

Circulating Levels of Inflammatory Cytokines and Risk of Colorectal Adenomas

Sangmi Kim,¹ Temitope O. Keku,² Christopher Martin,² Joseph Galanko,² John T. Woosley,³ Jane C. Schroeder,¹ Jessie A. Satia,¹ Susan Halabi,⁴ and Robert S. Sandler^{1,2}

¹Department of Epidemiology, School of Public Health, ²Center for Gastrointestinal Biology and Disease, and ³Department of Pathology, University of North Carolina, Chapel Hill, North Carolina and ⁴Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, North Carolina

Abstract

The association between obesity and colorectal neoplasia may be mediated by inflammation. Circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) are elevated in the obese. Adipose tissue can produce and release the inflammatory cytokines that are potentially procarcinogenic. We examined circulating levels of CRP, IL-6, and TNF- α in relation to risk factors and the prevalence of colorectal adenomas. Plasma levels of CRP, IL-6, and TNF- α were quantified in 873 participants (242 colorectal adenoma cases and 631 controls) in a colonoscopy-based cross-sectional study conducted between 1998 and 2002. Multivariable logistic regression was used to estimate associations between known risk factors for colorectal neoplasia and circulating levels of inflammatory cytokines and associations between inflammatory cytokines and colorectal adenomas. Several known risk factors for colorectal neoplasia were associated with higher levels of inflammatory cytokines, including older age, current smoking, and increasing adiposity. The prevalence of colorectal adenomas was associated with higher concentrations of IL-6 and TNF- α and, to a lesser degree, with CRP. For IL-6, adjusted odds ratios (OR) for colorectal adenomas were 1.79 [95% confidence interval (CI), 1.19–2.69] for the second highest plasma level and 1.85 (95% CI, 1.24–2.75) for the highest level compared with the reference level. A similar association was found with TNF- α , with adjusted ORs of 1.56 (95% CI, 1.03–2.36) and 1.66 (95% CI, 1.10–2.52), respectively. Our findings indicate that systemic inflammation might be involved in the early development of colorectal neoplasia. [Cancer Res 2008;68(1):323–8]

Introduction

Previous studies have shown that obesity is positively associated with colorectal adenomas and cancer (1). Possible mechanisms for the positive association between obesity and colorectal neoplasia include the obesity-induced insulin-related pathway (1) and inflammation (2, 3). Adipose tissue is now recognized as an endocrine organ rather than a simple fat storage site, and a wide range of inflammatory cytokines is released from adipose tissue, including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6; refs. 4, 5). Circulating levels of C-reactive protein (CRP), TNF- α , and IL-6 are elevated in the obese (6) and decrease after weight loss

among the same subjects (7, 8). Based on growing evidence suggesting procarcinogenic effects of the proinflammatory cytokines (9–11), we hypothesized that systemic inflammation might mediate the association between obesity and colorectal neoplasia. The aims of the present study were (1) to examine associations of levels of CRP, IL-6, and TNF- α and colorectal cancer risk factors [older age, high body mass index (BMI), and smoking] and protective factors [high physical activity and use of nonsteroidal anti-inflammatory drugs (NSAID)] and (2) to determine whether circulating levels of CRP, IL-6, and TNF- α were positively associated with prevalent colorectal adenomas.

Materials and Methods

Study population. Study participants were drawn from consecutive patients who underwent colonoscopy at the University of North Carolina Hospitals (Chapel Hill, NC) for a variety of indications, including abdominal pain, bleeding, and screening between August 1, 1998 and March 4, 2000 [Diet and Health Study (DHS) III] and for screening only between November 5, 2001 and December 20, 2002 (DHS IV), respectively. Patients were eligible to participate in the study if they were ≥ 30 years of age, could provide informed consent and complete a telephone interview, and had no known history of polyposis (>100 polyps), colon resection, colorectal cancer, colitis, or colorectal adenomas. Patients were excluded if they had inadequate preparation or an incomplete colonoscopic examination (cecum not reached). At the time of colonoscopy, all elevated lesions were biopsied or removed. Biopsy specimens were placed in formalin and submitted directly to pathology for sectioning and staining. A single experienced study pathologist (J.T.W.) examined all pathologic specimens and used a standardized form to record the total number of polyps and the maximum diameter (in millimeters), location, histologic type, and atypia grade of each polyp. Any polyp with tubular, tubulovillous, or villous pathology, or that had mixed adenomatous and hyperplastic characteristics, was classified as an adenoma.

