

## Research Article

## A Prospective Evaluation of C-reactive Protein Levels and Colorectal Adenoma Development

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## Abstract

**Background:** Inflammation is hypothesized to play a role in colorectal tumorigenesis. Circulating levels of C-reactive protein (CRP), a serologic marker of the inflammatory response, have been positively associated with colorectal cancer development in some studies; however, there are limited data on the relation of CRP with colorectal adenomas, established precursors of colorectal cancer.

**Methods:** A nested case-control investigation of CRP levels and incident colorectal adenoma was conducted in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, a randomized trial of 154,942 individuals designed to test the efficacy of flexible sigmoidoscopy on colorectal cancer mortality when performed once, and then repeated 3 to 5 years later. Serum CRP levels were measured in baseline blood specimens from participants who were free of polyps in the left-sided colorectum at the baseline screening procedure, but who were found at the subsequent screen to have at least one colorectal adenoma ( $n = 356$ ), and in a set of polyp-free, frequency-matched controls ( $n = 396$ ).

**Results:** In a multivariable logistic regression model that included established colorectal adenoma risk factors, a 1-unit increase in log CRP level was associated with a 15% reduction in risk of developing colorectal adenoma (OR = 0.85, 95% CI, 0.75–0.98,  $P_{\text{trend}} = 0.01$ ). This association did not differ according to body size, smoking behavior, gender, use of nonsteroidal antiinflammatory drugs, or adenoma location.

**Conclusions:** High circulating CRP levels may be protective against colorectal adenoma development.

**Impact:** Though at contrast with mechanistic data on inflammation and colorectal tumorigenesis, this finding is not inconsistent with prior results on CRP and colorectal adenoma and warrants further investigation. *Cancer Epidemiol Biomarkers Prev*; 20(3); 537–44. ©2011 AACR.

## Introduction

Colorectal cancer is the third most common malignancy among women and men in the United States, with an estimated total of 143,000 cases in 2010 (1). The majority of colorectal cancers evolve from colorectal adenomas (the so-called adenoma-carcinoma sequence) and colorectal cancer and adenoma exhibit similar risk factor profiles. Both laboratory and observational evidence support a role for inflammation in colorectal tumorigenesis.

Inflammation exerts tumor-promoting effects by damaging DNA (2), stimulating angiogenesis, promoting cell proliferation, and inhibiting apoptosis (3). Further, precursor lesions of colorectal cancer, such as colorectal adenomas frequently exhibit inflammatory histologic features (4). Patients with inflammatory colonic diseases such as Crohn's disease and ulcerative colitis have a higher risk of developing colorectal cancer (5), while regular use of nonsteroidal antiinflammatory drugs (NSAID) such as aspirin confers a 40% to 50% reduction in colorectal cancer risk (6). Finally, common variants in genes that encode inflammatory cytokines have been associated with both colorectal cancer (7–9) and adenoma (10).

Prospective data on the association between inflammatory markers and colorectal neoplasia are limited. C-reactive protein (CRP), a liver-derived acute phase protein that is routinely examined as a clinical biomarker of inflammation, has been examined prospectively in several colorectal cancer studies. Of the 8 published investigations to date, 3 demonstrated positive associations between circulating CRP levels and colorectal cancer incidence (11–13), whereas the remainder reported generally null (14–17) or even inverse (18) relationships. To

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our knowledge, only one study prospectively evaluated the association between CRP levels and colorectal adenoma and the findings of that investigation were essentially null, and even suggested a potentially inverse relationship in a subgroup analysis (19).

Given the prior inconsistent literature on CRP and risk of colorectal cancer, we sought to address whether CRP levels are associated with colorectal tumorigenesis at an earlier stage in the natural history of the disease. We therefore investigated the relation of serum CRP levels with development of colorectal adenoma among 154,942 men and women enrolled in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening (PLCO) trial, a large randomized cancer screening trial.

