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Regular Aspirin Use Associates With Lower Risk of Colorectal Cancers With Low Numbers of Tumor-Infiltrating Lymphocytes

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This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e17. Learning Objective: Upon completion of this examination, successful learners will be able to: (1) Evaluate data on lifestyle factors and medication use in relation to tumor biomarkers. (2) Utilize official symbols for genes and gene products that are designated by the Human Genome Organization (HUGO). (3) Interpret findings from a prospective cohort study design, as compared to cross-sectional study designs.

BACKGROUND & AIMS: Aspirin use reduces colorectal cancer risk. Aspirin, a nonsteroidal anti-inflammatory drug, inhibits prostaglandin-endoperoxide synthase 2 (PTGS2 or cyclooxygenase-2); PTGS2 promotes inflammation and suppresses T-cell–mediated adaptive immunity. We investigated whether the inverse association of aspirin use with colorectal carcinoma risk was stronger for tumors with lower degrees of lymphocytic infiltrates than for tumors with higher degrees of lymphocytic infiltrates. **METHODS:** We collected aspirin use data biennially from participants in the Nurses' Health Study and Health Professionals Follow-up Study. Participants were asked whether they took aspirin in most weeks, the number of tablets taken per week, and years of aspirin use. We collected available tumor specimens (n = 1458) from pathology laboratories in the United States. A pathologist confirmed the diagnosis of colorectal adenocarcinoma (excluding anal squamous cell carcinoma), and evaluated histopathology features, including patterns and degrees of lymphocytic infiltrates within and around tumor areas. Person-years of follow-up evaluation were accrued from the date of return of questionnaires until dates of colorectal cancer diagnosis, death, or the end of follow-up evaluation (June 2010). Duplication-method Cox proportional hazards regression was used to assess the association of aspirin with the incidence of colorectal carcinoma subgroups according to the degree of tumor-infiltrating lymphocytes (TILs), intratumoral periglandular reaction, peritumoral reaction, or Crohn's-like reaction. **RESULTS:** We documented 1458 rectal and colon cancers. The inverse association between regular aspirin use and colorectal cancer risk significantly differed by concentrations of TILs ($P_{\text{heterogeneity}} = .007$). Compared with nonregular use, regular aspirin use was associated with a lower risk of tumors that had low levels of TILs (relative risk, 0.72; 95% confidence interval, 0.63–0.81), and strength of the association depended on aspirin dose and duration (both $P_{\text{trend}} < .001$). In contrast, aspirin use was not associated with a risk of tumors having intermediate or high levels of TILs. This differential association was consistent regardless of the status of tumor microsatellite instability, mutations in *BRAF*, or expression of PTGS2. Regular aspirin use was associated with a lower risk of tumors that contained low levels of $CD3^+$ T cells, $CD8^+$ T cells, or CD45RO $(PTPRC)^+$ T cells (measured by immunohistochemistry and computer-assisted image analysis). **CONCLUSIONS:** Based on data from the prospective cohort studies, regular use of aspirin is associated with a lower risk of colorectal carcinomas with low concentrations of TILs. These findings indicate that the immune response in the tumor microenvironment could be involved in the chemopreventive effects of aspirin.

Keywords: Immunoprevention; Molecular Pathological Epidemiology; NSAID; Pharmacoepidemiology.

CLINICAL AT

Colorectal cancer is the second leading cause of cancer death in the United States.[1] Evidence from epidemiologic studies and clinical trials suggests that aspirin can reduce the risk of colorectal cancer[2,3]; however, the mechanisms remain incompletely understood.[4–7] Despite the well-recognized importance of the complex interactions between neoplastic and immune cells in the tumor microenvironment,[8–11] whether the antitumor effect of aspirin might differ by immune status in the tumor microenvironment has been underexplored.

We previously showed that the benefit of aspirin might be stronger for colorectal cancers with overexpression of prostaglandin-endoperoxide synthase 2 (PTGS2 or cyclooxygenase-2) compared with colorectal cancers lacking PTGS2 overexpression.[12] In other words, aspirin appears to inhibit the development of tumors dependent at least in part on PTGS2 for their growth. Given evidence supporting a role of PTGS2 of tumor cells in suppressing T-cell–mediated antitumor immunity,[13–15] we further postulated that aspirin's role in enhancing antitumor immune responses also may underlie its anticancer benefit. Thus, we would expect that the inverse association between aspirin use and colorectal cancer risk might be stronger for tumors that arise owing to greater suppression of antitumor immunity as reflected by low-level lymphocytic infiltrates compared with tumors with more robust antitumor immunity as reflected by high-level lymphocytic infiltrates.

To examine this hypothesis, we took a unique approach of integrating longitudinal data on aspirin use with analyses of immune cells in incident cancer tissue, using the resources of 2 large prospective cohort studies. We investigated the association of regular aspirin use with the risk of colorectal cancer according to the pattern and intensity of histopathologic lymphocytic reactions. As an exploratory analysis, we also examined T-cell densities in tumor tissue using cases with available tissue microarray and image analysis data. In addition, our existing tumor characteristics data enabled us to control for key tumor tissue biomarkers, including PTGS2 expression, *BRAF* mutation, and microsatellite instability (MSI) status (the latter of which has been associated with immune response in colorectal cancer[16,17]).

Methods

Study Population

We used 2 ongoing prospective cohort studies: the Nurses' Health Study (NHS), a cohort study of 121,700 US female nurses aged 30–55 at enrollment in 1976, and the Health Professionals Follow-up Study (HPFS), a cohort study of 51,529 US male health professionals aged 40–75 at enrollment in 1986 (Figure 1). Participants have been mailed questionnaires at enrollment, and every 2 years thereafter, to collect data on demographics, lifestyle factors, medical history, and disease outcomes, and every 4 years to update dietary intake. The follow-up rates in both cohorts have been greater than 90%. The institutional review boards of the Harvard T.H. Chan School of Public Health and Partners Healthcare approved the study protocol.

