>
<td>Smith et al. (38)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>−1082</td>
<td>125</td>
<td>100</td>
<td>Susceptibility</td>
<td>−1082 AA</td>
<td>Giordani et al. (39)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>−592</td>
<td>500</td>
<td>500</td>
<td>Protection</td>
<td>−592 AA</td>
<td>Langsenlehner et al. (40)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>−3575, −1349, −1082, −592, +19, +117</td>
<td>2000</td>
<td>2000</td>
<td>No</td>
<td>—</td>
<td>Bulpert et al. (41)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>−1082</td>
<td>77</td>
<td>69</td>
<td>Susceptibility</td>
<td>−1082 AG</td>
<td>Stanczuk et al. (42)</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>−1082, −819, −592</td>
<td>220</td>
<td>230</td>
<td>Susceptibility, advanced stage</td>
<td>GCC (1 or 2 copies)</td>
<td>Wu et al. (44)</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>−1082</td>
<td>150</td>
<td>220</td>
<td>Association with EBV – noncardiac gastric cancer</td>
<td>−1082 G allele</td>
<td>Wu et al. (45)</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>−1082, −819, −592</td>
<td>188</td>
<td>212</td>
<td>Susceptibility</td>
<td>ATA haplotype</td>
<td>El-Omar et al. (46)</td>
</tr>
<tr>
<td>Squamous cell carcinoma of skin (post renal transplant)</td>
<td>−1082, −819, −592</td>
<td>70</td>
<td>70</td>
<td>Susceptibility</td>
<td>GCC haplotype</td>
<td>Alamantine et al. (47)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma (post HBV infection)</td>
<td>−1082, −819, −592, +117</td>
<td>230</td>
<td>792</td>
<td>Susceptibility, accelerated age of onset</td>
<td>ACCT haplotype</td>
<td>Shin et al. (48)</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>−1082</td>
<td>166</td>
<td>161</td>
<td>Susceptibility</td>
<td>−1082 AA</td>
<td>Hovanesek et al. (49)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>IL−10G, IL−10R microsatellites</td>
<td>73</td>
<td>109</td>
<td>Protection</td>
<td>112/114, IL−10R 114/116E</td>
<td>Zheng et al. (50)</td>
</tr>
<tr>
<td>Myelodysplasia Acute myeloid leukemia</td>
<td>−1082, −819, −592</td>
<td>150</td>
<td>Up to 1000</td>
<td>No</td>
<td>—</td>
<td>Gowans et al. (51)</td>
</tr>
<tr>
<td>Non-Hodgkins lymphoma</td>
<td>−1082, −819, −592</td>
<td>126</td>
<td>302</td>
<td>Susceptibility to aggressive disease</td>
<td>−1082 AA, ATA, ACC haplotypes</td>
<td>Cunningham et al. (52)</td>
</tr>
<tr>
<td>Non-Hodgkins lymphoma (in AIDS patients)</td>
<td>−592</td>
<td>139</td>
<td>1011</td>
<td>Susceptibility</td>
<td>−592 CC</td>
<td>Breen et al. (53)</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>−1082, −819, −592</td>
<td>199</td>
<td>112</td>
<td>Susceptibility</td>
<td>−1082 G</td>
<td>Lech-Maranda et al. (54)</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>−1082, −592</td>
<td>149</td>
<td>111</td>
<td>No</td>
<td>—</td>
<td>Munro et al. (55)</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>−1082</td>
<td>135</td>
<td>—</td>
<td>Protection from poor response to prednisone treatment</td>
<td>−1082 GG</td>
<td>Lauten et al. (56)</td>
</tr>
</tbody>
</table>
In prostate cancer, our group has reported associations between the \(IL10\) – 1082 AA “low expression” genotype and susceptibility to prostate cancer (36). However, a second study has failed to confirm this association (37), although patients were recruited to this study as part of a screening trial and so may have had less advanced disease.