## Materials and Methods

### Study design

The case-control study described here was nested within the screening arm of the PLCO trial (20). The PLCO trial is a large, randomized controlled trial designed to test the efficacy of cancer screening and to investigate etiology and early markers of cancer (21–23). From November 1993 until July 2001, 154,942 men and women aged 55 to 74 years of age, who had no prior history of prostate, lung, colorectal or ovarian cancer were enrolled at 10 U.S. sites (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St. Louis, MO and Washington, D.C). Exclusion criteria included current treatment for cancer (other than basal cell and squamous cell skin cancer); prior total colectomy, pneumonectomy, prostatectomy, or bilateral oophorectomy; participation in another cancer screening or primary prevention study; and recent use of finasteride (Proscar) or tamoxifen (Nolvadex). Beginning in April 1995, men who reported more than 1 serum prostate-specific antigen (PSA) test, and men and women who reported any lower gastrointestinal procedure (proctoscopy, sigmoidoscopy, barium enema, or colonoscopy) within 3 years prior to study enrollment, were excluded. Those randomly assigned to the screening arm ( $n = 77,465$ ) were offered a flexible sigmoidoscopy examination of the left-sided colorectum at study entry ( $T_0$ ), of which 83% (64,658) were compliant. The baseline sigmoidoscopy procedure was successfully completed (insertion to at least 50 cm within greater than 90% of mucosa visible or a suspect lesion identified) in 57,559 (89%) of the 64,658 participants that underwent screening. Participants had a repeat sigmoidoscopy either 3 or 5 ( $T_3$  or  $T_5$ ) years after the  $T_0$  screen. If neoplastic lesions were detected during the sigmoidoscopy procedure, participants were referred for subsequent colonoscopic examination. In addition, all participants provided a blood sample at the baseline screen ( $T_0$ ), and then every year after the baseline screen. Blood samples were collected at the clinical centers and were processed and frozen within 2 hours of blood draw and stored at  $-70^{\circ}\text{C}$ . All study participants completed a

risk factor and dietary questionnaire at baseline. All available medical and pathologic reports on follow-up were obtained and coded by trained medical record abstractors. The institutional review boards of the U.S. National Cancer Institute and the 10 screening centers approved the study and all participants provided informed consent.

### Study sample

Participants for this nested case-control study were selected from individuals assigned to the screening arm of the PLCO trial between November 1993 and September 1999 who had undergone a successful sigmoidoscopy at  $T_0$  and who met the following inclusion criteria: (i) Completion of the baseline risk factor questionnaire; (ii) Provided a blood sample for use in etiologic studies; (iii) No self-reported history of Crohn's disease, ulcerative colitis, familial polyposis, Gardner's syndrome, colorectal polyps or cancer (except basal cell skin cancer). Cases were identified from all participants who had a negative sigmoidoscopy result at the  $T_0$  screen but who were found to have a left-sided colorectal adenoma at the subsequent ( $T_3$  or  $T_5$ ) screen. Controls were defined as individuals who tested negative at the  $T_0$  sigmoidoscopy screen (i.e., no polyp or other suspect lesion detected), and were also found to be negative at the subsequent screen. Cases ( $n = 356$ ) were then frequency-matched to controls ( $n = 396$ ) by age at study entry (categorized as 55–59, 60–64, 65–69, and 70–74 years), gender, fiscal year at study entry, race (categorized as White, Black, Hispanic, Asian, Pacific Islander), screening center, study protocol ( $T_3$  or  $T_5$  rescreen), and season of blood draw (categorized as May–September or October–April).

A random sample of 30 control participants with available blood specimens from the  $T_0$ ,  $T_1$ , and  $T_5$  screens were selected to investigate the reproducibility of CRP measurements over a 5-year period. In addition, to investigate whether CRP levels change significantly during colorectal adenoma development, a random sample of 50 selected incident cases were further assayed for serum CRP levels at the  $T_3$  or  $T_5$  screen (i.e., whenever an adenoma was detected).

### Laboratory assays

Specimens were assayed in batches of 40 with cases and matched control sets arranged within the same batch. Specimens from participants obtained at multiple time points were assayed together in the same batch and for quality control purposes, blinded repeat samples ( $n = 90$ ) were embedded within and between batches to test for assay reproducibility. CRP levels were quantified using a solid phase chemiluminescent immunometric assay using the Immulite analyzer (Siemens Medical Solutions Diagnostics). The solid phase comprises a bead coated with an anti-CRP antibody and the liquid phase consists of alkaline phosphatase conjugated to rabbit polyclonal anti-CRP antibody in buffer. Each specimen was diluted 1:100 and was incubated together with the coated bead

for 30 minutes. During this incubation period, CRP forms an antibody sandwich complex with the anti-ligand antibody on the bead and enzyme-conjugated monoclonal murine anti-ligand antibody. Unbound sample and enzyme conjugate are then removed by centrifugation. Finally, chemiluminescent substrate is added and a signal is generated in proportion to the bound enzyme. The concentration of CRP in each sample is then derived from a standard curve. The intrabatch and interbatch coefficients of variation were 5.9% and 8.1%, respectively.

