Inflammation-Related Gene Polymorphisms and Colorectal Adenoma

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Abstract

Chronic inflammation has been reported to be a risk factor for colorectal neoplasia. The propensity to mount an inflammatory response is modified by germ line variation in cytokine and other inflammation-related genes. We hypothesized that a proinflammatory genotype would be positively associated with colorectal adenoma, a precursor of colorectal cancer. We investigated the association of colorectal adenoma with 19 single nucleotide polymorphisms in a range of important proinflammatory (IL1B, IL6, IL8, TNF, and LTA) and anti-inflammatory (IL4, IL10, and IL13) cytokines and other inflammation-related genes (PTGS2 and PPARG) in a case-control study of risk factors for colorectal polyphs in which all participants (ages 18-74 years) had undergone colonoscopy or sigmoidoscopy. The study sample comprised 244 cases of colorectal adenoma and 231 polypl-free controls. Compared with being homozygous for the common allele, heterozygosity at the IL1B −31 (C>T) locus was associated with an odds ratio (OR) for colorectal adenoma of 1.8 (95% confidence interval (95% CI), 1.2-2.9). Homozygous carriers of the IL8 −251-A allele were at 2.7-fold increased risk of adenoma (95% CI, 1.5-4.9) compared with homozygosity for the common T allele, whereas carriage of at least one IL8 −251-A allele conferred a 1.5 increased odds of disease (95% CI, 1.0-2.4). Among non-steroidal anti-inflammatory drug users, there was a statistically significant association between the IL10 −819/T T genotype and adenoma compared with the common IL10 −819/C/C genotype (OR, 3.9; 95% CI, 1.1-13.6), which was not evident among nonsteroidal anti-inflammatory drug users (OR, 0.7; 95% CI, 0.3-1.5; Pinteraction = 0.01). These exploratory data provide evidence that polymorphic variation in genes that regulate inflammation could alter risk for colorectal adenoma. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1126–31)

Introduction

Both mechanistic and observational data implicate chronic inflammation in the etiology of colorectal cancer. At the cellular level, the colonic epithelium is exposed to a range of toxic and pathogenic challenges, including the balance between intestinal microflora. In turn, a shift in microflora can result in a change in immune response, including the induction of inflammation (1). A notable hallmark is the release of proinflammatory cytokines by infiltrating lymphocytes. These can lead to the generation of reactive oxygen species and other genotoxic compounds in the epithelial environment. Cytokines are peptide mediators that act between immunocompetent and hematopoietic cells and between the immune and neuroendocrine systems. They are synthesized by activated cells and exert their biological activities upon binding to specific receptors expressed on target cells with subsequent proinflammatory or anti-inflammatory consequences (see Table 1). There is evidence to suggest that cytokines are involved in the control of cancer development, and that they may be relevant for gastrointestinal tumors. The genes that encode these peptides are polymorphic, and the most common variant is the single nucleotide polymorphism (SNP), of which many have been identified in the regulatory regions of cytokine genes. For example, the IL1B −1080-T allele, which lies in the promoter region of this proinflammatory cytokine, is associated with enhanced interleukin-1β (IL1β) expression and has been linked to increased risk of gastric cancer in response to Helicobacter pylori infection (2, 3). Furthermore, common variants of IL6, IL8, and IL10 have been associated with colorectal cancer risk (4, 5).

Stimulation of arachidonic acid metabolism is a critical step in the induction of inflammation. Prostaglandin H synthase (PTGS; also known as cyclooxygenase) catalyzes the conversion of arachidonic acid to prostaglandin precursors, which function as autocrine and paracrine mediators of a range of cell functions, including vasodilation and nociception. This enzyme is the pharmacologic target for nonsteroidal anti-inflammatory drugs (NSAID), and inhibition of PTGS2 accounts for at least some of the anti-inflammatory properties of these drugs. Elevated levels of PTGS2, the inducible form of the enzyme, have been shown in colon cancer tissue (6), and this enzyme has proangiogenic, pro-proliferative, and anti-apoptotic effects (7). Allelic variants of PTGS2 have been associated with colorectal neoplasia in some (8-10) but not all studies (11, 12). It was recently reported that a variant within the PTGS2 promoter region has been associated with colorectal adenoma among non-NSAID users (8).

The peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that functions as a transcriptional regulator of metabolism. Implicated in the pathophysiology of obesity and insulin resistance, PPARγ binds small molecules, such as fatty acids, and is required for the accumulation of adipose tissue. PPARγ also possesses anti-inflammatory properties. It has been suggested that PPARγ binds nuclear factor-κB, activator protein 1, and signal transducers and activators of transcription factors, thereby inhibiting initiation of the inflammatory response (13). Natural ligands and drug agonists...
Table 1. List of studied genes and SNPs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Polymorphism</th>
<th>dbSNP no.</th>
<th>Variant phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1B</td>
<td>Proinflammatory, primary initiator of inflammatory response</td>
<td>−31C&gt;T</td>
<td>rs1143627</td>
<td>C allele: increased expression (2)</td>
</tr>
<tr>
<td>IL4</td>
<td>Regulates antibody production, attenuates inflammatory response</td>
<td>Ex5+14C&gt;T</td>
<td>rs1143634</td>
<td></td>
</tr>
<tr>
<td>IL5</td>
<td>Hematopoietic growth factor, promotes growth and differentiation of eosinophils</td>
<td>−1098G&gt;T</td>
<td>rs2243250</td>
<td></td>
</tr>
<tr>
<td>IL6</td>
<td>Proinflammatory, initiator of inflammatory response, growth factor for cancer cells</td>
<td>−174C&gt;G</td>
<td>rs1800795</td>
<td>G allele: increased expression (31)</td>
</tr>
<tr>
<td>IL8</td>
<td>Proinflammatory chemokine, activates neutrophils</td>
<td>−251A&gt;T</td>
<td>rs4073</td>
<td>A allele: increased expression (22)</td>
</tr>
<tr>
<td>IL10</td>
<td>Anti-inflammatory, regulates T-cell and macrophage function</td>
<td>−819C&gt;T</td>
<td>rs1800871</td>
<td>In LD with −592 C&gt;A SNP which is linked to reduced expression (27, 28)</td>
</tr>
<tr>
<td>IL13</td>
<td>Anti-inflammatory, suppresses IL1, IL6, and IL8 production</td>
<td>−1082A&gt;G</td>
<td>rs1800896</td>
<td>A allele: reduced expression (32)</td>
</tr>
<tr>
<td>LTA</td>
<td>Proinflammatory, stimulates prostaglandin synthesis, induces reactive oxygen species production in neutrophils</td>
<td>IVS3−24T&gt;C</td>
<td>rs1295686</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IVS1−82G&gt;C</td>
<td>rs746868</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>Proinflammatory, key immunomediator</td>
<td>IVS1+90G&gt;A</td>
<td>rs909253</td>
<td>A allele: higher transcriptional activation (33, 34)</td>
</tr>
<tr>
<td>PPARG</td>
<td>Anti-inflammatory, tumor suppressor and promoter properties</td>
<td>Ex4−49C&gt;G (Pro12Ala)</td>
<td>rs1801282</td>
<td>Ala allele: reduced ligand binding (35)</td>
</tr>
<tr>
<td>PTGS2</td>
<td>Converts arachidonic acid to prostaglandins, thereby promoting inflammatory response</td>
<td>−765C&gt;G</td>
<td>rs20417</td>
<td>C allele: reduced expression (36)</td>
</tr>
<tr>
<td>(COX2)</td>
<td></td>
<td>Ex3−8G&gt;C</td>
<td>rs5277</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IVS5−275G&gt;T</td>
<td>rs20432</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ex10+837C&gt;T</td>
<td>rs5275</td>
<td></td>
</tr>
</tbody>
</table>

of PPARα have been shown to reduce intestinal inflammation in both humans and rodent models (14, 15). Furthermore, the formation of aberrant crypt foci by chemical induction is inhibited by PPARα ligands (16). A common PPARG SNP in exon 12 that leads to a nonsynonymous amino acid substitution has been associated with colorectal cancer and adenoma (4, 17).

Based on emerging evidence that chronic inflammation is related to colorectal neoplasia, and that the expression and function of a number of important cytokines and other inflammation-related enzymes is under genetic control, we hypothesized that a proinflammatory genetic profile is associated with increased susceptibility to colorectal adenoma, an established precursor of colorectal cancer. We investigated the relation between 19 polymorphisms in IL1B, IL4, IL5, IL6, IL8, IL10, IL13, LTA, and TNF as well as the PTGS2 and PPARG genes with colorectal adenoma.

Materials and Methods

Study Sample. We genotyped participants from a case-control study of colorectal adenomas conducted at the National Naval Medical Center (Bethesda, MD); cases were patients who were diagnosed with colorectal adenoma at sigmoidoscopy (18%) or colonoscopy (82%) between April 1994 and September 1996 (details of this study are described elsewhere; refs. 18, 19). Controls were selected among individuals confirmed as polyp-free during sigmoidoscopic screening and were frequency matched to cases by age (±5 years) and gender. To be eligible for the study, cases and controls had to be residents of the study area, between ages 18 and 74 years, and had never been diagnosed with Crohn’s disease, ulcerative colitis, colorectal neoplasms, or cancer except nonmelanoma skin cancer.