Assessment of Aspirin Use

A detailed description of the collection of information on aspirin use has been published previously.[12] Briefly, in the NHS, aspirin use first was assessed in 1980 and every 2 years thereafter, except in 1986. NHS participants were asked whether they took aspirin in most weeks, the number of tablets taken per week, and years of aspirin use. We updated the information on the number of aspirin tablets taken per week (in categories) every 2 years. Consistent with our prior analyses,[12,18] regular aspirin users were defined as women who reported consumption of 2 or more aspirin tablets per week and nonregular users were defined as women who used fewer than 2 tablets per week, or no aspirin. In the HPFS, in 1986 and every 2 years thereafter, participants were asked whether they used aspirin 2 or more times per week. Beginning in 1994, the mean number of tablets taken per week was assessed. For both cohorts, participants were asked specifically about standard-dose (325 mg) aspirin tablets. Beginning in 1994, to reflect secular trends in aspirin use, participants also were asked to convert intake of 4 low-dose (81 mg) aspirin (baby aspirin) tablets to 1 standard aspirin tablet in their responses. Since 2000, we asked about low-dose aspirin use separately in both cohorts. The major reasons for aspirin use were arthritis and other musculoskeletal pain, headache, and cardiovascular disease prevention. In addition, we also collected updated information on regular use ($\geq$2 times/wk) of other nonsteroidal anti-inflammatory drugs (NSAIDs) (including Motrin, Advil, Nuprin, Indocin, Dolobid, Aleve, Naprosyn, Anaprox, Relafen, and Ketoprofen).

Ascertainment of Colorectal Cancer Cases

We requested written permission to acquire medical records and pathology reports from participants who reported colorectal cancer on biennial questionnaires. We identified unreported lethal colorectal cancer cases through the National Death Index and next-of-kin. For all deaths attributable to colorectal cancer, we requested permission from next-of-kin to review medical records. A study physician, blinded to exposure information, reviewed records to extract information on anatomic location and stage. Cases related to inflammatory bowel diseases and those related to polyposis syndromes were excluded from the current analyses.

Tumor Immunity and Molecular Analyses

We collected available tumor specimens (n = 1458) from pathology laboratories across the United States (Figure 1). In each case, a study pathologist (S.O.) confirmed the diagnosis of colorectal carcinoma (excluding anal squamous cell carcinoma),

*Authors share co-first authorship; §Authors share co-senior authorship.

Abbreviations used in this paper: **AHEI, Alternate Healthy Eating Index; CI, confidence interval; HPFS, Health Professionals Follow-up Study; MHT, menopausal hormone therapy; MSI, microsatellite instability; NHS, Nurses' Health Study; NSAID, nonsteroidal anti-inflammatory drug; PTGS2, prostaglandin-endoperoxide synthase 2; RR, relative risk; TIL, tumor-infiltrating lymphocytes.**

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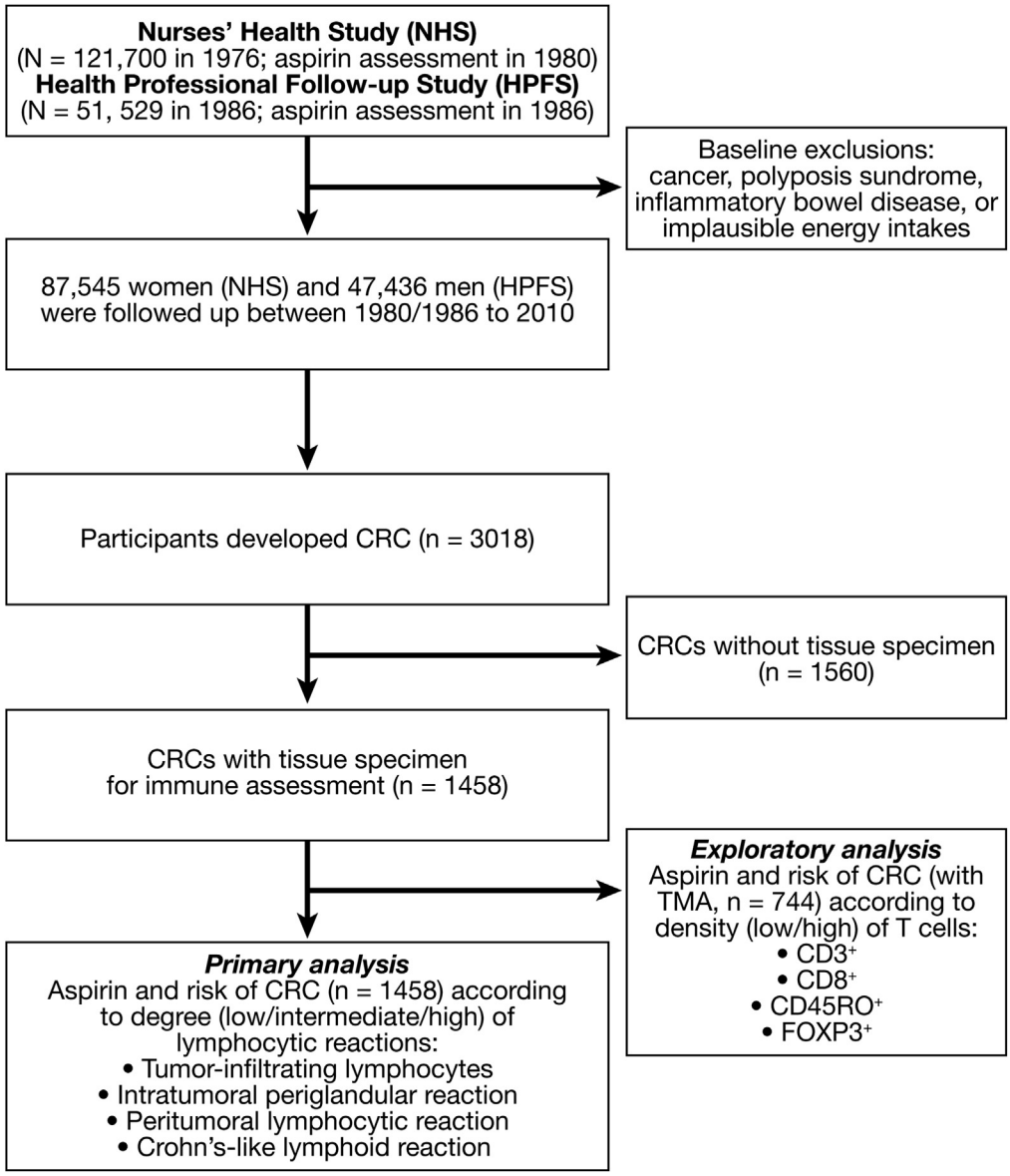

