Inflammatory Cytokine Gene Polymorphisms, Nonsteroidal Anti-Inflammatory Drug Use, and Risk of Adenoma Polyp Recurrence in the Polyp Prevention Trial

Leah B. Sansbury,1,2 Andrew W. Bergen,3 Kay L. Wanke,3 Binbing Yu,4 Neil E. Caporaso,3 Nilanjana Chatterjee,5 Luke Ratnasinghe,6 Arthur Schatzkin,7 Teresa A. Lehman,8 Aravind Kalidindi,8 Ramakrishna Modali,8 and Elaine Lanza2

1Cancer Prevention Fellowship Program, Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; 2Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; 3Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institute of Health, Bethesda, Maryland; 4Information Management Services, Inc., Silver Spring, Maryland; 5Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute; 6Division of Molecular Epidemiology, National Center for Toxicology Research, Jefferson, Arkansas; Arkansas Cancer Research Center; and Department of Surgery, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arizona; 7Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute; and 8BioServe Biotechnologies, Ltd., Laurel, Maryland

Abstract

Background: Pro- and anti-inflammatory cytokine genes may be important in the maintenance and progression of colorectal cancer. It is possible that single-nucleotide polymorphisms in inflammatory genes may play a role in chronic colonic inflammation and development of colorectal adenomas. Furthermore, common variants in cytokine genes may modify the anti-inflammatory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) in the prevention of colorectal cancer.

Methods: We examined the association between cytokine gene polymorphisms and risk of recurrent adenomas among 1,723 participants in the Polyp Prevention Trial. We used logistic regression to calculate odds ratios (OR) for the association between genotype, NSAID use, and risk of adenoma recurrence.

Results: Cytokine gene polymorphisms were not statistically significantly associated with risk of adenoma recurrence in our study. We observed statistically significant interactions between NSAID use, IL-10 −1082 G>A genotype, and risk of adenoma recurrence (P = 0.01) and multiple adenoma recurrence (P = 0.01). Carriers of the IL-10 −1082 G>A variant allele who were non-NSAID users had a statistically significant decreased risk of multiple adenoma recurrence (OR, 0.43; 95% confidence interval, 0.24-0.77) as well as a nonsignificant 30% decreased risk of any adenoma recurrence. In contrast, NSAID users who were carriers of the IL-10 −1082 G>A variant allele were at an increased risk of any adenoma recurrence (OR, 1.55; 95% confidence interval, 1.00-2.43).

Conclusion: These findings suggest that individuals who are carriers of the IL-10 −1082 G>A variant allele may not benefit from the chemoprotective effect of NSAIDs on adenoma polyp recurrence. (Cancer Epidemiol Biomarkers Prev 2006;15(3):494–501)

Introduction

The colorectal adenoma is considered the main precursor lesion of colorectal cancer, and its removal at colonoscopy is thought to reduce colorectal mortality (1). However, the majority of Americans go unscreened and nearly 57% of colorectal cancers are diagnosed with either regional or distant disease (2). Furthermore, it is estimated that 30% to 40% of adults of ages ≥60 years have prevalent colorectal adenomatous polyps and individuals with a history of adenoma are at increased risk of colorectal cancer, even with routine colonoscopic exams (3, 4). Identifying modifiable risk factors that affect the development and recurrence of these precancerous lesions is vital for colorectal cancer prevention strategies.

Chronic inflammation is a risk factor for many cancers, including colon cancer, and data from experimental and observational studies suggest that inflammation acts early in the carcinogenic pathway of colorectal cancer, possibly promoting the progression of colorectal adenomas to adenocarcinoma (5-15). The inflammatory response to cellular stresses, injury and infection, results from increased mucosal production of proinflammatory cytokines (16, 17). Proinflammatory cytokines, such as tumor necrosis factor α and the interleukins (IL-1β, IL-6, and IL-8), play a key role in angiogenesis, inhibition of apoptosis, and cell proliferation (17-20). These cytokines induce expression of cyclooxygenase-2 (COX-2), one of the key enzymes in the production of prostaglandins (21). COX-2 mRNA and protein are present in both colorectal adenomas and adenocarcinomas, and thus support a role of inflammation early in the carcinogenic pathway of colorectal cancer (5-15).