The participation rates were 84% for the cases (244 of the 289 eligible cases identified) and 74% for the controls (231 of 314 eligible controls). The main reason for nonparticipation was subject refusal (12% of cases and 21% of controls) followed by illness (3% of cases and 4% of controls). Three cases with familial adenomatous polyposis syndrome were excluded from the study. All 231 control subjects had been verified by sigmoidoscopy. Of the 244 cases, 210 (86.1%) had undergone colonoscopic examination, and 34 (13.9%) had undergone sigmoidoscopic examination. The average time between colorectal examination and blood draw was 3 days for cases and 23 days for controls, and the time between colorectal examination and collection of questionnaire data was 2.4 and 3.3 months for cases and controls, respectively.

Genotyping and Statistical Analysis. SNPs were selected on the basis of functional data related to changes induced in the expression of the cytokine and prevalence (>5% minor allele frequency in Caucasians). For PTGS2, a SNP in a putative promoter sequence variant that has been associated with differential PTGS2 expression was genotyped. In addition, three SNPs with >5% prevalence in Caucasian populations and spaced at approximately regular intervals across the gene region were selected for genotyping. A SNP that gives rise to a nonsynonymous amino acid change in PPARα and has been associated with colorectal neoplasia was also genotyped (see Table 1). Genotyping of IL6, IL8, PPARG, and PTGS2 was performed according to the methodology of Landi et al. (4). Primer sequences for the PTGS2 genotyping probes are available upon request. All remaining genotyping was done using Taqman assays, and detailed protocols can be accessed at http://snp500cancer.nci.nih.gov/assays. To ensure reproducibility of genotyping methods, multiple blinded quality control samples (n = 30) from two individuals were embedded among the case-control samples. For all genotypes tested, the quality control samples indicated a reproducibility rate of 100%.

Differences in genotype distributions between cases and controls were ascertained by the χ² statistic. Association between genotypes and colorectal adenoma was calculated as odds ratios (OR) with 95% confidence intervals (95% CI) by unconditional logistic regression and were adjusted for...
In total, 244 cases of colorectal adenoma and 231 polyp-free controls participated in this study. Demographic and other selected characteristics of the study population are presented in Table 2. The distribution of gender differed among the case and control groups; males were overrepresented in the case category. The median age of cases and controls were 60 and 57 years, respectively. Overall, cases had a higher body mass index (P = 0.04) and were more likely to be current smokers (P = 0.01) compared with controls. Regular use of NSAIDs was more common among controls compared with cases (P = 0.002). Inclusion of body mass index, NSAID use, and smoking status did not cause a material change in the risk estimates; thus, these variables were not included as covariates in the final regression models.

Among the control group, all genotypes were distributed in accordance with Hardy-Weinberg equilibrium except for two SNPs: IL1B −31 (P = 0.004) and IL10 −1082 (P = 0.003). Table 3 provides genotype distributions and ORs for possible associations between genotypes and colorectal adenoma. Compared with homozygosity for the common allele, heterozygosity at the IL1B −31 (C>T) locus was associated with an OR for colorectal adenoma of 1.8 (95% CI, 1.2-2.9), and carriage of an IL1B −31-C allele was associated with a 1.5-fold increased risk of colorectal adenoma (95% CI, 1.0-2.4). Haplotypes reconstructed from unphased genotype data using PHASE version 2.0 (20). Overall differences in haplotype distribution between cases and controls were assessed using the likelihood ratio test statistic. All P-values were two sided, and all models were adjusted for age, sex, and ethnicity. To account for the large number of comparisons made in this study, the false-positive report probability was calculated using a prior probability of association using the method of Wacholder et al. (21).

### Results

We observed statistically significant associations between alleles of proinflammatory cytokines IL1B and IL8 and colorectal adenoma. Furthermore, we identified a significant interaction between a polymorphism in the anti-inflammatory cytokine IL10 and NSAID use in colorectal adenoma risk.

### Discussion

We observed statistically significant associations between alleles of proinflammatory cytokines IL1B and IL8 and colorectal adenoma. Furthermore, we identified a significant interaction between a polymorphism in the anti-inflammatory cytokine IL10 and NSAID use in colorectal adenoma risk.