Figure 1. Flow diagram of the study population. CRC, colorectal cancer; TMA, tissue microarray.

and evaluated histopathologic features including patterns and degrees of lymphocytic infiltrates within and around tumor areas. Cases with preoperative treatment were excluded. There were no substantial differences in demographic or clinical features between cases with (n = 1458) and without (n = 1560) histopathologic immunity data (Supplementary Table 1). The 4 components of lymphocytic reaction, including tumor-infiltrating lymphocytes (TILs), intratumoral periglandular reaction, peritumoral lymphocytic reaction, and Crohn's-like lymphoid reaction were recorded as previously described.[17] Each component was evaluated as low, intermediate, or high, and an agreement study between independent reviews of more than 400 cases by 2 pathologists (S.O. and J. Glickman) showed a good concordance.[17] We constructed tissue microarrays among a subset of cases (n = 744), and performed immunohistochemistry for $CD3^+$ cells, $CD8^+$ cells, $CD45RO^+$ (one of PTPRC protein isoforms) cells, and $FOXP3^+$ cells (Figure 1). We performed image analysis using an automated scanning microscope and the Ariol image analysis system (Genetix, San Jose, CA) to calculate the average density (cells/mm^2) of each T-cell subset in tumor tissue, as previously described.[19] We dichotomized cases according to the median cut-off point for each marker. We also analyzed MSI, *BRAF* mutation, and PTGS2 expression status, as previously described.[12]

Statistical Analysis

At baseline, we excluded participants who had cancer, polyposis syndrome, or inflammatory bowel disease, or reported implausible energy intakes (<600 or >3500 kcal/d for women, and <800 or >4200 kcal/d for men). Person-years of follow-up evaluation were accrued from the date of return of the 1980 questionnaire in the NHS and of the 1986 questionnaire in the HPFS until the date of either colorectal cancer diagnosis, death, or the end of the follow-up period (June 2010 for the NHS and January 2010 for the HPFS), whichever came first. We examined the association between regular aspirin use and risk of colorectal cancer cases with histopathologic

immunity data (n = 1458; 863 cases from nonregular users vs 595 cases from regular users) using a Cox proportional hazards regression model that censored cases without immunity data (n = 1560; 968 cases from nonregular users vs 592 cases from regular users) at their time of diagnosis. Duplication-method Cox proportional cause-specific hazards regression for competing risks data was used to assess the association of aspirin with tumor subgroups according to the degree (low, intermediate, or high) of each lymphocytic reaction pattern (TILs, intratumoral periglandular reaction, peritumoral reaction, or Crohn's-like reaction). When examining the association specific to 1 tumor subgroup, other subgroups were treated as competing events, and tumors of unknown subgroup (ie, tumors without immunity data) were censored. Hazard ratios as estimates for age-adjusted and multivariable-adjusted relative risks (RRs) with 95% confidence intervals (CIs) were computed. Our primary hypothesis test was a heterogeneity test on a difference in the RR for 1 subgroup (with a low reaction), the RR for another subgroup (with an intermediate reaction), and the RR for the third subgroup (with a high reaction) as an ordinal statistical trend.[20] Specifically, we assessed whether the magnitude of the subgroup-specific associations had an increasing or decreasing ordinal trend according to levels of lymphocytic reaction, with the statistical significance of this trend test (1 *df*) presented as $P_{\text{heterogeneity}}$. All other assessments were secondary analyses. To account for multiple hypotheses testing for the 4 lymphocytic reaction components, we used Bonferroni correction to adjust the statistical significance level to $\alpha = .012$ ($\approx 0.05/4$). All analyses were performed using SAS V.9.3 (SAS Institute, Inc, Cary, NC). All statistical tests were 2-sided.

The Cox models also were conditioned on age in months and calendar year of the questionnaire cycle (and sex/cohort in the combined cohort analysis). Departures from the proportional hazards assumption were tested by likelihood ratio tests comparing models with and without the interaction terms of age or follow-up cycle by aspirin exposures and no significant violation of the proportionality assumption was found ($P > .05$ for all tests). We used time-varying aspirin exposure and covariates (when applicable) such that each individual participant contributed person-time according to the aspirin and covariate data they provided on each biennial questionnaire. We adjusted for the following covariates in the multivariable models: family history of colorectal cancer (yes/no), history of diabetes (yes/no), body mass index (quartile), history of colonoscopy/sigmoidoscopy (yes/no; ever had a colonoscopy/sigmoidoscopy before study baseline and updated every 2 years during follow-up evaluation), smoking in pack-years (never, 0.1–4.9, 5–19.9, 20–39.9, and ≥40), physical activity (quartile), alcohol intake (0, 0.1–4.9, 5–14.9, 15–29.9, and ≥30 g/d), current multivitamin use (yes/no), regular use of other NSAIDs (yes/no), total energy intake (quartile), folate (quartile), calcium (quartile), red and processed meat intake (quartile), and the Alternate Healthy Eating Index (AHEI)-2010 without alcohol (quartile). For women, we additionally adjusted for menopausal status/menopausal hormone therapy (MHT) (premenopausal, postmenopausal and never use of MHT, postmenopausal and past use of MHT, and postmenopausal and current use of MHT). To capture potential confounding by diet, we adjusted for the AHEI-2010,[21] which features a higher consumption of vegetables (excluding potatoes), whole fruit, whole grains, nuts and legumes, long-chain omega-3 fatty acids, polyunsaturated fatty acids; and a lower consumption of sugar-sweetened beverages, red/processed meat, sodium, trans fat, and moderate alcohol consumption. Adherence to the AHEI-2010 has been associated with a reduced risk of cardiovascular disease, diabetes, and cancer in our cohorts.[22] Because alcohol was included as a separate term in our model, we used a modified AHEI-2010 without alcohol consumption.