### Statistical methods

The baseline characteristics of cases and controls were compared using the Wilcoxon rank-sum test (for continuous data) or Pearson's chi-square (for categorical data; SAS statistical software, version 9.1; SAS Institute). Because the CRP values were not normally distributed in the control population, they were logarithmically transformed to approximate normality. Intraindividual and interindividual variances were estimated for the case and control sets with multiple CRP measurements using random effects models and the intraclass correlation coefficient (ICC) was derived from these values. Logistic regression was used to estimate odds ratios (OR) and 95% CI for the association between CRP levels and incidence of colorectal adenoma. CRP values were analyzed as both a continuous, log-transformed variable and as a 4-level categorical variable based on the distribution of quartile cut-points in the control population. Multivariable models were adjusted for an *a priori*-determined set of colorectal neoplasia risk factors, namely, body mass index (BMI, categorized as <25 kg/m<sup>2</sup> (referent), 25–30 kg/m<sup>2</sup> and >30 kg/m<sup>2</sup>); regular use of NSAIDs (yes/no), family history of colorectal cancer (yes/no), self-reported history of diabetes (yes/no), educational attainment (no college education/some college education and above), use of hormone therapy (HT, females only; never/ever), and smoking (categorized as never, former or current smoker). Inclusion of other potential covariates such as alcohol consumption, physical activity, and intake of dietary fiber, calcium, folate, vitamin D, saturated fat and red meat, frequency of NSAID use (use per month) and smoking intensity (cigarettes per day), did not lead to any appreciable change in the risk estimates (defined as a 10% or greater change in the beta coefficients) and were therefore not considered in the final multivariable models. Further, inclusion of the matching factors as additional covariates had no impact on the risk estimates. Significance tests for trend were calculated using a single ordinal variable corresponding to the median values for each CRP quartile which was entered into the multivariable model as a continuous variable.

Analyses stratified according to smoking status (never,  $n = 362$ /ever,  $n = 390$ ), BMI (<25,  $n = 240$ ;  $\geq 25$  kg/m<sup>2</sup>,  $n = 512$ ), gender (Males,  $n = 491$ ; Females,  $n = 261$ ), NSAID use (user,  $n = 434$ /non-user,  $n = 318$ ), were performed and tests of heterogeneity were conducted by entering a cross product term of CRP with the variable of interest

into the multivariable model, the coefficient of which was evaluated with the Wald test. The association of CRP with colorectal adenoma was also evaluated according to adenoma location (left colon vs. rectum) or advanced histology (defined as  $\geq 1$  cm, high-grade dysplasia or villous components) All statistical tests were 2-sided and a  $P < 0.05$  was considered statistically significant.

### Results

Selected baseline characteristics of the colorectal adenoma and polyp-free control groups are compared in Table 1. Relative to the controls, the colorectal adenoma cases were more likely to have reported a family history of colorectal cancer and a higher consumption of alcoholic drinks but did not differ significantly with respect to any of the other colorectal adenoma risk factors. The median level of CRP was 1.6 mg/L in the cases and 1.8 mg/L in the controls ( $P = 0.29$ ).

Table 2 presents distributions of known colorectal adenoma risk factors across quartiles of CRP among the control participants. CRP levels were positively related to BMI, female gender, diabetes, use of HT (in women), regular use of NSAIDs, and a history of hypertension. Physical activity and consumption of total calories, folate, saturated fat, fiber, and calcium were inversely associated with CRP levels.