Although the findings presented here are exploratory, the study is limited by its small size and the relatively large number of statistical comparisons, which together increase the probability that false positives have been featured. For the IL1B −31-C and IL8 −251-A alleles, we had relatively high prior probabilities that they would be associated with colorectal adenoma, based on findings for other cancer sites together with preliminary evidence that the tested variants could have functional consequences (2-4, 22). We applied the method of Wacholder et al., in which the false-positive report probability is calculated using a prior probability of association, observed P, and the statistical power to estimate the variability or “noteworthiness” of the findings (21). For carriage of the IL1B −31-C and IL8 −251-A alleles, we calculated that the false-positive report probability associated with the risk estimates for these alleles would be in excess of the moderate prior probability (0.1) that a true association exists. Therefore, we are cautious in our interpretation of these data. However, we view these findings as exploratory and give the emerging evidence on chronic inflammation and colorectal carcinogenesis and the apparent functional role these cytokines play in the innate immune response; further study of these variants in relation to colorectal polyps is warranted.

In addition to statistical bias, additional bias may also lie in study design. In total, 86% of the cases underwent a full

### Table 2. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 244)</th>
<th>Controls (n = 231)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (%)</td>
<td>22.5</td>
<td>36.4</td>
<td>0.0009†</td>
</tr>
<tr>
<td>Age (y)</td>
<td>60 (55.5-74.5)</td>
<td>57 (43-71)</td>
<td>0.07</td>
</tr>
<tr>
<td>Non-White (%)</td>
<td>11.1</td>
<td>9.1</td>
<td>0.48†</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.5 (21.9-31.2)</td>
<td>25.8 (21.1-30.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>11.1</td>
<td>4.8</td>
<td>0.01†</td>
</tr>
<tr>
<td>Regular smoker (%)</td>
<td>26 (6-46)</td>
<td>18 (0-36)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>20 (0.8-39.2)</td>
<td>20 (0-40)</td>
<td>0.012</td>
</tr>
<tr>
<td>NSAID use (%)</td>
<td>51.6</td>
<td>65.4</td>
<td>0.002‡</td>
</tr>
<tr>
<td>Family history of colon cancer (%)</td>
<td>16.8</td>
<td>11.9</td>
<td>0.13</td>
</tr>
</tbody>
</table>

NOTE: All values are medians (interquartile range) unless otherwise indicated.

†Ps derived from the Wilcoxon signed rank sum test unless otherwise indicated.

‡Denotes regular use of aspirin or other NSAIDs.
IL10 

IL6 

IL4 

IL1B –584 

IL7 –74 

IL6 –174 

IL8 –251 

IL10 –819 

IL13 –1069 

IL13 IVS3 –24 

LTA IVS1 –82 

Table 3. Genotype distributions and genotypic risks for association with colorectal adenoma for cytokine and other inflammation-related genes (Cont’d)

**Genotype** | **Cases, n (%)** | **Controls, n (%)** | **OR* (95% CI)**
--- | --- | --- | ---
**G/G** | 35 (15.5) | 29 (13.9) | 1.2 (0.7-2.2) |
**G** | 136 (60.2) | 114 (57.9) | 1.0 (0.8-1.7) |
**G/G** | 146 (67.3) | 139 (68.8) | 1.0 |
**A** | 59 (27.2) | 57 (28.2) | 1.0 (0.7-1.6) |
**A/A** | 12 (5.5) | 6 (3.0) | 1.9 (0.7-5.4) |
**G** | 71 (32.7) | 63 (31.2) | 1.1 (0.7-1.7) |
**A** | 41 (20.7) | 37 (19.1) | 1.1 (0.7-1.8) |
**C** | 4 (2.0) | 1 (0.5) | 3.2 (0.3-31.1) |
**G** | 45 (22.7) | 38 (21.5) | 1.2 (0.7-1.9) |
**G** | 153 (73.3) | 146 (79.4) | 1.0 |
**C** | 54 (25.7) | 52 (26.5) | 0.9 (0.6-1.4) |
**C/C** | 5 (2.4) | 3 (1.5) | 1.3 (0.3-5.9) |
**G** | 59 (28.1) | 55 (28.0) | 0.9 (0.6-1.5) |
**G** | 154 (73.3) | 142 (72.1) | 1.0 |
**G** | 52 (24.8) | 51 (25.9) | 1.0 (0.6-1.6) |
**G/C** | 4 (1.9) | 4 (2.0) | 1.2 (0.3-5.1) |
**G** | 56 (26.7) | 55 (27.9) | 1.0 (0.6-1.6) |
**G** | 149 (71.0) | 136 (69.0) | 1.0 |
**G** | 54 (25.7) | 57 (28.9) | 0.8 (0.5-1.3) |
**G/G** | 7 (3.3) | 4 (2.1) | 1.4 (0.4-5.1) |
**G** | 61 (29.0) | 61 (30.9) | 0.9 (0.6-1.3) |
**C/C** | 92 (43.8) | 77 (39.1) | 1.0 |
**C/C** | 88 (41.9) | 102 (51.8) | 0.7 (0.5-1.1) |
**C/T** | 30 (14.3) | 18 (9.1) | 1.2 (0.6-2.4) |
**G** | 118 (56.2) | 120 (60.9) | 0.8 (0.5-1.2) |