Further support for an inflammatory role in colon cancer progression comes from recent results of randomized clinical trials investigating the use of aspirin in the prevention of adenoma polyp recurrence (22-24). Similar to the observed findings from 24 case-control studies of nonsteroidal anti-inflammatory drugs (NSAID) and colon cancer, these studies observed a significant decrease in risk for adenoma recurrence among individuals who took aspirin compared with those who took only placebo (11, 22, 23, 25-27). However, the reported reduction in risk of colorectal adenoma and cancer by NSAID use never exceeds 50% (27, 28), suggesting that nonresponders to NSAIDs may attenuate the effect of NSAIDs in the prevention of colorectal cancer. Thus, it is possible that factors...
that differ among individuals, such as dietary or lifestyle characteristics, as well as individual genetic variations in inflammatory genes may modify response to inflammation or to the chemopreventive effect of NSAIDs. Differences in individual lifestyle characteristics and genetic variations may, in turn, modify the association between NSAID use and risk of colorectal cancer.

Single-nucleotide polymorphisms (SNP) in the cytokine genes have been associated with changes in gene expression and may mediate differential expression of cytokine alleles by influencing the binding affinity of transcription factors and the exacerbation of tissue damage or altered cell growth (16, 29). Data show that SNPs in the proinflammatory IL-1β, IL-6, and IL-8 genes and in the anti-inflammatory IL-10 gene result in changes in biological functions of the inflammation pathway and have been associated with a number of inflammatory diseases, including inflammatory bowel disease, arthritis, and Alzheimer's disease (29-33), as well as a number of cancers including colon, stomach, breast, and liver cancer and melanoma of the skin (34-40).

Recent association studies investigating inflammatory gene polymorphisms and risk of colorectal cancer and adenomas have been mixed (34, 35). Landi et al. (34) reported a significant increased risk of colon cancer among carriers of the IL-6 −174 C allele and a significant decreased risk of colorectal cancer among individuals with the variant PARP Pro12Ala genotype and among carriers of the IL-8 −251 A allele (34). Recent data from a case-control study of colon cancer in Scotland did not observe an association between polymorphisms in the IL-1, IL-10, TNF-α, and TGF-β genes and colon cancer risk, but they did report a statistically significant interaction between the IL-10 −592 C/A polymorphism, aspirin use, and risk of colon cancer (35). Finally, a recent report investigating the association between the COX-2 765 G>C promoter variant, which is also involved in the inflammation-mediated carcinogenic pathway of colon cancer, and risk of colorectal adenomas observed a significant interaction between COX-2 765 G>C genotype, NSAID use, and risk of colorectal adenomas (41).

To our knowledge, no one has reported on the association between variants in cytokine genes and risk of colorectal adenoma recurrence, as well as the possible modification of the association between cytokine gene SNPs and susceptibility to adenoma recurrence by NSAIDs. We therefore investigated the association between proinflammatory cytokine SNPs in the inflammation-mediated carcinogenic pathway of colon cancer, and risk of colorectal adenomas observed a significant interaction between COX-2 765 G>C genotype, NSAID use, and risk of colorectal adenomas (41).

Subjects and Methods

Study Population. Participants in this study were from the Polyp Prevention Trial, a multicenter randomized clinical trial to evaluate the effects of a high-fiber, high fruit and vegetable, low-fat diet on the recurrence of colorectal polyps. Men and women, ages ≥35 years and with at least one histologically confirmed adenoma removed in the prior 6 months, were randomized to the dietary intervention group or control group for 4 years. Eligible participants had no history of colorectal cancer, surgical resection of adenomas, or inflammatory bowel disease; weighed no more than 150% of the recommended level; were not taking lipid-lowering drugs; and had no medical conditions or dietary restrictions that would limit their compliance with the protocol. A total of 2,079 participants were enrolled in the trial, with 1,037 randomized to the intervention diet and 1,042 assigned to their usual diet. The study was completed by 1,905 participants (91.6%), 958 in the intervention group and 947 in the control group.

All participants received a colonoscopy 1 year (T1) and 4 years (T4) after randomization. The 1-year colonoscopy served to detect and remove any lesions missed at the baseline colonoscopy (T0). The participants were then followed for 4 years after randomization, at which time the subjects returned to their usual endoscopist for colonoscopy. A detailed description of the study design, dietary intervention, study population, and end-point assessment is reported elsewhere (42-44).

For the purposes of this analysis, the outcome of “any recurrence” was defined as those Polyp Prevention Trial participants who had any recurrence by any endoscopic procedure during the 3 years following the 1-year colonoscopy. We examined a subset of cases with “multiple adenoma recurrence” who were individuals with >1 adenoma identified at defined intestinal sites during their follow-up endoscopic procedure (n = 381). We did not have enough power to investigate the association between genotype and risk of advanced adenoma recurrence (n = 125), defined by any adenoma >1 cm, had evidence of high-grade dysplasia, or >25% villous elements. Controls are those participants who did not have a polyp recurrence at the end of the 4 years of follow-up.