Reproducibility of serum CRP measurements over a 6-year period was assessed in both the colorectal adenoma and control groups. For the controls ( $n = 30$ ), median CRP levels measured at the 3 time points ( $T_0$ ,  $T_1$ , and  $T_5$ ) were essentially constant, and did not differ significantly from each other ( $P = 0.35$ ). For the case series ( $n = 50$ ), median CRP levels decreased from 1.6 mg/L at baseline ( $T_0$ ) to 1.2 mg/L at either  $T_3$  or  $T_5$  (i.e., whenever the adenoma was diagnosed), however, these values were also not significantly different ( $p = 0.37$ ; ref. Table 3). Further, the ICC values for both the case and control series were similar (0.70 and 0.83, respectively) indicating that the within-individual fluctuations in CRP levels were similar in the 2 groups.

Table 4 provides ORs and 95% CIs for the association between serum CRP levels and colorectal adenoma incidence. In a multivariable model that included established colorectal adenoma risk factors, a 1-unit increase in log-CRP concentration was associated with a statistically significant 15% reduction in risk of developing colorectal adenoma (OR = 0.85, 95% CI, 0.74–0.98,  $P_{\text{trend}} = 0.01$ ). Similarly, when analyzed as a categorical variable, a statistically significant inverse trend was observed across quartiles of CRP level (OR comparing extreme quartiles [OR<sub>q4-q1</sub>] = 0.65; 95% CI, 0.41–1.03;  $P_{\text{trend}} = 0.03$ ). The inverse association of CRP with colorectal adenoma incidence was not different when analyses were restricted to advanced adenomas (multivariate OR per unit change in log CRP = 0.83; 95% CI, 0.69–1.00) and was slightly weaker when restricted to colon adenomas only (multivariate OR per unit change in log CRP = 0.93; 95% CI,

**Table 1.** Baseline characteristics of incident colorectal adenoma cases and matched controls in the PLCO trial

Characteristic	Cases (n = 356)	Controls (n = 396)	P <sup>a</sup>
Age, y	62.7 (5.0)	62.8 (5.0)	Matched
Female, %	34.8	34.6	Matched
Caucasian, %	89.6	90.4	Matched
Family history of colorectal cancer, %	12.1	7.8	0.05
Some college education and above, %	71.0	70.0	0.77
Diabetes, %	6.4	7.0	0.74
BMI at baseline, kg/m <sup>2</sup>	27.4 (4.5)	27.2 (4.6)	0.36
Hours spent in vigorous physical activity per week, %			
0	15.0	11.0	0.55
1–2	30.0	30.0	
≥3	55.0	59.0	
Cigarette smoking status			
Never, %	45.0	51.0	0.18
Current, %	9.0	7.0	
Former, %	46.0	43.0	
Ever use of female hormone therapy, % <sup>b</sup>	59.0	54.0	0.81
Use of NSAIDs, %	54.0	61.0	0.29
Daily intake			
Alcohol, g	15.2 (26.4)	11.4 (26.7)	0.03
Folate, μg	399 (200)	403 (199)	0.73
Red meat, g	89.7 (73.6)	81.6 (68.9)	0.37
Energy, kcal	2,197 (866)	2,229 (930)	0.94
Saturated fat, g	24.1 (12.3)	24.5 (14.7)	0.47
Fiber, g	23.9 (12.3)	24.6 (14.8)	0.37
Calcium, mg	997 (544)	1,026 (590)	0.91
CRP, mg/L <sup>c</sup>	1.6 (0.3–3.0)	1.8 (0.1–3.4)	0.29
Adenoma characteristics			
Advanced adenoma (n)	143		
Non-advanced adenoma	144		
Unknown	69		
Multiple adenomas present (n)	55		
Non-multiple adenomas	274		
Unknown	27		
Adenoma Location <sup>d</sup> (n)			
Colon	305		
Rectum	90		
Follow-up year when adenoma detected (n)			
Year 3	109		
Year 5	247		

Values are means (SD) unless otherwise stated.

<sup>a</sup>P-values derived from *t* test for continuous data and Pearson's chi-square for categorical data.

<sup>b</sup>In females only.

<sup>c</sup>Median levels (and inter-quartile range).

<sup>d</sup>Total number exceeds 396 due to presence of multiple adenomas for some subjects.