We further examined the associations of dose (tablets/wk) and duration (years) of aspirin use with risk of colorectal cancer according to levels of TILs. Tests for linear trend were performed using the median of each category of aspirin dose or duration as a continuous variable. Histopathologic lymphoid reactions including TILs have been associated with MSI-high colorectal cancers,[16,17] and we previously showed that the inverse association between regular aspirin use and colorectal cancer risk differed by *BRAF* mutation status[18] and PTGS2 expression level.[12] Thus, we conducted secondary analyses to examine the association between regular aspirin use and colorectal cancer risk according to the levels (low vs intermediate/high) of TILs stratified by MSI, *BRAF*, or PTGS2 status. We also examined the association between aspirin use and risk of colorectal cancer according to levels of TILs and stage (I/II vs III/IV). As an exploratory analysis, we examined the association between regular aspirin use, levels of TILs, and colorectal cancer–specific mortality (up to January 2012). We also examined the association of regular use of any NSAIDs including aspirin with risk of colorectal cancer according to the components of a lymphocytic reaction.

In a subset of cases (n = 744) with tissue microarray data, we examined whether the association between regular aspirin use and colorectal cancer might differ by densities of $CD3^+$, $CD8^+$, $CD45RO^+$, or $FOXP3^+$ cells.

Results

During 30 years of follow-up evaluation with 3,397,324 person-years, we documented 1458 colorectal cancers with available tissue for characterization of patterns and degrees of lymphocytic infiltrates in tumor tissue. Participants reporting regular aspirin use were more likely to have a history of diabetes, regularly use other NSAIDs or multivitamins, and consume alcohol (Table 1). Men who used aspirin regularly also were more likely to have a lower gastrointestinal endoscopy. Postmenopausal women who used aspirin regularly were more likely to use MHT. Consistent with our prior analyses over earlier follow-up evaluation,[12] regular aspirin use was associated with a significantly lower risk of colorectal cancer compared with nonregular use (RR, 0.78; 95% CI, 0.70–0.87), with similar associations in women and men (Table 2).

In testing our primary hypothesis, the inverse association of regular aspirin use with risk of colorectal cancer significantly differed by the density of TILs after correction for multiple testing ($P_{\text{heterogeneity}} = .007$, with an adjusted α level of .012) (Table 2). Compared with nonregular use, regular aspirin use was associated with a lower risk of the tumor subgroup with low-level TILs (RR, 0.72; 95% CI, 0.63–0.81), but not with risk of tumor subgroups with

Table 1. Age-Standardized Characteristics According to Person-Years of Regular Aspirin Use

Characteristic	NHS		HPFS		Combined	
	Nonregular users	Regular users	Nonregular users	Regular users	Nonregular users	Regular users
Age, *y*[a]	59.1 (51.3–66.7)	61.3 (53.3–69.2)	61.3 (53.2–69.6)	65.4 (58.3–73.1)	59.6 (51.8–67.4)	62.8 (54.8–70.6)
Family history of cancer, %	13	13	12	12	13	13
History of diabetes, %	6.2	8.1	6.3	8.2	6.3	8.1
BMI, kg/m^2	24.0 (21.9–27.1)	24.6 (22.2–28.0)	25.2 (23.5–27.3)	25.5 (23.8–27.6)	24.4 (22.3–27.2)	25.0 (22.7–27.8)
Postmenopause, %	76	78	-	-	77	77
Menopausal hormone therapy, %	26	31	-	-	26	31
History of colonoscopy/sigmoidoscopy, %	36	38	47	57	39	44
Current use of multivitamin, %	48	55	40	54	46	54
Regular use of NSAIDs, %	25	34	13	17	20	27
Physical activity, MET h/wk	11.9 (5.4–22.1)	11.3 (5.1–21.3)	21.2 (9.4–40.3)	23.2 (11.3–41.7)	14.3 (6.3–27.6)	14.9 (6.6–28.8)
Pack-year among ever smokers	18 (7–35)	20 (7–37)	20 (10–35)	20 (10–35)	19 (8–35)	20 (8–36)
Total calorie, *kcal/d*	1625 (1349–1936)	1664 (1386–1977)	1890 (1557–2303)	1924 (1586–2323)	1690 (1395–2036)	1736 (1436–2086)
Alcohol intake, *g/d*	1.9 (0.2–7.6)	2.2 (0.3–8.1)	5.4 (0.9–14.3)	7.0 (1.5–16.3)	2.5 (0.3–9.6)	3.3 (0.5–11.2)
Red and processed meat, *servings/wk*	6.0 (4.0–8.4)	6.2 (4.3–8.6)	5.7 (3.2–8.8)	5.5 (3.2–8.5)	5.9 (3.9–8.5)	6.0 (4.0–8.6)
Calcium, *mg/d*	856 (648–1112)	888 (672–1147)	830 (656–1093)	863 (691–1115)	850 (651–1109)	878 (678–1136)
Folate, $\mu g/d$	366 (263–520)	393 (277–549)	446 (334–645)	510 (370–701)	389 (282–550)	427 (302–596)
AHEI, 2010[b]	46.0 (39.6–52.9)	45.4 (39.0–52.0)	47.5 (40.6–54.8)	48.1 (41.4–55.0)	46.5 (39.9–53.4)	46.2 (39.7–53.0)

BMI, body mass index; MET, metabolic equivalent task.
[a]All values other than age have been standardized directly to age distribution (in 5-year age groups) of all the participants. Medians (25th–75th percentile) are presented for continuous variables.
[b]Without alcohol intake.