Among the 1,905 participants who completed the Polyp Prevention Trial, 1,723 (90.4%) of the participants, 673 (89.3%) cases and 1,050 (91.2%) controls, had available DNA for genotyping. The analysis is limited to those participants identifying themselves as African American or Caucasian, as those participants endorsing “other” race were excluded due to small numbers (n = 43). The study was approved by the institutional review boards of the National Cancer Institute and those of the collaborating centers. All subjects provided written informed consent.

Data Collection and Variable Coding. Demographic characteristics, dietary intake, medical history, and health-related behavior information, including NSAID use, were collected in-person by a trained interviewer at the baseline visit and at each of four annual visits. The questionnaires collected information such as age, sex, education, race, income, and first-degree family history of colon cancer. In addition, data were collected on lifestyle factors such as physical activity, tobacco use, medication use, and medical history. Also at these visits, participants completed a Four Day Food Record and Food Frequency Questionnaire, as well as the Block Health Habits and History Questionnaire (45, 46), which was modified to account for the intake of high-fiber, low-fat foods.

Participants were also questioned about their use of various medications, including the use of NSAIDs. Information on prescription and nonprescription NSAID use was ascertained at each visit by asking participants if they were currently taking any medication, including NSAIDs, on a regular basis (defined as once per month or more frequently). In addition, participants were asked to bring any prescription or nonprescription medication with them to each visit for the interviewers to verify the medication name and dose. For the purposes of this analysis, regular NSAID use includes both aspirin and nonaspirin NSAIDs.

For continuous covariates, median cut points were determined on the basis of distributions among the entire cohort. These covariates included total energy intake, percent of calories from fat, total fiber intake, servings of fruits and vegetables per day, and physical activity. Type of recurrence, any adenoma and multiple adenomas, was determined from hospital pathology reports and confirmed by the study pathologists.
Additional covariates evaluated for confounding included regular vitamin/mineral supplement use (>1 week over the last year, <1 week over the last year); cigarette smoking (never, former, or current); education level (high school graduate, >high school graduate); first-degree relative with colon cancer (yes, no); age (continuous); multiple polyps diagnosed at baseline, body mass index, and physical activity. Body mass index was computed based on measured weight and height at the baseline interview and categorized as normal (<24.9), overweight (25.0-29.9), and obese (>30.0); (ref. 47). Physical activity was measured by asking participants how much time during the past year they typically spent on weekends and on weekdays in moderate or vigorous activity for combined occupational, nonoccupational, and nonwork/weekend activities. Data are expressed in terms of average hours per week spent in either moderate or vigorous activities of all types.

SNP Selection

We identified both proinflammatory and anti-inflammatory genes that have a role in inflammation and choose variants in these genes that were either reported to result in a functional change of the SNP or associated with inflammatory disease or cancer. We limited our selection to SNPs with a reported frequency of >10% to adequately examine the main effect of the SNP given our sample size. We assessed three SNPs in three different proinflammatory genes, IL-1β −511 C>T (rs16944), IL-6 −174 G>C (rs1800795), and IL-8 −251 T>A (rs4073), and two SNPs in the anti-inflammatory gene IL-10, −819 C>T (rs1800871) and −1082 G>A (rs1800896).

SNP Genotyping. Genotyping was done by BioServe Biotechnologies, Ltd. (Laurel, MD) via a two-step PCR process and mass spectrometry (Masscode, Qiagen Genomics, Bothel, WA) as described by Kokoris et al. (48). A 2-μL PCR master mix containing 1.73 μL of water, 0.2 μL of 10× buffer (Qiagen), 0.04 μL of 10 mmol/L deoxynucleotide triphosphates (Roche Applied Science, Indianapolis, IN), 0.01 μL of 100% formamide, and 0.02 μL of 5 units/μL HotStarTaq (Qiagen) was added to 3.5 ng of genomic DNA and external primers. Touchdown PCR protocol was used, with an additional 20 cycles with annealing at 50°C instead of 25 cycles. The second PCR used two allele-specific primers, differing at their 5’ ends by a tag-specific sequence and at their 3′ ends with the complementary base of the two possible alleles, and two universally tagged primers with a photo-technically cleavable mass spectrometry tags with 5’ end sequences identical to the allele-specific primers. A primer mix of 0.25 μL (0.01 μL of a 50 μmol/L stock of each allele specific primer, and 0.23 μL of a 44× stock of universally tagged primer mix) and 7.75 μL of master mix [6.478 μL of water, 1.0 μL of 10× mix (300 mmol/L Tris-HCl pH 8.7, 17 mmol/L MgCl₂, 360 mmol/L KCl, 40 mmol/L (NH₄)₂SO₄), 0.16 μL of 10 mmol/L each deoxynucleotide triphosphates (Roche), 0.04 μL of 100% formamide, and 0.072 μL of 5 units/μL HotFireTaq (Solis Biodyne, Tartu, Estonia)] were added and a touchdown PCR identical to the locus-specific PCR was done. The allele-specific PCR products were pooled from 384-well plates and purified using a 96-well QIAquick (Qiagen) plate according to the instructions of the manufacturer.