0.81–1.07). In addition, the relation between CRP level and colorectal adenoma incidence persisted following exclusion of individuals with very high CRP levels (>10 mg/L, *n* = 80; multivariate OR per unit change in log CRP = 0.90, 95% CI, 0.77–1.05) or when CRP levels

were categorized according to cutpoints proposed clinically for cardiovascular disease prevention (≥3mg/L compared with <3mg/L; multivariate OR = 0.77, 95% CI, 0.56–1.04). Finally, we observed no difference in the association of CRP level with colorectal adenoma

**Table 2.** Distribution of selected colorectal adenoma risk factors by quartile of CRP among control (polyp-free) individuals ( $n = 396$ )

	Quartile of CRP (mg/L)			
	<0.8	0.8–1.8	1.8–4.0	≥4.0
Age, y	62.5 (0.5)	62.6 (0.5)	63.6 (0.5)	62.4 (0.5)
BMI, kg/m <sup>2</sup>	25.0 (0.4)	27.3 (0.4)	27.3 (0.4)	29.1 (0.4)
Female, %	24.6	28.9	37.1	50.0
Caucasian, %	82.4	93.0	92.3	92.4
Family history of CRC, %	8.0	8.0	12.9	10.6
College education, %	46.0	38.8	33.5	35.9
Diabetes, %	4.8	5.5	6.7	9.4
≥3 hours physical activity/week	36.9	24.4	22.2	19.4
Current Smoker, %	5.4	4.5	11.3	8.2
Ever use of HT (women)	51.0	53.0	47.0	70.0
Regular use of NSAID, %	48.7	57.7	60.3	62.9
Hypertension, %	23.9	34.3	31.3	38.7
Alcohol intake, g/day	12.2 (2.7)	12.2 (2.8)	12.2 (2.7)	8.9 (2.7)
Folate intake, μg/day	437 (20)	427 (21)	391 (20)	360 (20)
Red meat intake, g/day	74.5 (7.0)	86.9 (7.2)	81.2 (7.0)	84.0 (7.0)
Energy intake, kcal/day	2,346 (94)	2,300 (97)	2,212 (94)	2,059 (94)
Saturated fat, g/day	26.0 (1.5)	25.2 (1.5)	24.6 (1.5)	22.9 (1.5)
Fiber, g/day	28.3 (1.1)	26.6 (1.1)	23.5 (1.1)	23.2 (1.1)
Calcium, mg/day	1,028 (60)	1,101 (61)	1,046 (60)	932 (60)

NOTE: Values are means ( $\pm$ SD) unless otherwise stated.

following stratification by BMI, smoking status, gender, or NSAID use (data not shown).

## Discussion

In this prospective, sigmoidoscopy-based investigation of colorectal adenoma, circulating levels of CRP, a systemic marker of inflammation, were inversely associated with colorectal adenoma incidence. These data suggest that healthy individuals with elevated levels of CRP may be at lower risk of developing colorectal adenomas—a

result that contrasts with findings from several studies of CRP and colorectal cancer incidence and with mechanistic studies of inflammation and colorectal tumorigenesis.

A number of epidemiologic studies have investigated the association of prediagnostic CRP concentrations and colorectal cancer risk and have yielded inconsistent results. However, a recent meta-analysis, which summarized data from 8 independent prospective investigations, concluded that CRP was a weak, positive risk factor for colorectal cancer (summary relative

**Table 3.** Median levels and interquartile ranges for CRP levels in incident colorectal adenoma cases and controls sampled at multiple time points

	Screen			P <sup>a</sup>
	T <sub>0</sub>	T <sub>1</sub>	T <sub>5</sub>	
Controls ( $n = 30$ )				
CRP, mg/L	1.2 (0.1–2.3)	0.9 (0.3–1.5)	1.2 (0.1–2.3)	0.35
Adenoma cases ( $n = 50$ )				
CRP, mg/L	1.6 (0.6–2.5)	1.2 (0.2–2.3)	N/A	0.37

N/A; Not applicable

<sup>a</sup>For difference in median values assessed using Friedman's  $\chi^2$  statistic<sup>b</sup>Whenever the colorectal adenoma was diagnosed.