Data collection. DHS research staff weighed all subjects and measured their height and waist and hip circumferences before colonoscopy. Information about demographic characteristics, education, medical history, NSAID use, smoking, and other lifestyle exposures was collected by telephone interview within 12 weeks of the colonoscopy using a structured questionnaire. Dietary intake was assessed using the Block food frequency questionnaire (DHS III; ref. 12) and the National Cancer Institute quantitative food frequency questionnaire (DHS IV; ref. 13). Physical activity was estimated by computing weekly energy expenditure in metabolic equivalents (MET; a standard measure of activity) based on the duration and intensity of various occupational and nonoccupational activities during typical days in the previous year. This study was approved by the institutional review board at the University of North Carolina School of Medicine.

Samples for analyses. There were 2,162 patients (926 for the DHS III and 1,236 for the DHS IV) who met the eligibility criteria described above. Overall, 84.3% of the eligible patients ($n = 1,822$) were asked to participate in the study; of these, 89.6% ($n = 1,633$) agreed. Telephone interviews

Requests for reprints: Robert S. Sandler, University of North Carolina, CB#7555, 4111 Bioinformatics Building, Chapel Hill, NC 27599-7555. Phone: 919-966-0090; Fax: 919-966-2478; E-mail: rsandler@med.unc.edu.

©2008 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-07-2924

Table 1. Selected characteristics of colorectal adenoma cases and controls, DHS, 1998 to 2002

Characteristics	Cases (n = 242)		Controls (n = 631)		P*
	No.	%	No.	%	
Age (y)					
30–49	45	18.6	173	27.5	
50–64	125	51.7	335	53.2	
≥65	72	29.8	122	19.4	0.001
Median age	58		54		
Sex					
Male	144	59.5	250	39.6	
Female	98	40.5	381	60.4	<0.001
Regular use of NSAIDs					
Yes	100	45.3	323	55.3	
No	121	54.8	261	44.7	0.011
Smoking status					
Never	95	43.4	299	51.2	
Past smokers	88	40.2	208	35.6	
Current smokers	36	16.4	77	13.2	0.13
BMI (kg/m ²)					
<25	80	33.6	248	40.6	
25–29.9	90	37.8	213	34.9	
≥30	68	28.6	150	24.6	0.131
Median BMI	26.84		26.15		
Abdominal obesity [†]					
Normal	34	19.2	131	25.8	
Level 1	47	26.6	116	22.9	
Level 2	96	54.2	260	51.3	0.187
Comorbidity [‡]					
Yes	144	65.2	336	57.6	
No	77	34.8	247	42.4	0.052
Physical activity (average MET-min/d)					
1st tertile (<2,400)	76	39.8	175	32.5	
2nd tertile (2,400–15,269)	65	34.0	183	34.0	
3rd tertile (≥15,270)	50	26.2	180	33.5	0.104
Median physical activity	2,670		2,973		
Total energy intake (kcal/d)					
1st tertile (1,389.11)	61	29.5	183	33.3	
2nd tertile (1,389.11–1,935.63)	64	30.9	183	33.3	
3rd tertile (≥1,935.64)	82	39.6	184	33.5	0.279
Median energy intake	1,757.72		1,598.18		
Total fat intake (g/d)					
1st tertile (47.05)	64	30.9	183	33.3	
2nd tertile (47.05–72.88)	60	29.0	183	33.3	
3rd tertile (≥72.89)	83	40.1	184	33.5	0.223
Median fat intake	63.85		59.57		

*P values are based on χ^2 statistics.

[†] Defined based on waist circumference. In women, <80 (cm) defined as normal, 80 to 87.9 as action level 1, and ≥88 as action level 2 abdominal obesity. In men, <94 defined as normal, 94 to 101.9 as action level 1, and ≥102 as action level 2 abdominal obesity.

[‡] Defined as presence of arthritis, diabetes, hypertension, or heart attack.

were completed with 75.5% of the subjects who consented to participate (n = 1,233). The final study sample consisted of 873 participants (327 for the DHS III and 546 for the DHS IV) with plasma samples for cytokine assays. There were slightly more men in the final study sample (45% versus 40%; P < 0.04) compared with those who were interviewed but not included in cytokine assays. However, there were no significant differences ($\alpha = 0.05$) in other demographic characteristics, such as age and race.