An auto-injector HTS PAL (CTC Analytics, Zwingen, Switzerland) was used to load the samples into the mass spectrometer. Before the samples reached the mass spectrometer, they were passed through a photolysis unit containing a mercury lamp emitting UV light at 254 nm. An Agilent 1100 series LC/MSD with an APCI ionization chamber was used to analyze the samples. The single quadrupole filters and channels the ions according to their mass/charge ratios. Datagen software was used to make automatic or manual calls of the SNP alleles.

The quality control used for this high-throughput genotyping consists of repeated assays on ~10% of randomly selected samples from each experiment as well as the inclusion of blinded controls. The genotyping results of the DNA as a “sample” and as a “quality control duplicated sample” were compared. The quality control concordance rate between duplicate samples for this analysis was ≥98%.

Data Analysis. We estimated allele frequencies (number of alleles / number of chromosomes) and genotype frequencies (number of participants with genotype / total number of participants) among individuals without an adenoma recurrence and those with any adenoma recurrence or with multiple adenoma recurrence. Each SNP was tested in the entire cohort to ensure that observed genotype frequencies exhibited Hardy-Weinberg equilibrium.

Unconditional logistic regression was used to determine odds ratios (OR) and 95% confidence intervals (95% CI) for the association between genotype and risk of adenoma recurrence after 4 years in the trial, as well as risk of multiple adenoma recurrence, using the PROC LOGISTIC function of the software package SAS (version 8.1, SAS Institute, Cary, NC), adjusting for age, race, sex, and body mass index. For the association of polymorphisms, homozygosity for the most frequent allele was set as the reference category, and ORs were calculated comparing the heterozygote to the reference category (homozygote for the common allele) and the homozygote for the rarer allele to the reference category using dummy variables. For the stratified analyses by regular NSAID use, we combined the heterozygote and the homozygote for the rare allele into a dominant model to increase statistical power. A two-sided significance level of 5% was used for these analyses.

As previously mentioned, we were missing available DNA on 168 participants and thus were not able to include them in our genotyping analysis. χ² statistics was used to determine whether significant differences in categorical variables were present between participants with genotype data and those without genotype data (data not shown). In our study, individuals with a family history of colon cancer were more likely to have genotype data compared with those without a family history (P = 0.03) and individuals who were current smokers were less likely to have genotype data (P = 0.02). However, these differences in distribution of covariates were nondifferential by disease status.

Several potential confounders were identified from a review of the literature and from previous publications using this data and were retained in models based on a ≥10% change in the β coefficients for genotype (homozygous wild-type set as the reference genotype) between the crude and the adjusted models. The multivariate adjusted models for genotype and risk of all outcome categories of polyp recurrence were adjusted for age, race, sex, and body mass index.

To assess heterogeneity in main effect of the genotype, we conducted stratified analyses by sex and by NSAID use. For our stratification by NSAID use, we defined regular NSAID users as individuals who reported current, regular NSAID use for three or more study year visits compared with individuals who reported no use over the entire study period (at baseline and at all four study year visits). We evaluated departures from expectations for multiplicative joint effects using the log-likelihood ratio test comparing the change in deviance (−2 log likelihood) between the model that included the interaction term to the model with only the main effects (α = 0.05, likelihood ratio test).
Demographic and lifestyle characteristics of the study participants are presented in Table 1. There were 673 participants (39.1%) who had at least one adenoma recurrence at 4 years and 1,050 (60.9%) participants who did not. The mean age of the study population at baseline was 61 years and 90% of participants are presented in Table 1. There were 673 participants (39.1%) who had at least one adenoma recurrence at 4 years and 1,050 (60.9%) participants who did not. The mean age of the study population at baseline was 61 years and 90% of participants who had at least one adenoma recurrence were more likely to be older, to be male, and less likely to report current, regular NSAID use compared with individuals who had no recurrence at 4 years (Table 1).