Table 2. Regular Aspirin Use and Risk of Colorectal Cancer Overall and by Components of Lymphocytic Reaction

	NHS		HPFS		Combined		
	Nonregular users	Regular users	Nonregular users	Regular users	Nonregular users	Regular users	$P_{heterogeneity}$[b]
Total colorectal cancer							
Person-years	1,455,499	966,281	519,815	455,729	1,975,314	1,422,010	
Cases, *n*	526	304	337	291	863	595	
Age-adjusted RR (95% CI)	1 (reference)	0.75 (0.65–0.87)	1 (reference)	0.82 (0.70–0.97)	1 (reference)	0.78 (0.70–0.87)	
Multivariable RR (95% CI)[a]	1 (reference)	0.75 (0.65–0.87)	1 (reference)	0.83 (0.71–0.98)	1 (reference)	0.78 (0.70–0.87)	
TILs							
Low							
Cases, n	387	204	278	218	665	422	
Age-adjusted RR (95% CI)	1 (reference)	0.69 (0.58–0.82)	1 (reference)	0.75 (0.62–0.89)	1 (reference)	0.72 (0.63–0.81)	.007
Multivariable RR (95% CI)[a]	1 (reference)	0.69 (0.58–0.82)	1 (reference)	0.75 (0.63–0.91)	1 (reference)	0.72 (0.63–0.81)	.007
Intermediate							
Cases, n	83	59	39	40	122	99	
Age-adjusted RR (95% CI)	1 (reference)	0.91 (0.65–1.27)	1 (reference)	1.01 (0.64–1.59)	1 (reference)	0.94 (0.72–1.23)	
Multivariable RR (95% CI)[a]	1 (reference)	0.91 (0.65–1.27)	1 (reference)	1.02 (0.65–1.61)	1 (reference)	0.95 (0.72–1.24)	
High							
Cases, n	55	40	19	32	74	72	
Age-adjusted RR (95% CI)	1 (reference)	0.90 (0.60–1.35)	1 (reference)	1.56 (0.88–2.78)	1 (reference)	1.08 (0.78–1.51)	
Multivariable RR (95% CI)[a]	1 (reference)	0.91 (0.60–1.37)	1 (reference)	1.57 (0.88–2.79)	1 (reference)	1.09 (0.78–1.51)	
Intratumoral periglandular reaction							
Low							
Cases, n	68	45	41	37	109	82	
Age-adjusted RR (95% CI)	1 (reference)	0.85 (0.58–1.24)	1 (reference)	0.71 (0.46–1.12)	1 (reference)	0.79 (0.59–1.05)	.37
Multivariable RR (95% CI)[a]	1 (reference)	0.84 (0.57–1.23)	1 (reference)	0.72 (0.46–1.13)	1 (reference)	0.78 (0.59–1.05)	.36
Intermediate							
Cases, n	385	217	266	209	651	426	
Age-adjusted RR (95% CI)	1 (reference)	0.74 (0.63–0.88)	1 (reference)	0.78 (0.65–0.94)	1 (reference)	0.76 (0.67–0.86)	
Multivariable RR (95% CI)[a]	1 (reference)	0.74 (0.62–0.87)	1 (reference)	0.79 (0.66–0.96)	1 (reference)	0.76 (0.67–0.86)	
High							
Cases, n	69	42	30	45	99	87	
Age-adjusted RR (95% CI)	1 (reference)	0.76 (0.52–1.12)	1 (reference)	1.33 (0.83–2.12)	1 (reference)	0.95 (0.71–1.28)	
Multivariable RR (95% CI)[a]	1 (reference)	0.77 (0.52–1.13)	1 (reference)	1.31 (0.82–2.10)	1 (reference)	0.95 (0.71–1.27)	

Table 2. Continued

	NHS		HPFS		Combined		
	Nonregular users	Regular users	Nonregular users	Regular users	Nonregular users	Regular users	$P_{heterogeneity}$[b]
Peritumoral lymphocytic reaction							
Low							
Cases, n	72	43	44	46	116	89	
Age-adjusted RR (95% CI)	1 (reference)	0.76 (0.52–1.11)	1 (reference)	0.84 (0.55–1.28)	1 (reference)	0.80 (0.60–1.05)	.15
Multivariable RR (95% CI)[a]	1 (reference)	0.76 (0.52–1.11)	1 (reference)	0.84 (0.55–1.28)	1 (reference)	0.79 (0.60–1.05)	.15
Intermediate							
Cases, n	370	206	256	180	626	386	
Age-adjusted RR (95% CI)	1 (reference)	0.73 (0.62–0.87)	1 (reference)	0.71 (0.59–0.87)	1 (reference)	0.73 (0.64–0.83)	
Multivariable RR (95% CI)[a]	1 (reference)	0.73 (0.61–0.87)	1 (reference)	0.73 (0.60–0.88)	1 (reference)	0.73 (0.64–0.83)	
High							
Cases, n	78	55	36	62	114	117	
Age-adjusted RR (95% CI)	1 (reference)	0.87 (0.62–1.23)	1 (reference)	1.44 (0.95–2.19)	1 (reference)	1.07 (0.83–1.40)	
Multivariable RR (95% CI)[a]	1 (reference)	0.88 (0.62–1.24)	1 (reference)	1.43 (0.94–2.17)	1 (reference)	1.07 (0.82–1.39)	
Crohn's-like lymphoid reaction							
Low							
Cases, n	339	186	203	171	542	357	
Age-adjusted RR (95% CI)	1 (reference)	0.71 (0.60–0.85)	1 (reference)	0.78 (0.63–0.96)	1 (reference)	0.74 (0.65–0.85)	.36
Multivariable RR (95% CI)[a]	1 (reference)	0.71 (0.59–0.85)	1 (reference)	0.78 (0.63–0.97)	1 (reference)	0.74 (0.64–0.85)	.42
Intermediate							
Cases, n	72	39	49	45	121	84	
Age-adjusted RR (95% CI)	1 (reference)	0.69 (0.46–1.02)	1 (reference)	0.97 (0.64–1.47)	1 (reference)	0.81 (0.61–1.07)	
Multivariable RR (95% CI)[a]	1 (reference)	0.68 (0.46–1.01)	1 (reference)	0.95 (0.62–1.43)	1 (reference)	0.80 (0.60–1.06)	
High							
Cases, n	32	21	15	18	47	39	
Age-adjusted RR (95% CI)	1 (reference)	0.80 (0.46–1.39)	1 (reference)	1.07 (0.53–2.14)	1 (reference)	0.89 (0.58–1.37)	
Multivariable RR (95% CI)[a]	1 (reference)	0.80 (0.46–1.39)	1 (reference)	1.02 (0.51–2.07)	1 (reference)	0.87 (0.57–1.34)	

[a]Adjusted for family history of colorectal cancer (yes/no), history of diabetes (yes/no), body mass index (quartile), history of colonoscopy/sigmoidoscopy (yes/no), smoking in pack-years (never, 0.1–4.9, 5–19.9, 20–39.9, and $\geq$40), physical activity (quartile), alcohol intake (0, 0.1–4.9, 5–14.9, 15–29.9, and $\geq$30 g/d), current multivitamin use (yes/no), regular use of NSAIDs (yes/no), total energy intake (quartile), folate (quartile), calcium (quartile), red and processed meat intake (quartile), and Alternate Healthy Eating Index-2010 without alcohol (quartile). For women, we additionally adjusted for menopause status/MHT (premenopausal, postmenopausal and never use of MHT, postmenopausal and past use of MHT, and postmenopausal and current use of MHT). The Cox models also were conditioned on age in months, calendar year of the questionnaire cycle, and sex/cohort (in the combined cohort analysis).