Finally, 1 study of NHL reported that genotypes and haplotypes associated with low IL-10 expression are a risk factor for aggressive disease, whereas a second study suggested that genotypes associated with increased IL-10 expression are a risk factor for the development of the disease in patients with AIDS. A third study in diffuse large B-cell lymphoma (the most frequent form of NHL in North America and Western Europe) suggests that although the \(IL10\) – 1082 G (high expression) allele may be a risk factor for disease susceptibility (at a marginal level of significance), this same allele is protective in patients with NHL.

Taken together, these apparently conflicting data may reflect the dual biological function of IL-10 as an antiinflammatory (potentially cancer-promoting) and antiangiogenic (potentially cancer-inhibiting) cytokine in relation to the biological growth patterns of the cancer in question. Alternatively, association between \(IL10\) genotype and IL-10 expression may differ between tissues and disease states and antigenic and nonantigenic stimuli. In any event, at this stage, all of the above findings should be regarded as highly preliminary because of the small sample sizes of most of the studies, limited numbers of \(IL10\) polymorphisms examined, and differing ethnicity of the study groups. In addition, only a few studies examined levels of IL-10 production in vivo in the subjects genotyped.

The preliminary data obtained thus far indicate that much larger studies are required on a number of common cancers to confirm initial results, extend studies to include more detailed genotype and haplotype analysis, and combine genotype and gene expression studies in the same subjects. In this way, our understanding of the biological role of IL-10 in tumor development will be greatly aided, with implications for cytokine immunotherapy in cancer. In addition, although results from studies of \(IL10\) polymorphism and breast cancer are at best conflicting, further studies of other cytokine and related growth factor polymorphisms in this disease are certainly merited. A number of studies investigated \(TNFA\) (38) and other cytokine SNPs (57,58) in breast cancer, also with conflicting findings, but more convincingly a number of studies investigated transforming growth factor-\(\beta\) SNPs and reported predisposing effects (57–60), including a large case-control study (61).

**Future prospects**

Only a relatively small literature exists in this field, and few consensus associations with therapeutically useful indicators, such as markers of prognosis or response to therapy, have emerged. For many polymorphisms, only a single study in a given cancer has been performed. Even when this is not the case, most studies are based on small numbers of cases and controls, which may be population rather than matched controls. Studies based on limited numbers of subjects often result in unreliable conclusions as a result of type 1 or type 2 errors. Very often such studies have been purely of a case-control nature, and markers of prognosis and disease-free survival have not been examined. In addition, very often only a single SNP per gene has been examined, making it difficult to exclude a role for other polymorphisms in the genes concerned in cancer susceptibility or prognosis. Even when positive associations are reported, these may be the result of linkage disequilibrium between the SNP under investigation and the actual causative SNP, which may be known or unknown at the time of study. The results for \(IL10\) polymorphisms and cancer considered in this article illustrate these points.

Despite the limitations of most published studies, the preliminary literature indicates that definitive studies of selected immune polymorphisms are required in certain cancers. These studies should have a robust design: collection of peripheral blood DNA samples from sufficient cases of the cancer in question, ideally 20,000, but upwards of 5000 would still constitute a major advance. For example, to determine whether a rare polymorphism with a population frequency of 1% confers an odds ratio for disease of 1.5 (effectively an increased risk of 50% for a rare disease), ~8000 cases and controls are required to achieve 80% power at a 5% level of significance (62). Even larger numbers of cases and controls would be required to demonstrate more modest odds ratios with similar statistical power. Collection of definitive clinical and pathological data for all cases, with full follow-up, including periodic assessment of therapeutic responses, must be an integral part of such an approach. Recruitment of appropriately matched control subjects is equally important. The same is true of careful selection of SNPs to cover the whole length of a candidate gene sequence so that areas of association can be defined and informative haplotypes constructed. Emerging genotyping technologies will facilitate such definitive, comprehensive studies. Finally, to be fully informative, immunogenetic studies must be integrated with other approaches, especially gene expression analyses and proteomics.

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