Genotype and allele frequencies along with associations of the cytokine SNPs are shown in Table 2. Variant allele frequencies in our cohort were similar to distributions among controls in previously reported case-control studies (34, 36). Among all of the SNPs analyzed, the genotype frequencies among the entire cohort were in Hardy-Weinberg equilibrium, except for IL-1β, which exhibited a borderline deviation from Hardy-Weinberg equilibrium (χ² = 4.2; P = 0.04). In our cohort, n = 773 (48%) had the heterozygote genotype IL-1β –511 when we expected n = 736 (46%) based on allele frequencies. There was a similar shift of observed and expected genotypes for the homozygote wild-type (41% observed, 42% expected) and the homozygote variant genotype (11% observed, 12% expected). We do not attribute this deviation from Hardy-Weinberg equilibrium as due to laboratory or genotyping error because the overall concordance rate for blinded quality controls was >98% and the overall allelic call rate for this SNP was 94%.

Overall, there were no statistically significant associations between any of the cytokine SNPs investigated in this study and risk of adenoma recurrence (Table 2). Furthermore, investigation of IL-10 haplotypes constructed from IL-10 –819 C>T and IL-10 –1082 G>A SNPs did not add any explanatory power; thus, only the results of the IL-10 genotype associations are presented. We investigated the association between cytokine genotype and risk of adenoma recurrence, stratified by gender, and did not observe any differences in patterns of the main effect of genotype or statistically significant interactions by gender (data not shown).

Previously, we reported an inverse association between NSAID use and adenoma recurrence in the entire Polyp Prevention Trial cohort of 1995 (11). Similarly, we found current, regular NSAID use for at least 3 years was inversely associated with risk of adenoma recurrence (OR, 0.70; 95% CI, 0.55-0.90) and multiple adenoma recurrence (OR, 0.55; 95% CI, 0.38-0.80) in our cohort of 1,723 Polyp Prevention Trial participants. Therefore, we examined the association of the cytokine polymorphisms and risk of adenoma recurrence separately among non-NSAID users and by regular NSAID use reported for at least 3 years over the study period (Table 3). In our stratified analyses, we observed a borderline significant increased risk of any adenoma recurrence among carriers of the IL-10 –1082 G>A variant allele among regular NSAID users (OR, 1.55; 95% CI, 1.00-2.43), as well as a suggestion of a
Table 2. Cytokine genotype frequencies by adenomatous polyp recurrence and adjusted ORs and 95% CIs for adenomatous polyp recurrence among participants in the Polyp Prevention Trial

<table>
<thead>
<tr>
<th>Cytokine Gene Polymorphisms</th>
<th>Total</th>
<th>No recurrence</th>
<th>Adenoma recurrence</th>
<th>Multiple recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>OR* (95% CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td><strong>IL-1β – 511</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>641 (40.8)</td>
<td>385 (40.1)</td>
<td>1.0</td>
<td>118 (45.0)</td>
</tr>
<tr>
<td>T/T</td>
<td>178 (11.3)</td>
<td>110 (11.5)</td>
<td>0.92 (0.74-1.15)</td>
<td>43 (13.0)</td>
</tr>
<tr>
<td>C/T + T/T</td>
<td>929 (59.2)</td>
<td>574 (59.9)</td>
<td>0.92 (0.74-1.14)</td>
<td>34 (11.0)</td>
</tr>
<tr>
<td><strong>IL-6 – 174</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>578 (37.5)</td>
<td>361 (38.3)</td>
<td>1.0</td>
<td>90 (35.6)</td>
</tr>
<tr>
<td>A/A</td>
<td>734 (47.6)</td>
<td>428 (45.4)</td>
<td>1.25 (0.99-1.57)</td>
<td>136 (53.8)</td>
</tr>
<tr>
<td>C/C</td>
<td>231 (15.0)</td>
<td>153 (16.2)</td>
<td>0.85 (0.61-1.19)</td>
<td>27 (10.7)</td>
</tr>
<tr>
<td>G/C + C/C</td>
<td>965 (62.5)</td>
<td>581 (61.7)</td>
<td>1.14 (0.91-1.42)</td>
<td>163 (64.4)</td>
</tr>
<tr>
<td><strong>IL-8 – 251</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>429 (27.5)</td>
<td>272 (28.4)</td>
<td>0.99 (0.85-1.15)</td>
<td>149 (56.2)</td>
</tr>
<tr>
<td>A/A</td>
<td>746 (47.9)</td>
<td>447 (46.7)</td>
<td>1.18 (0.92-1.52)</td>
<td>125 (48.6)</td>
</tr>
<tr>
<td>A/T + T/T</td>
<td>1,130 (72.5)</td>
<td>686 (71.6)</td>
<td>1.14 (0.90-1.45)</td>
<td>188 (73.2)</td>
</tr>
<tr>
<td><strong>IL-10 – 819</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>921 (57.6)</td>
<td>568 (58.3)</td>
<td>1.0</td>
<td>149 (56.2)</td>
</tr>
<tr>
<td>T/T</td>
<td>585 (36.6)</td>
<td>352 (36.2)</td>
<td>1.05 (0.85-1.31)</td>
<td>109 (39.6)</td>
</tr>
<tr>
<td>G/G + C/G</td>
<td>677 (42.4)</td>
<td>407 (41.7)</td>
<td>1.13 (0.72-1.76)</td>
<td>114 (43.8)</td>
</tr>
<tr>
<td><strong>IL-10 – 1082</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>333 (20.7)</td>
<td>204 (20.8)</td>
<td>1.0</td>
<td>61 (22.8)</td>
</tr>
<tr>
<td>G/A</td>
<td>831 (51.7)</td>
<td>511 (52.1)</td>
<td>0.98 (0.75-1.27)</td>
<td>131 (48.9)</td>
</tr>
<tr>
<td>A/A</td>
<td>442 (27.5)</td>
<td>265 (27.0)</td>
<td>1.05 (0.85-1.31)</td>
<td>105 (39.6)</td>
</tr>
</tbody>
</table>