[b]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to the subtyping marker using a trend test with 1 *df*, and the statistical significance of this test was presented as $P_{heterogeneity}$.

intermediate-level TILs (RR, 0.95; 95% CI, 0.72–1.24) or high-level TILs (RR, 1.09; 95% CI, 0.78–1.51). The differential association was observed similarly in women and men. Although similar differential associations of aspirin use with colorectal cancer risk according to levels of intratumoral periglandular reaction (and peritumoral reaction) were observed, the differences were not statistically significant ($P_{heterogeneity} \geq .15$) (Table 2).

We further explored the heterogeneous association according to the degree of TILs across tablets of aspirin consumed each week and duration of aspirin use. We observed a lower risk of TIL-low colorectal cancer with increasing aspirin dosage per week ($P_{trend} < .001$). In contrast, aspirin dosage per week was not associated significantly with tumors with intermediate or high-level TILs ($P_{trend} > .28$) (Table 3). Similarly, the inverse association of aspirin with TIL-low colorectal cancer risk became stronger with longer duration of use ($P_{trend} < .001$), but duration of aspirin use was not associated significantly with colorectal cancer with intermediate or high-level TILs ($P_{trend} > .5$) (Table 4).

The differential association between regular aspirin use and risk of colorectal cancer according to levels of TILs appeared to be consistent in strata of tumor MSI status, tumor *BRAF* mutation status, PTGS2 expression status (Table 5), and stage (I/II vs III/IV) (Supplementary Table 2), although statistical power was limited in these subgroup analyses.

Regular aspirin use was not associated differentially with colorectal cancer–specific mortality according to the degree of TILs (low vs intermediate/high) ($P_{interaction} = .17$) (Supplementary Table 3). Nonetheless, it is difficult to determine the lack of statistical interaction owing to limited statistical power. Additional studies are warranted to examine the interactive effects of aspirin and TILs that may modify clinical outcome of colorectal cancer patients.

Statistical power was limited in our cohorts to examine the association between nonaspirin NSAIDs and colorectal cancer according to tumor immunity status. We thus analyzed the association between any NSAIDs including aspirin and risk of colorectal cancer according to components of the lymphocytic reaction (Supplementary Table 4). The findings generally were consistent with the findings in our primary analysis of regular aspirin use as an exposure variable.

In a subset of cases with tissue microarray data, inverse associations of regular aspirin use with cancer risk were observed for tumors with low densities of $CD3^+$ (RR, 0.73; 95% CI, 0.58–0.91), $CD8^+$ (RR, 0.73; 95% CI, 0.58–0.91), and $CD45RO^+$ cells (RR, 0.74; 95% CI, 0.60–0.92), but not for tumors with high densities of $CD3^+$, $CD8^+$, or $CD45RO^+$ cells (Table 6). The association of aspirin with colorectal cancer risk appeared to be similar according to tumor $FOXP3^+$ cell density.

Discussion

In 2 large prospective cohort studies, we observed an inverse association between regular aspirin use and risk of colorectal cancers with low-level TILs, but not with risk of colorectal cancers with high-level TILs. The apparent benefit

Table 3. Dose of Regular Aspirin Use and Risk of Colorectal Cancer Overall and by Tumor-Infiltrating Lymphocytes

	Tablets/wk					
	0	0.5–1.5	2–5	≥6	P_{trend}[a]	$P_{heterogeneity}$[b]
Total colorectal cancer						
Person-years	625,399	1,148,842	680,674	599,431		
Cases, n	226	544	293	236		
Age-adjusted RR (95% CI)	1 (reference)	1.09 (0.92–1.28)	0.87 (0.73–1.03)	0.78 (0.65–0.94)	<.001	
Multivariable RR (95% CI)[c]	1 (reference)	1.07 (0.91–1.26)	0.86 (0.72–1.03)	0.76 (0.63–0.92)	<.001	
Tumor-infiltrating lymphocytes						
Low						
Cases, n	178	399	215	164		
Age-adjusted RR (95% CI)	1 (reference)	1.05 (0.87–1.26)	0.83 (0.68–1.01)	0.70 (0.56–0.87)	<.001	.04
Multivariable RR (95% CI)[c]	1 (reference)	1.03 (0.86–1.25)	0.82 (0.67–1.01)	0.69 (0.55–0.85)	<.001	.04
Intermediate						
Cases, n	30	89	47	40		
Age-adjusted RR (95% CI)	1 (reference)	1.20 (0.78–1.84)	0.98 (0.61–1.57)	0.94 (0.58–1.52)	.31	
Multivariable RR (95% CI)[c]	1 (reference)	1.18 (0.77–1.82)	0.97 (0.61–1.55)	0.92 (0.57–1.49)	.29	
High						
Cases, n	18	56	31	32		
Age-adjusted RR (95% CI)	1 (reference)	1.30 (0.75–2.25)	1.05 (0.58–1.89)	1.26 (0.70–2.26)	.83	
Multivariable RR (95% CI)[c]	1 (reference)	1.28 (0.74–2.21)	1.05 (0.58–1.90)	1.24 (0.69–2.23)	.85	

[a]Tests for trend were conducted using the median value of each category as a continuous variable.
[b]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to levels of TILs, using a trend test with 1 *df*, and the statistical significance of this test was presented as $P_{heterogeneity}$.
[c]Adjusted for the same set of covariates as in Table 2.