**Table 4.** OR and 95% CI of colorectal adenoma according to quartiles of the distribution of serum CRP concentration in the PLCO Cancer Screening Trial

	Quartile of CRP				<i>P</i> <sub>trend</sub> <sup>a</sup>
	<0.8	0.8–1.8	1.8–4.0	≥4.0	
Median, mg/L	0.5	1.3	2.6	6.4	
Interquartile range, mg/L	0.3–0.7	1.1–1.6	2.0–3.2	3.8–9.1	
All colorectal adenomas					
Cases/controls, <i>n</i>	88/99	102/99	91/99	75/99	
OR (95% CI) <sup>b</sup>	1.00	1.16 (0.78–1.73)	1.08 (0.72–1.61)	0.81 (0.53–1.23)	0.15
OR (95% CI) <sup>c</sup>	1.00	1.08 (0.72–1.64)	0.93 (0.61–1.43)	0.65 (0.41–1.03)	0.03

<sup>a</sup>Entered into the model as a single ordinal variable with values corresponding to the median of each quartile of the CRP distribution.

<sup>b</sup>Univariate model

<sup>c</sup>Adjusted for cigarette smoking status, BMI at baseline (overweight, obese), use of NSAIDs, diabetes, use of hormone therapy (females only), family history of colorectal cancer, and educational attainment.

risk per one unit change in natural log-transformed CRP, 1.12 (95% CI, 1.01–1.25; ref. 24). The inverse association between CRP levels and colorectal adenoma risk observed in the current investigation was surprising, given our *a priori* hypothesis that predicted a positive relationship, in keeping with the tumorigenic effects of inflammation in the colonic mucosa and the aforementioned findings from prospective studies of CRP levels and colorectal cancer risk. However, the results from the current study, though unexpected, are in fact somewhat concurrent with a previous investigation of CRP levels and incident colorectal adenoma development that was conducted within the CLUE II cohort (19). In the CLUE II study, which evaluated prediagnostic CRP concentrations and incident adenoma risk among 135 colorectal adenoma cases and 269 matched controls, CRP levels were also inversely, albeit nonsignificantly, associated with colorectal adenoma risk. In that investigation, the inverse association between CRP levels and colorectal adenoma appeared to be stronger for proximal and tubular adenomas. For the latter, individuals in the highest quartile of CRP had a statistically significant 70% reduction in risk of developing a tubular adenoma than those in the lowest CRP quartile. Although these findings are based on a relatively small number of cases, the consistency of the CLUE II data with the results of the current investigation are notable. In addition, our data are partially supported by the results of a recent case-control study conducted in Hawaii which reported that a polymorphism in the *CRP* gene which is associated with higher circulating CRP levels, was inversely associated with colorectal adenoma prevalence (25).

If the observed inverse association between CRP and colorectal adenoma development is indeed real, it may suggest that an elevated systemic inflammatory state is somehow protective in the early stages of colorectal tumorigenesis. This hypothesis stands in direct contrast

to mechanistic and observational evidence, which point towards tumorigenic effects of inflammation. However, there are data to demonstrate that neoplastic lesions can be targeted for removal by the immune system (26, 27). In colon cancer patients specifically, atopic dermatitis is accompanied by upregulation of cytokines that stimulate T-cell infiltration against colon tumor cells (28). Further, circulating levels of eosinophils and mast cells are associated with better prognosis (29) and the type and density of T-cells adjacent to colon tumor cells was shown to be a better predictor of survival than traditional staging (30). Therefore, it may be hypothesized that an individual with a heightened immune response may be at lower risk of developing early neoplastic lesions because they are targeted and removed more effectively than in an individual with a weaker response. This hypothesis is also supported by epidemiologic data which have revealed inverse associations between allergies and cancer risk (31). Allergies are associated with enhanced immunosurveillance and the increased immunoglobulin E (IgE) levels which accompany an allergic response have been shown to decrease tumor development and increase survival time in murine models (32–34). In a recent investigation nested within the Iowa Women's Health Study, a statistically significant reduced incidence of colorectal cancer was observed among allergy-sufferers compared with those with no reported allergies, suggesting that heightened immunosurveillance associated with allergic response may indeed be protective against colorectal tumorigenesis (35). In addition, CRP itself may play a direct role in the elimination of neoplastic lesions through its function as an opsonin. CRP plays a key role in tissue repair and maintenance of tissue homeostasis by binding to damaged or apoptotic cells and targeting them for removal by immune cells (36). Therefore, higher CRP levels may lead to more efficient elimination of precancerous cells and reduced incidence of colorectal adenoma.