NOTE: Adenomatous polyp recurrence diagnosed after year 1 in the study through postintervention at year 4.

*Multivariate OR and 95% CI adjusted by age, race, sex, and body mass index. Three participants with no recurrence and two participants with an adenomatous polyp recurrence with missing interview data were excluded from models.

Table 3. ORs and 95% CIs for interactions between cytokine genotypes and regular NSAID use and adenomatous polyp recurrence among participants of the Polyp Prevention Trial

<table>
<thead>
<tr>
<th>Cytokine Gene Polymorphisms</th>
<th>Regular NSAID use*</th>
<th>No NSAID use*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%) OR* (95% CI)</td>
</tr>
<tr>
<td><strong>IL-1β – 511</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>143 (41.7)</td>
<td>82 (41.2)</td>
</tr>
<tr>
<td>T/T</td>
<td>200 (58.3)</td>
<td>117 (58.8)</td>
</tr>
<tr>
<td>C/T + T/T</td>
<td>126 (37.6)</td>
<td>72 (37.7)</td>
</tr>
<tr>
<td><strong>IL-6 – 174</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>209 (62.4)</td>
<td>119 (62.3)</td>
</tr>
<tr>
<td>A/G + A/A</td>
<td>1,273 (79.3)</td>
<td>776 (79.2)</td>
</tr>
</tbody>
</table>

NOTE: Adenomatous polyp recurrence diagnosed after year 1 in the study through postintervention at year 4.

*Regular NSAID use includes current use of NSAIDs on a regular basis (>1 per month) at three or more yearly study visits. The reference group of no NSAID use includes individuals who reported no current use of NSAIDs on a regular basis (<1 per month) at all five study visits.

Multivariate OR and 95% CI adjusted by age, race, sex, and body mass index. Three participants with no recurrence and two participants with an adenomatous polyp recurrence with missing interview data were excluded from models.
We investigated the associations between several cytokine gene polymorphisms and risk of recurrent adenomatous polyps. Although we failed to observe main effects of a series of SNPs in pro- and anti-inflammatory cytokines, we observed a statistically significant interaction between the IL-10 –1082 G>A variant allele and regular NSAID use (Pinteraction = 0.01), as well as risk of multiple adenoma recurrence (Pinteraction = 0.01), compared with individuals who were homozygous for the common G allele and who were non-NSAID users. The observed results in Table 4 suggest that, compared with non-regular NSAID users with the IL-10 –1082 GG common genotype, non-regular NSAID users with any IL-10 –1082 G>A variant allele are at decreased risk of multiple adenoma recurrence (OR, 0.44; 95% CI, 0.25-0.77), as are those individuals with the IL-10 –1082 GG common genotype who take NSAIDs regularly (OR, 0.24; 95% CI, 0.11-0.53). However, individuals with the IL-10 –1082 G>A variant allele do not gain further benefit in their reduction of risk of multiple adenoma recurrence by taking NSAIDs regularly (OR, 0.34; 95% CI, 0.19-0.61). We observed similar patterns and magnitude of the main effect of the IL-10 –1082 G>A genotype when we stratified by NSAID use only at baseline, as well as significant interactions between NSAID use at baseline, IL-10 –1082 G>A genotype, and risk of adenoma recurrence (data not shown). We also investigated interactions and associations stratified by NSAID use for the entire study period (baseline and all four study visits) and we also observed similar patterns of association of the main effect if IL-10 –1082 G>A genotype and risk of adenoma recurrence, but the associations were not statistically significant (data not shown).