Table 4. Duration of Regular Aspirin Use and Risk of Colorectal Cancer Overall and by Tumor-Infiltrating Lymphocytes

	Years of regular aspirin use						
	0	1–5	6–10	11–15	≥16	P_{trend}[a]	$P_{heterogeneity}$[b]
Total colorectal cancer							
Person-years	1,246,298	628,463	585,563	278,593	644,154		
Cases, n	486	296	306	128	236		
Age-adjusted RR (95% CI)	1 (reference)	1.00 (0.87–1.16)	0.92 (0.79–1.06)	0.74 (0.61–0.91)	0.74 (0.63–0.87)	<.001	
Multivariable RR (95% CI)[c]	1 (reference)	1.01 (0.88–1.18)	0.93 (0.80–1.08)	0.75 (0.61–0.92)	0.74 (0.62–0.87)	<.001	
Tumor-infiltrating lymphocytes							
Low							
Cases, n	385	223	233	80	164		
Age-adjusted RR (95% CI)	1 (reference)	0.95 (0.81–1.13)	0.88 (0.75–1.04)	0.60 (0.47–0.77)	0.68 (0.56–0.82)	<.001	.03
Multivariable RR (95% CI)[c]	1 (reference)	0.96 (0.81–1.14)	0.90 (0.76–1.06)	0.61 (0.47–0.78)	0.68 (0.56–0.82)	<.001	.04
Intermediate							
Cases, n	60	44	43	30	44		
Age-adjusted RR (95% CI)	1 (reference)	1.20 (0.81–1.78)	1.04 (0.70–1.56)	1.32 (0.84–2.08)	0.90 (0.60–1.35)	.52	
Multivariable RR (95% CI)[c]	1 (reference)	1.22 (0.82–1.82)	1.06 (0.71–1.58)	1.34 (0.85–2.10)	0.90 (0.60–1.36)	.52	
High							
Cases, n	41	29	30	18	28		
Age-adjusted RR (95% CI)	1 (reference)	1.18 (0.73–1.90)	1.09 (0.67–1.76)	1.11 (0.63–1.97)	1.02 (0.62–1.69)	.96	
Multivariable RR (95% CI)[c]	1 (reference)	1.18 (0.73–1.92)	1.10 (0.68–1.78)	1.13 (0.64–2.00)	1.01 (0.61–1.68)	.95	

[a]Tests for trend were conducted using the median value of each category as a continuous variable.
[b]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to levels of TILs, using a trend test with 1 *df*, and the statistical significance of this test was presented as $P_{heterogeneity}$.
[c]Adjusted for the same set of covariates as in Table 2.

of aspirin use for tumors with low-level TILs increased with dose and duration of aspirin use. Our findings provide population-based evidence for the role of host immunity in mediating the effect of aspirin in colorectal cancer chemoprevention. Aspirin, through either prostaglandin-dependent or prostaglandin-independent pathways, may enhance antitumor immunity, thereby exerting a stronger effect on tumors that depend more strongly on suppression of tumor immune response for their growth. Overall, these results improve our understanding of the mechanisms through which aspirin may exert its antineoplastic effects and also provide broad support for the potential of exploiting immune mechanisms for disease prevention (ie, immunoprevention).[23,24] Nonetheless, further functional studies to elucidate the immune mechanisms of the antitumor effect of aspirin more fully are warranted.

The observed differential association of aspirin and colorectal cancer according to tumor immunity status is biologically plausible. Evidence suggests that aspirin may exert multiple effects on different components of innate and adaptive immunity through modulation of immune and inflammatory cytokines.[23,25–29] For example, aspirin may induce tolerogenic activity in dendritic cells and inhibit their subsequent immunostimulatory function.[30] In addition, aspirin can induce apoptosis in neutrophils and monocytes,[31] and trigger a lipoxin-driven immune counter-regulation.[32] For T lymphocytes, aspirin can disrupt the integrin- and SELL (selectin L)–mediated binding of T cells to the endothelium,[33,34] directly suppress T-cell activation or proliferation, and/or inhibit cytokine production related to the T-cell–mediated adaptive immune response.[25] Our data support the possibility that aspirin may cooperate with the host immune system, in particular, lymphocytes, to interrupt the development or growth of colorectal neoplasia.

Integrated analysis of tumor characteristics is increasingly important in cancer research.[35–37] Tumor MSI status should be analyzed in the current study of aspirin use and risk of colorectal cancer according to lymphocytic infiltrates because MSI-high tumor cells have many frameshift mutations in coding sequences throughout the genome, resulting in abundant neoantigens that elicit intense and more diverse immune responses.[16,38–41] Recently, some MSI-high colorectal cancers have been shown to respond to immunotherapy blocking the PDCD1 (programmed cell death-1) immune checkpoint, supporting the importance of the interplay between MSI-high tumor cells and immune cells.[42] However, MSI status is not the sole determinant of tumoral immune response because the levels of tumor-infiltrating T cells overlap considerably between MSI-high and microsatellite stable colorectal cancers.[17,19,43,44] In the current study, we found that the differential association between aspirin and cancer risk according to levels of TILs appeared to be largely independent of MSI status, further supporting a distinct role of host immunity in mediating the association between aspirin and colorectal cancer.