Laboratory methods. Specimen collection and handling conditions were similar for DHS III and IV. Fasting blood samples were collected from participants at the time of colonoscopy. Plasma was extracted from blood

samples and stored in aliquots at -80°C until analyses. Plasma concentrations of inflammatory cytokines were quantified using commercially available ultrasensitive ELISA kits for human CRP (Biosource) and human IL-6 and TNF- α (Diagnostic System Laboratories, Inc.). Minimum detection levels were 1.6 ng/mL for CRP, 0.104 pg/mL for IL-6, and 0.09 pg/mL for TNF- α according to the manufacturers. All assays were run in duplicate, and levels were classified according to the average of each pair of measurements. The intraassay and interassay coefficients of variation were 2.8% and 0.19% for CRP at 100 ng/mL, 11.3% and 16.9% for IL-6 at 0.16 pg/mL, and 5% and 11.2% for TNF- α at 1 pg/mL, respectively.

Statistical analysis. Selected characteristics were compared between cases and controls, and χ^2 tests were used to assess differences in proportions. Median and interquartile ranges (IQR) for each inflammatory cytokine were calculated according to case/control status. Mann-Whitney *U* test *P* values were calculated to evaluate the difference in circulating levels of each inflammatory cytokine by case/control status because levels of inflammatory cytokines were not normally distributed on raw or log-transformed scales. Spearman's rank test was used to evaluate correlations between circulating levels of three inflammatory cytokines.

Logistic regression was used to evaluate associations between risk factors for colorectal neoplasia and high levels of inflammatory cytokines (CRP, IL-6, and TNF- α , dichotomized as described below) after adjustment for age (30–49, 50–64, or ≥ 65 years) and sex. Subjects were classified as having high CRP or TNF- α if their measured levels were greater than or equal to the value of the 66th percentile in the distribution of each cytokine among controls. For IL-6, 630 subjects (50% of cases and 65% of controls) had values below the detection limit; therefore, we classified subjects as having high IL-6 if their levels were greater than or equal to the median value among controls with detectable values (0.3571 pg/mL).

Risk factors for colorectal neoplasia that were evaluated for associations with inflammatory cytokines were age at colonoscopy (30–49, 50–64, or ≥ 65 years), sex, regular use of NSAIDs (use three or more times per week during the past 5 years), smoking status (current, former, or never), physical activity (average levels in the prior year categorized into tertiles based on the distribution among controls), average daily total energy and fat intakes in the prior year (tertiles based on distribution among controls), and obesity (measured by BMI or waist circumference). BMI was categorized based on the WHO definitions (14) as obese (BMI, ≥ 30 kg/m²), overweight (BMI,

25–29 kg/m²), and normal weight or underweight (BMI, <25 kg/m²). Waist circumference was categorized according to the American Diabetes Association criteria for abdominal obesity as action level 1 (men, ≥ 94 cm; women, ≥ 80 cm), action level 2 (men, ≥ 102 cm; women, ≥ 88 cm), or normal (15).

Odds ratios (OR) and 95% confidence intervals (95% CI) for associations between colorectal adenomas and each inflammatory cytokine were estimated using unconditional logistic regression models. CRP and TNF- α were categorized based on tertile distributions among controls, with the lowest tertile serving as the referent exposure category for each cytokine. For IL-6, subjects with values below the detection limit in the assay were the referent exposure category, and the remaining subjects were categorized into two groups using the median IL-6 level among controls with detectable values (0.3571 pg/mL) as a cut point.

Based on a directed acyclic graph (16), age (30–49, 50–54, 55–59, 60–64, 65–69, 70–74, or ≥ 75 years), sex, smoking status, regular use of NSAIDs, comorbidity (defined as presence of arthritis, diabetes, hypertension, or heart attack), study phase (DHS III or DHS IV), daily total energy and fat intakes, physical activity, and BMI were considered as potential confounding factors. To determine which covariates should be entered in the final multivariable models, we constructed a full model with all potential confounders and assessed change in β coefficients for high levels of inflammatory cytokines versus the reference categories in relation to occurrence of colorectal adenomas. Age and sex were included in all models, and other covariates were retained if the β coefficient for any cytokine changed by >10% when they were removed. Final models for each inflammatory cytokine included age (30–49, 50–54, 55–59, 60–64, 65–69, 70–74, or ≥ 75 years), sex, and BMI (<25, 25–29.9, or 30 kg/m²).