### Discussion

We investigated the associations between several cytokine gene polymorphisms and risk of recurrent adenomatous polyps. Although we failed to observe main effects of a series of SNPs in pro- and anti-inflammatory cytokines, we observed a statistically significant interaction between the IL-10 –1082 G>A genotype, regular NSAID use, and risk of adenoma recurrence (P = 0.01) and multiple adenoma recurrence (P = 0.01). Specifically, we observed an increase in risk for any adenoma and multiple adenoma recurrence among regular NSAID users who were carriers of the IL-10 –1082 G>A variant allele; among non-NSAID users, there was a nonsignificant decrease in risk for any adenoma recurrence and a significant decreased risk for multiple adenoma recurrence in those who were carriers of the IL-10 –1082 G>A variant allele. Although a few reports have investigated the association between cytokine polymorphisms and risk of colon cancer with mixed results (34, 35), our study is among the first to investigate the association between the cytokine gene polymorphisms IL-1β –511 C>T, IL-6 –174 G>C, IL-8 –251 T>A, IL-10 –818 C>T, and –1082 G>A and risk of colorectal adenoma recurrence. These data suggest that the IL-10 genotype may play a role in the progression of inflammation-associated colon cancer and that this association may be modified by NSAID use.

IL-10, which is produced by a variety of cells, including T lymphocytes, B lymphocytes, and monocytes, has been identified as a cytokine with important anti-inflammatory and immunosuppressive properties, which plays a major role in inhibiting the synthesis of proinflammatory cytokines including IL-1β, IL-6, IL-8, and IL-12 (49-51). Recent reports observed that carriers of the IL-10 –1082 A allele produced significantly lower levels of in vitro secretions of IL-10 compared with individuals with the IL-10 –1082 G>G genotype (51), whereas the IL-10 A [TCATA] haplotype formed by polymorphisms at positions –3575, –2763, –1082, –819, and –592 in the promoter of the IL-10 gene has been associated with an increased level of circulating IL-10 (52). Low IL-10 levels are associated with risk for prostate, cervical, noncardia gastric cancers, melanoma, and lymphoma (53). However, other studies show that high levels of IL-10 may actually be a risk factor for other cancers, hepatocellular, ovarian, melanoma, lymphoma, and myeloma (50). The IL-10 –592 C>A promoter polymorphism has been associated with a reduced breast cancer risk (37) and the IL-10 –1082 G>A polymorphism was associated with increased risk of noncardia gastric cancer (36). Currently, the role of IL-10 in cancer remains unresolved (53).
Recent evidence suggests that NSAID use may modify the association between polymorphisms in inflammatory genes and risk of colorectal cancer (34, 35) and colorectal adenomas (41). Macarthur et al. (35) investigated the association between the IL-10 –1082 G>A SNP and risk of colorectal cancer in a small population-based case-control study in Northeast Scotland. In their study, compared with individuals with the IL-10 –1082 GG genotype, carriers of the variant IL-10 –1082 A allele who used aspirin had a nonstatistically significant reduced risk of colorectal cancer (35). These differences in results may reflect real differences in the gene-drug association and their effect at different stages of disease (i.e., adenoma recurrence versus invasive colorectal cancer), or may be due to limited sample size, differences in aspirin/NSAID exposure in vitro or in vivo, or due to chance. However, our data mimic IL-10-deficient mice that develop spontaneous chronic inflammatory bowel disease, a known risk factor for colorectal cancer (49, 54). IL-10-deficient mice have increased production of proinflammatory cytokines and several studies report that IL-10 –1082 A allele mice treated with NSAIDs develop progressive, severe colitis much faster than IL-10/–/– mice not treated with NSAIDs (49). On the other hand, NSAID-treated wild-type mice did not develop colitis and their colonic epithelium had no evidence of hyperplasia or ulcerations (49). Microscopic examination of NSAID-treated IL-10/–/– mice revealed severe inflammatory infiltrates in their colonic mucosa and increased mRNA expression of inflammatory cytokines and COX-2 expression compared with NSAID-treated wild-type mice (49). It seems that inhibition of prostaglandin production was central to the development of NSAID-induced colitis. These findings may help to explain our observed findings that individuals who used NSAIDs and were carriers of the IL-10 –1082 A allele, which is associated with a decreased production of the IL-10 anti-inflammatory cytokine and proposed, subsequent increased production of proinflammatory cytokines, were at a significant increased risk of multiple adenoma recurrence as well as a suggested increased risk of any adenoma recurrence.