The findings of this study may, however, raise questions regarding the utility of CRP as a biomarker of colonic inflammation. CRP synthesis occurs in the liver in response to proinflammatory cytokines such as IL-6 and TNF- $\alpha$ , which are released by phagocytes and adipocytes. CRP levels are routinely measured in clinical settings to evaluate a patient's overall inflammatory state and are considered a sensitive, yet non-specific, marker of chronic inflammation. However, CRP is also an acute phase protein and circulating levels of CRP can be influenced by a wide range of exogenous and endogenous factors, and it is not known to what degree circulating CRP levels reflect low-grade inflammation in the colonic mucosa. A serologic biomarker that is specific to colonic inflammation has yet to be identified; however, a recent cross-sectional study of colorectal adenomas reported a significant positive relationship between circulating levels of IL-6 and TNF- $\alpha$  and colorectal adenoma prevalence, and a weaker, non-significant association for CRP (37). Unfortunately, the current study lacks data on IL-6 and TNF- $\alpha$  levels but the assessment of these factors in relation to incident colorectal adenoma could prove highly informative. In addition, further work is required to ascertain the degree to which circulating CRP level is indicative of colonic inflammation, whereas future prospective studies of colorectal adenoma may wish to explore the additional inflammatory parameters and cytokines that have been mechanistically linked to colorectal tumorigenesis to more accurately evaluate the role of inflammation in the early stages of colorectal cancer.

Given that CRP levels can be influenced by a broad range of factors, it is also plausible that the current findings are the result of bias or confounding by unrecognized parameters. One possibility is that individuals with high levels of CRP may be more likely to be at higher risk for cardiovascular disease or other diseases associated with obesity and the metabolic syndrome, and they may have been using medications such as NSAIDs or statins, which are protective against colorectal adenomas. Indeed, in our study population, NSAID use was more common among those with the highest CRP levels; however, the inverse association between CRP levels and colorectal adenoma incidence did not differ according to use of NSAIDs. We note, however, that the NSAID data was only collected at the baseline sigmoidoscopy, and assessment of NSAIDs and other lifestyle factors at multiple time points may have allowed more accurate classification of habitual exposure. Further, data on other medications that may be relevant, such as statin use, were also not available, however, we feel it is unlikely that our findings were significantly influenced by statin use due to the relatively low prevalence of use at the time the baseline blood specimens were collected (38). We also considered the fact that some individuals had very high levels of CRP (>10 mg/L)-indicative of acute inflammation at any site in the body, and speculated that these subjects might be driving the inverse association between

CRP and colorectal adenoma. We, therefore, performed a sensitivity analysis, whereby we evaluated the relation of CRP with colorectal adenoma following exclusion of all subjects with CRP less than 10 mg/L ( $n = 80$ ), and found the results to be essentially unaltered. Similarly, we also analyzed the data using CRP cutpoints that have been proposed clinically for cardiovascular disease prevention ( $\geq 3$ mg/L compared with  $< 3$ mg/L) and likewise observed no overall change in the findings (data not shown).

Strengths of the study include a population of subjects with incident colorectal adenomas and matched controls that had undergone a flexible sigmoidoscopy and were known to be free of neoplasia in the left-sided colorectum at the baseline screen. A limitation is lack of data on polyp status in the right-sided colorectum as this was not evaluated during the screening procedure. The availability of serum from multiple time points in a subset of both the case and control groups enabled us to perform an assessment of the temporal reproducibility of CRP levels in both the cases and the controls. The relative stability of CRP levels over a 5-year period in both the case and control groups suggests that a one-time measurement of CRP provides an accurate estimate of medium-term levels and that the development of colorectal adenoma is not associated with any significant changes in circulating CRP levels.

In summary, we found CRP levels to be inversely related to the development of colorectal adenoma-data that is not inconsistent with the majority of results from other studies of CRP and colorectal adenoma risk. This finding suggests that an elevated systemic inflammatory response (as evaluated by CRP levels) may be protective against the early stages of colorectal tumorigenesis, hypothetically through enhanced immunosurveillance.

#### Disclosure of Potential Conflicts of Interest

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