Table 2. Multivariable associations of risk factors for colorectal neoplasia and high levels of inflammatory cytokines, DHS, 1998 to 2002

Risk factors	OR* (95% CI)		
	CRP ($\geq 12,013.1$ ng/mL)	IL-6 (≥ 0.3571 pg/mL)	TNF- α (≥ 2.2358 pg/mL)
Age (y)			
50–64	1.39 (0.97–2.0)	1.6 (1.02–2.5)	1.16 (0.82–1.65)
≥ 65	1.62 (1.06–2.48)	2.17 (1.31–3.59)	1.94 (1.29–2.91)
Female sex	2.25 (1.67–3.04)	0.85 (0.61–1.19)	0.94 (0.71–1.25)
Regular use of NSAIDs	1.19 (0.88–1.62)	1.47 (1.03–2.12)	1.34 (1.0–1.81)
Smoking status			
Current	2.05 (1.31–3.23)	2.59 (1.6–4.2)	1.23 (0.79–1.91)
Past	0.85 (0.61–1.2)	1.01 (0.67–1.51)	0.93 (0.67–1.29)
BMI (kg/m ²)			
25–29.9	1.94 (1.34–2.83)	1.85 (1.22–2.83)	1.46 (1.04–2.05)
≥ 30	5.36 (3.61–7.96)	2.47 (1.58–3.85)	1.91 (1.32–2.76)
Abdominal obesity [†]			
Level 1	1.93 (1.10–3.39)	1.56 (0.79–3.06)	1.25 (0.76–2.04)
Level 2	4.72 (2.91–7.65)	3.14 (1.75–5.61)	1.94 (1.27–2.96)
Physical activity (MET-min/d)			
2nd tertile [‡]	0.74 (0.5–1.09)	0.71 (0.44–1.14)	0.69 (0.47–1.01)
3rd tertile [‡]	0.65 (0.43–0.99)	1.09 (0.68–1.74)	0.73 (0.49–1.09)
Daily energy intake (kcal)			
2nd tertile [‡]	1.06 (0.72–1.56)	0.73 (0.45–1.17)	0.80 (0.55–1.16)
3rd tertile [‡]	1.45 (0.97–2.16)	1.16 (0.74–1.81)	1.02 (0.7–1.49)
Total daily fat intake (g)			
2nd tertile [‡]	1.01 (0.68–1.5)	0.92 (0.57–1.48)	0.86 (0.58–1.25)
3rd tertile [‡]	1.48 (1.0–2.19)	1.29 (0.82–2.04)	1.24 (0.85–1.81)

*Adjusted for age (30–49, 50–64, or ≥ 65 y) and sex.

[†] Defined based on waist circumference. In women, <80 (cm) defined as normal, 80 to 87.9 as action level 1, and ≥ 88 as action level 2 abdominal obesity. In men, <94 defined as normal, 94 to 101.9 as action level 1, and ≥ 102 as action level 2 abdominal obesity.

[‡] Tertiles are based on the distribution among controls.

Table 3. Crude and adjusted ORs and 95% CIs for associations between colorectal adenomas and plasma levels of inflammatory cytokines, DHS, 1998 to 2002

	Cases		Controls		OR (95% CI)	Adjusted* OR (95% CI)
	No.	%	No.	%		
CRP (ng/mL)						
<2,916.03	71	30.34	204	33.28	1.	1.
2,916.03–12,013	75	32.05	204	33.28	1.06 (0.72–1.54)	0.99 (0.66–1.49)
≥12,013.1	88	37.61	205	33.44	1.23 (0.85–1.78)	1.47 (0.96–2.25)
IL-6 (pg/mL)						
0	122	50.83	403	64.86	1.	1.
<0.3571	54	22.5	110	17.49	1.64 (1.12–2.41)	1.79 (1.19–2.69)
≥0.3571	64	26.67	111	17.65	1.93 (1.33–2.79)	1.85 (1.24–2.75)
TNF-α (pg/mL)						
<1.3877	53	22.36	209	33.28	1.	1.
1.3877–2.2357	89	37.55	209	33.28	1.68 (1.14–2.48)	1.56 (1.03–2.36)
≥2.2358	95	40.08	210	33.44	1.78 (1.21–2.63)	1.66 (1.10–2.52)

*Adjusted for age (30–49, 50–54, 55–59, 60–64, 65–69, 70–74, or ≥75 y), sex, and BMI (<25, 25–29.9, or ≥30 kg/m²).