SNPs in the IL-6 gene proinflammatory genes have been associated with changes in cytokine production and inflammatory diseases (30-33, 55, 56). Landi et al. (34), reported that the IL-6 –174 C allele was associated with increased risk of colorectal cancer but only in subjects who did not habitually take NSAIDs. We did not observe any interactions between IL-6 genotype, NSAID use, and risk of adenoma recurrence in our study. Functional studies investigating the biological role of the substitution IL-6 –174 G>C have been mixed. Reports indicate that the –174 C allele is associated with lower and higher levels of expression of IL-6 in vitro and in vivo (33, 55, 57). Few studies have reported on the functional role of the IL-8 –251 T/A SNP, but one case-control study, investigating the role of IL-8 and ulcerative colitis, observed significantly higher IL-8 concentrations in patients with active ulcerative colitis compared with controls (31), whereas the IL-8 –251 A allele was associated with a decreased risk of colorectal cancer in one study (34).

There are several strengths to our study. First, we are among the first to report on the association between several cytokine polymorphisms and risk of colorectal adenoma recurrence using a substantial sample adequate to avoid the false positives that plague smaller studies and to investigate effect modification. Data for this analysis came from participants in a large dietary randomized trial in which we were able to assess confounding and joint effects by several dietary and lifestyle factors, including NSAID use. Second, due to the prospective study design, all participants had complete ascertainment of recurrent polyp because all participants received a full colonoscopy at the end of the trial intervention period, which also minimized the chance for misclassification of adenoma status compared with sigmoidoscopy of proximal adenomas.

Some limitations of the study should be noted. First, generalizability of these findings may be limited, as all of the participants had a history of an adenoma, the majority of the participants were male, and >90% self-identified as White. However, it is estimated that close to 40% of adults of ages ≥60 years have at least one prevalent polyp; therefore, these findings may be generalizable to a number of individuals at risk for colorectal cancer. Second, we did have limited power to detect joint effects and there were few participants who were regular NSAID users for the entire study period, with only 13% reporting regular NSAID use at baseline and at all four study visits. It is also possible that other functional or regulatory SNPs in linkage disequilibrium with the selected SNPs in this study account for the observed results. However, strong functional data seem to support the role of the IL-10 –1082 G/A SNP in altering plasma cytokine concentrations and risk of cancer (29, 58). Finally, it is plausible that variants in drug metabolism genes or in the COX-2 gene may modify or inhibit the association between NSAIDs and colon cancer and may explain some of the observed differences in the association between NSAID use and risk of adenoma recurrence (41, 59, 60).

In summary, our results add to recent reports that suggest NSAID use may not be beneficial among individuals with certain inflammatory genotypes and, given our data, may even increase an individual’s risk for colorectal adenomas. Specifically, our study provides evidence that carriers of the IL-10 –1082 A variant allele exhibit decreased risk for recurrent adenomas among non-NSAID users. These results suggest that the IL-10 –1082 A allele is a potential genotype identifying individuals who may not benefit from the chemoprevention of colorectal cancer by NSAIDs. Verification of this finding in other population-based samples and further investigations of its biological role as an effect modifier of the NSAID-colon cancer association are warranted. Future studies investigating the role of variants in inflammatory genes that modify the chemoprotective effect of NSAIDs in colon carcinogenesis may help to elucidate the biological mechanisms of the disease and identify individuals who may respond best to these chemopreventive agents, as well as aid in the development of public health and clinical intervention programs aimed at preventing colorectal cancer.

References


Inflammatory Cytokine Gene Polymorphisms, Nonsteroidal Anti-Inflammatory Drug Use, and Risk of Adenoma Polyp Recurrence in the Polyp Prevention Trial

Leah B. Sansbury, Andrew W. Bergen, Kay L. Wanke, et al.