**A/G** | 101 (44.7) | 92 (44.0) | 1.0 |

**A/G** | 101 (44.7) | 92 (44.0) | 1.0 |

**A/G** | 101 (44.7) | 92 (44.0) | 1.0 |

**A/G** | 101 (44.7) | 92 (44.0) | 1.0 |

**A/G** | 101 (44.7) | 92 (44.0) | 1.0 |
frequencies under Hardy-Weinberg equilibrium and observed frequencies among the controls revealed fewer heterozygotes than expected. This raises the possibility that the positive association observed between the IL1B −31C>T genotype and adenoma was due to chance. In an attempt to circumvent this problem, we compared the observed genotype distributions among the cases with the expected distributions among the controls. This yielded attenuated risk estimates for the heterozygous genotype (OR, 0.7; 95% CI, 0.5-1.1) but an enhanced, yet nonsignificant, association for homozygosity for the less common allele (OR, 1.3; 95% CI, 0.7-2.4).

IL8 is a potent chemokine for neutrophils, recruiting them to sites of infection and regulating leukocyte trafficking through peripheral lymphoid tissues. High concentrations of IL8 have been detected in the colonic lumen of ulcerative colitis patients, and rectal dialysate from these patients is capable of activating neutrophils in vitro (23). Three well-characterized SNPs in the 5′-promoter sequence have been analyzed and shown to alter expression of the gene under laboratory conditions (24). Specifically, there is evidence to suggest enhanced IL8 expression among carriers of the IL8 −251-A allele (22). In our study, we observed a 3-fold increase in risk for colorectal adenoma among IL8 −251-A/A carriers. Interestingly, a previous colorectal cancer study conducted among a Spanish population found an inverse association between carriage of the IL8 −251-A allele and colorectal cancer (4); however, studies on other cancers of the gastrointestinal tract have found reported positive associations between the IL8 −251-A allele and disease risk (25).

We detected an increased risk of adenoma for carriers of the IL10 −819-T/T genotype among nonusers of NSAIDs, whereas this association was not apparent among those reporting use of NSAIDs (OR, 1.1; 95% CI, 0.7-1.7). The lack of association observed for the remaining polymorphisms may not only reflect the small sample size but may also reflect the small number of samples in the subgroups. The low number of cases with heterozygosity at the 765G > C promoter polymorphism (765G/C0) has also been associated with lower levels of IL10, were at reduced risk of colorectal adenoma among IL10 626-A allele, which has been associated with lower levels of IL10 (27, 28), is it plausible that allele is associated with increased levels of basal inflammation in the colon, which is modified by NSAID use. This is consistent with the findings of the previous study of IL10 polymorphisms and colorectal cancer, in which carriers of the IL10 −626-A allele, which has been associated with lower levels of IL10, were at reduced risk of colorectal cancer but only among habitual users of aspirin (5). It is also possible that difference in potential anti-inflammatory effect of NSAIDs, the association of cytokine polymorphisms with colorectal adenoma is masked among NSAID users, and the NSAIDs, the association of cytokine polymorphisms with colorectal adenoma is masked among NSAID users, and the interaction between cytokine polymorphisms and colorectal cancer (22). In conclusion, we have observed that SNPs in key cytokine genes could be important risk factors for colorectal adenoma. Specifically, we observed several SNPs in IL1B, IL8, and IL10, known to alter risk or functional expression of the gene that seems to confer increased susceptibility to colorectal adenomas. Further analysis of the tested SNPs in these genes, either by haplotype analysis or a pairwise comparison approach, is needed to confirm the current findings. Moreover,

a more thorough analysis of common variants in these genes and related genes in the same pathways of inflammation are required to determine the contribution of inflammatory genes to colorectal adenomas, precursor lesions to colorectal carcinoma. Our data support the contention that large, well-planned studies that examine inflammation-related genes in relation to the colorectal adenoma-carcinoma sequence could uncover important mechanistic pathways and could lead to new intervention or prevention strategies.

References


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