Levels of each inflammatory cytokine were compared between case subgroups defined according to villous histology (villous or nonvillous), the size of the largest adenoma (<10 mm or ≥10 mm in diameter), and the presence of multiple adenomas (one, or two or more adenomas). Participants with more than one adenoma were classified based on the most advanced or largest adenoma, respectively. Mann-Whitney *U* tests were used to assess median differences in cytokine levels between case subtypes.

All statistical tests were two sided, with an α level of 0.05. All analyses were performed using Stata version 9.0.

Results

Selected characteristics of colorectal adenoma cases and controls are shown in Table 1. The median age was 58 years in cases and 54 years in controls. Compared with controls, cases were more likely to be male and were less likely to have used NSAIDs regularly in the past 5 years. Cases were also more likely to self-report comorbid conditions (arthritis, hypertension, heart attack, or diabetes.) Although associations did not reach statistical significance, cases were also more likely to be current smokers, obese (based on both BMI and waist circumference), and less physically active and to have had higher total energy and fat intakes than controls.

The median level of TNF- α was 1.962 pg/mL (IQR, 1.419–2.277) in cases and 1.843 pg/mL (IQR, 1.199–2.470) in controls ($P = 0.0034$, Mann-Whitney *U* test). For CRP, the median concentration was 7,582.4 ng/mL (IQR, 2,376.9–16,823.3) in cases and 5,699.04 ng/mL (IQR, 2,066.3–15,646.4) in controls ($P = 0.2547$, Mann-Whitney *U* test). Median IL-6 levels were zero in both cases and controls; however, the Mann-Whitney *U* test ($P < 0.001$) indicated that the IL-6 concentration of a randomly selected case was higher than would be expected by chance alone compared with the IL-6 concentration of a randomly selected control. Plasma levels of all three cytokine were correlated [Spearman's (Rho) correlation coefficients of 0.3431 between CRP and IL-6, 0.3239 between IL-6 and TNF- α , and 0.2028 between CRP and TNF- α ; all P values <0.001].

Many known risk factors for colorectal neoplasia were positively associated with high levels of CRP, IL-6, and TNF- α , including older

age, current smoking, and higher adiposity (both defined as BMI and waist circumference; Table 2). Higher BMI was positively related to high levels of inflammatory cytokines, particularly for CRP (OR, 1.94; 95% CI, 1.34–2.83 for BMI of 25–29.9 kg/m² and OR, 5.36; 95% CI, 3.61–7.96 for BMI of ≥30 kg/m²). This association did not vary between men and women (P for interaction between BMI and sex in relation to high levels of CRP = 0.45). Similarly, positive associations were found between levels of abdominal adiposity and high levels of inflammatory cytokines, although estimates were less precise due to observations with missing waist circumference data ($n = 189$).

Subjects in the highest tertiles of total energy and fat intakes were more likely to have high CRP than those in the lowest tertiles of intakes. Subjects in the highest tertile of fat intake were also more likely to have high IL-6 and TNF- α than those in the lowest tertile of fat intake. Prevalence of high CRP and TNF- α was inversely associated with physical activity above the reference level. Although regular use of NSAIDs is a generally accepted protective factor for colorectal neoplasia, regular users of NSAIDs in this study had a slightly increased prevalence of high levels of inflammatory cytokines relative to nonregular users. Sex was not associated with IL-6 or TNF- α , but women were more likely than men to have high CRP.

Overall, the prevalence of colorectal adenomas was positively associated with IL-6 and TNF- α above reference levels (Table 3). Specifically, for IL-6, adjusted ORs for colorectal adenomas were 1.79 (95% CI, 1.19–2.69) for the second highest category and 1.85 (95% CI, 1.24–2.75) for the highest category compared with the reference category. A similar association was found with TNF- α , for which adjusted ORs for the second and third highest levels were 1.56 (95% CI, 1.03–2.36) and 1.66 (95% CI, 1.10–2.52), respectively. The prevalence of colorectal adenomas was also slightly increased in association with the highest category of circulating CRP only (adjusted OR, 1.47; 95% CI, 0.96–2.25).

To evaluate whether higher levels of circulating inflammatory cytokines were associated with advanced pathologic features of colorectal adenomas, we performed separate analyses comparing

adenoma subtypes. Of 242 adenoma cases, 22 (9%) had adenomas with villous histology, 56 (23%) had adenomas ≥ 10 mm in diameter, and 48 (20%) had more than one adenoma. The median CRP level was 11,480.34 ng/mL (IQR, 5,760.25–25,263.24) for adenoma with villous histology and 7,050.6 ng/mL (IQR, 2,302.28–16,504.8) for adenomas with no villous component ($P < 0.04$, Mann-Whitney U test). There was no significant difference in median concentrations of IL-6 and TNF- α according to villous histology. In addition, levels of inflammatory cytokines were not associated with large adenomas or multiple adenomas.

Discussion

In this colonoscopy-based cross-sectional study of colorectal adenomas, circulating levels of IL-6 and TNF- α , and to a lesser degree CRP, were positively associated with the prevalence of colorectal adenomas. Several known risk factors for colorectal neoplasia also were associated with high levels of inflammatory cytokines, specifically older age, current smoking, increasing adiposity, physical inactivity, and higher caloric and fat intake.

Previous studies have not evaluated associations between cytokine levels and colorectal adenomas, but several have evaluated associations with colorectal cancer, with mixed results. Our findings for adenomas agree with results for colorectal cancer from a nested case-control study in the CLUEII cohort, in which Erlinger et al. (17) found a positive association with the highest quartile of CRP at baseline compared with the lowest quartile. Two prospective studies based on a Japanese population (18) and a cohort of Finish male smokers (19) also support an association between CRP and colorectal cancer, but there was no clear relationship between CRP and colorectal cancer in the Women's Health Study (20) or in the Japan Collaborative Cohort Study (21). Few studies have evaluated plasma levels of IL-6 or TNF- α in relation to colorectal neoplasia. Among older adults (aged 70–79 years) participating in the Health Aging and Body Composition study, IL-6 and TNF- α as well as CRP were positively associated with incident cancers and cancer deaths (22). However, cancer site-specific estimates of associations with each cytokine were not presented.

Accumulating evidence suggests that systemic inflammation might be a plausible mechanism for colon carcinogenesis. Studies have shown that genetic variations in inflammation-related genes, such as *IL-6*, *IL-8*, and *IL-10*, are associated with susceptibility to colorectal cancer and adenomas (3, 9). IL-6 seems to stimulate cell growth and inhibit apoptosis (2, 11). TNF- α is a key cytokine that is involved in the regulation of cytokines during inflammatory responses (10). Although TNF- α was first identified as a host-induced substance that is selectively toxic to tumor cells at high doses (23), at physiologic levels TNF- α promotes cellular proliferation and inhibits apoptosis, at least partly by inducing nuclear factor- κ B (10). CRP up-regulates the expression of adhesion molecules and stimulates the release of IL-1, IL-6, IL-18, and TNF- α from mononuclear phagocytes (24).

With regard to associations between inflammatory cytokines and risk factors for colorectal neoplasia, our findings are largely in agreement with previous studies. Obesity is a known risk factor for colorectal neoplasia and has recently been characterized as a state of low-grade systemic inflammation (6). Circulating levels of inflammatory cytokines were elevated in obese individuals compared with lean persons (25), and levels have been shown to decrease after weight loss (7, 8). It is now recognized that adipose

tissue can synthesize and release cytokines, such as TNF- α and IL-6 (26). Strong positive associations between obesity and levels of the proinflammatory cytokines in our study were consistent with expectations given that adipose tissue is a source of cytokines. CRP and IL-6 levels increase with chronological age (27, 28), but it remains unclear whether this occurs as a consequence of aging or is simply a reflection of underlying health conditions that are more common with increasing age (29). Smoking also has been associated with elevated levels of CRP and IL-6 (30–32). Although the effect of smoking on inflammatory cytokines seemed to persist for several years after smoking cessation in one study (31), only current (not past) smoking was associated with high levels of inflammatory cytokines in our study. In addition, high levels of physical activity have been associated with decreased concentrations of CRP, IL-6, and TNF- α , independent of obesity (33).

Weak positive associations between regular use of NSAIDs and high levels of inflammatory cytokines were contrary to our expectations. This could have been due to confounding by indication because comorbidity (arthritis, diabetes, hypertension, or a history of heart attack) was related both to regular use of NSAIDs and to high levels of inflammatory cytokines. However, adjustment for comorbidity did not change the direction of associations, although the strength of the associations was slightly attenuated. Alternatively, inflammatory cytokines measured at the time of colonoscopy may not have reflected typical levels among regular NSAID users because it is recommended that patients abstain from NSAID use for 1 week before colonoscopy. A positive association between NSAIDs and cytokines might be evident if cytokine levels among regular users are elevated relative to nonusers in the absence of NSAID use. Finally, the protective effect of NSAIDs might not be exerted via modifying circulating cytokines, as we hypothesized. There have been inconsistent findings: whereas aspirin administration has been shown to reduce circulating levels of CRP by 29% and IL-6 by 37% in angina patients (34), Feldman et al. (35) did not detect any significant change in serum CRP levels with low-dose aspirin use.

Our study has several strengths. First, colorectal adenomas were completely ascertained by colonoscopy to the cecum and were reviewed by a single experienced pathologist. In addition, detailed information on exposure history enabled the assessment of a wide range of potential confounding factors as well as the evaluation of relations between inflammatory cytokines and risk factors for colorectal neoplasia.

The temporal ambiguity inherent in a cross-sectional study is a limitation, but it is unlikely that adenomas themselves would cause a systemic increase in inflammatory cytokines. In our study, the median size of the largest adenoma was only 5 mm, and macrophage infiltration, which is uncommon in adenomas in general, is particularly rare in small adenomas (36). We recognize that a one-time measurement of circulating inflammatory cytokines may not represent an individual's inflammatory status during the development of adenomas and that measured levels may be influenced by diurnal or stress-induced variation. For example, patients in our study could have experienced a short-term increase in plasma levels of CRP, IL-6, and TNF- α given that they were awaiting colonoscopy, which may be a stressful event. However, stress-induced activation of cytokines (37) should not have differed by case/control status. In addition, although there is a report that TNF- α is significantly lower in the morning than in the evening (38), CRP and IL-6 are tightly regulated over time and are not affected by circadian variation (38, 39). Lastly, our finding

that IL-6 levels were below the detection limit in about 50% of cases and 65% of controls was consistent with expectations because it has been recognized that IL-6 is generally undetectable in healthy individuals without infection, trauma, or other inflammatory conditions (40).

We have shown associations between the prevalence of colorectal adenomas and increased levels of IL-6 and TNF- α and, to a lesser degree, CRP. These findings indicate that systemic inflammation might be involved in the early development of colorectal neoplasia.

References

- Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004;4:579–91.
- Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–45.
- Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 2005;7:211–7.
- Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc* 2001;60:329–39.
- Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* 2004;92:347–55.
- Das UN. Is obesity an inflammatory condition? *Nutrition* 2001;17:953–66.
- Esposito K, Pontillo A, Di Palo C, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003;289:1799–804.
- Dietrich M, Jialal I. The effect of weight loss on a stable biomarker of inflammation, C-reactive protein. *Nutr Rev* 2005;63:22–8.
- Gunter MJ, Canzian F, Landi S, Chanock SJ, Sinha R, Rothman N. Inflammation-related gene polymorphisms and colorectal adenoma. *Cancer Epidemiol Biomarkers Prev* 2006;15:1126–31.
- de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 2006;6:24–37.
- Lotem J, Sachs L. Different mechanisms for suppression of apoptosis by cytokines and calcium mobilizing compounds. *Proc Natl Acad Sci U S A* 1998;95:4601–6.
- Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol* 1986;124:453–69.
- Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. *Am J Epidemiol* 2001;154:1089–99.
- WHO Consultation on Obesity. Obesity: preventing and managing the global epidemic. Geneva: World Health Organization; 1997.
- Ardern CI, Janssen I, Ross R, Katzmarzyk PT. Development of health-related waist circumference thresholds within BMI categories. *Obes Res* 2004;12:1094–103.
- Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol* 2002;155:176–84.
- Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. *JAMA* 2004;291:585–90.
- Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S. Plasma C-reactive protein and risk of colorectal cancer in a nested case-control study: Japan Public Health Center-based prospective study. *Cancer Epidemiol Biomarkers Prev* 2006;15:690–5.
- Gunter MJ, Stolzenberg-Solomon R, Cross AJ, et al. A prospective study of serum C-reactive protein and colorectal cancer risk in men. *Cancer Res* 2006;66:2483–7.
- Zhang SM, Buring JE, Lee IM, Cook NR, Ridker PM. C-reactive protein levels are not associated with increased risk for colorectal cancer in women. *Ann Intern Med* 2005;142:425–32.
- Ito Y, Suzuki K, Tamakoshi K, et al. Colorectal cancer and serum C-reactive protein levels: a case-control study nested in the JACC Study. *J Epidemiol* 2005;15 Suppl 2: S185–9.
- Ilyasova D, Colbert LH, Harris TB, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer Epidemiol Biomarkers Prev* 2005;14:2413–8.
- Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 1975;72:3666–70.
- Black S, Kushner I, Samols D. C-reactive protein. *J Biol Chem* 2004;279:48487–90.
- Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115:911–9.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796–808.
- Forsey RJ, Thompson JM, Ernerudh J, et al. Plasma cytokine profiles in elderly humans. *Mech Ageing Dev* 2003;124:487–93.
- Morley JE, Baumgartner RN. Cytokine-related aging process. *J Gerontol A Biol Sci Med Sci* 2004;59:M924–9.
- Ferrucci L, Corsi A, Lauretani F, et al. The origins of age-related proinflammatory state. *Blood* 2005;105:2294–9.
- Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arterioscler Thromb Vasc Biol* 2002;22:1668–73.
- Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol* 2002;89:1117–9.
- Tracy RP, Psaty BM, Macy E, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vasc Biol* 1997;17:2167–76.
- Flynn MG, McFarlin BK, Markofski MM. State of the art reviews: the anti-inflammatory actions of exercise training. *Am J Lifestyle Med* 2007;1:220–35.
- Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouzias P, Nihoyannopoulos P. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation* 1999;100:793–8.
- Feldman M, Jialal I, Devaraj S, Cryer B. Effects of low-dose aspirin on serum C-reactive protein and thromboxane B2 concentrations: a placebo-controlled study using a highly sensitive C-reactive protein assay. *J Am Coll Cardiol* 2001;37:2036–41.
- Elder DJ, Baker JA, Banu NA, Moorghen M, Paraskeva C. Human colorectal adenomas demonstrate a size-dependent increase in epithelial cyclooxygenase-2 expression. *J Pathol* 2002;198:428–34.
- Miller GE, Rohleder N, Stetler C, Kirschbaum C. Clinical depression and regulation of the inflammatory response during acute stress. *Psychosom Med* 2005;67:679–87.
- DeRijk R, Michelson D, Karp B, et al. Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 β (IL-1 β), IL-6, and tumor necrosis factor- α (TNF α) production in humans: high sensitivity of TNF α and resistance of IL-6. *J Clin Endocrinol Metab* 1997;82:2182–91.
- Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clin Chem* 2001;47:426–30.
- Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med* 2000;51:245–70.

Acknowledgments

Received 8/1/2007; revised 10/5/2007; accepted 11/2/2007.

Grant support: Investigator-Sponsored Study Program of AstraZeneca IRUSE-SOM0516 and NIH grants P30 DK34987 and R01 CA 44684.

The funding source had no role in the collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Rosemary Link and Michael Goy (University of North Carolina Center for Gastrointestinal Biology and Disease ImmunoTechnologies Core, NIH grant P30 DK34987) for their expert advice and technical help with the immunoassays included in our study.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Circulating Levels of Inflammatory Cytokines and Risk of Colorectal Adenomas

Sangmi Kim, Temitope O. Keku, Christopher Martin, et al.

Cancer Res 2008;68:323-328.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/68/1/323>

Cited articles This article cites 39 articles, 13 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/68/1/323.full#ref-list-1>

Citing articles This article has been cited by 27 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/68/1/323.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/68/1/323>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.