Cancer immunity status reflects molecular interactions between tumor and immune cells, occurring in the tumor microenvironment.[45,46] Compared with the other components of lymphocytic reaction, lymphocytes in the TIL component are present close to surfaces of tumor cells and

Table 5. Regular Aspirin Use and Risk of Colorectal Cancer by Microsatellite Instability, *BRAF* Mutation, PTGS2 Expression, and Tumor-Infiltrating Lymphocytes

			Aspirin use	
			Nonregular users	Regular users
MSI	MSS	Cases, n	662	448
		Multivariable RR (95% CI)[a]	1 (reference)	0.78 (0.69–0.88)
	MSI-high	Cases, n	122	93
		Multivariable RR (95% CI)[a]	1 (reference)	0.84 (0.64–1.11)
	MSS	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n	528	345
		Multivariable RR (95% CI)[a]	1 (reference)	0.75 (0.66–0.87)
		Intermediate/high		
		Cases, n	110	84
		Multivariable RR (95% CI)[a]	1 (reference)	0.93 (0.70–1.25)
	MSI-high	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n	47	17
		Multivariable RR (95% CI)[a]	1 (reference)	0.41 (0.23–0.71)
		Intermediate/high		
		Cases, n	74	73
		Multivariable RR (95% CI)[a]	1 (reference)	1.03 (0.74–1.44)
BRAF mutation	Wild-type	Cases, n	682	455
		Multivariable RR (95% CI)[a]	1 (reference)	0.76 (0.68–0.86)
	Mutant	Cases, n	112	93
		Multivariable RR (95% CI)[a]	1 (reference)	0.94 (0.71–1.24)
	Wild-type	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n	519	338
		Multivariable RR (95% CI)[a]	1 (reference)	0.75 (0.65–0.86)
		Intermediate/high		
		Cases, n	135	98
		Multivariable RR (95% CI)[a]	1 (reference)	0.87 (0.66–1.13)
	Mutant	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n	64	30
		Multivariable RR (95% CI)[a]	1 (reference)	0.54 (0.35–0.84)
		Intermediate/high		
		Cases, n	48	58
		Multivariable RR (95% CI)[a]	1 (reference)	1.27 (0.86–1.88)
PTGS2 expression	Negative	Cases, n	267	213
		Multivariable RR (95% CI)[a]	1 (reference)	0.90 (0.75–1.08)
	Positive	Cases, n	488	292
		Multivariable RR (95% CI)[a]	1 (reference)	0.70 (0.61–0.82)
	Negative	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n	187	133
		Multivariable RR (95% CI)[a]	1 (reference)	0.79 (0.63–0.99)
		Intermediate/high		
		Cases, n	71	67
		Multivariable RR (95% CI)[a]	1 (reference)	1.07 (0.76–1.50)
	Positive	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n	378	203
		Multivariable RR (95% CI)[a]	1 (reference)	0.64 (0.53–0.76)
		Intermediate/high		
		Cases, n	89	76
		Multivariable RR (95% CI)[a]	1 (reference)	1.01 (0.74–1.37)

MSS, microsatellite stable.
[a]Adjusted for the same set of covariates as in Table 2.

Table 6. Regular Aspirin Use and Risk of Colorectal Cancer by Tumor-Infiltrating T-Cell Subset Density

	NHS		HPFS		Combined		
	Nonregular users	Regular users	Nonregular users	Regular users	Nonregular users	Regular users	$P_{heterogeneity}$[a]
Total colorectal cancer							
Person-years	1,455,729	966,385	519,658	455,572	1,975,387	1,421,957	
Cases, n	285	183	154	122	439	305	
Age-adjusted RR (95% CI)	1 (reference)	0.86 (0.71–1.03)	1 (reference)	0.83 (0.65–1.06)	1 (reference)	0.85 (0.73–0.98)	
Multivariable RR (95% CI)[b]	1 (reference)	0.85 (0.70–1.02)	1 (reference)	0.85 (0.67–1.09)	1 (reference)	0.85 (0.73–0.98)	
$CD3^+$ cells							
Low							
Age-adjusted RR (95% CI)	1 (reference)	0.80 (0.61–1.05)	1 (reference)	0.63 (0.43–0.91)	1 (reference)	0.73 (0.59–0.91)	0.04
Multivariable RR (95% CI)[b]	1 (reference)	0.79 (0.60–1.04)	1 (reference)	0.65 (0.44–0.94)	1 (reference)	0.73 (0.58–0.91)	0.03
High							
Age-adjusted RR (95% CI)	1 (reference)	0.95 (0.72–1.24)	1 (reference)	1.12 (0.80–1.57)	1 (reference)	1.01 (0.82–1.25)	
Multivariable RR (95% CI)[b]	1 (reference)	0.94 (0.72–1.24)	1 (reference)	1.14 (0.81–1.60)	1 (reference)	1.01 (0.82–1.25)	
$CD8^+$ cells							
Low							
Age-adjusted RR (95% CI)	1 (reference)	0.71 (0.55–0.93)	1 (reference)	0.76 (0.51–1.12)	1 (reference)	0.73 (0.58–0.91)	0.04
Multivariable RR (95% CI)[b]	1 (reference)	0.70 (0.53–0.92)	1 (reference)	0.78 (0.53–1.16)	1 (reference)	0.73 (0.58–0.91)	0.04
High							
Age-adjusted RR (95% CI)	1 (reference)	1.06 (0.81–1.41)	1 (reference)	0.92 (0.65–1.28)	1 (reference)	1.00 (0.81–1.24)	
Multivariable RR (95% CI)[b]	1 (reference)	1.06 (0.80–1.40)	1 (reference)	0.95 (0.68–1.33)	1 (reference)	1.00 (0.81–1.24)	
$CD45RO^+$ cells							
Low							
Age-adjusted RR (95% CI)	1 (reference)	0.72 (0.54–0.97)	1 (reference)	0.75 (0.54–1.04)	1 (reference)	0.74 (0.59–0.92)	0.05
Multivariable RR (95% CI)[b]	1 (reference)	0.72 (0.53–0.97)	1 (reference)	0.78 (0.56–1.08)	1 (reference)	0.74 (0.60–0.92)	0.06
High							
Age-adjusted RR (95% CI)	1 (reference)	0.98 (0.76–1.26)	1 (reference)	1.03 (0.70–1.51)	1 (reference)	0.99 (0.80–1.23)	
Multivariable RR (95% CI)[b]	1 (reference)	0.97 (0.75–1.25)	1 (reference)	1.06 (0.72–1.56)	1 (reference)	0.99 (0.80–1.22)	
$FOXP3^+$							
Low							
Age-adjusted RR (95% CI)	1 (reference)	0.82 (0.62–1.08)	1 (reference)	0.87 (0.61–1.23)	1 (reference)	0.84 (0.67–1.04)	0.89
Multivariable RR (95% CI)[b]	1 (reference)	0.80 (0.60–1.07)	1 (reference)	0.91 (0.64–1.29)	1 (reference)	0.84 (0.67–1.04)	0.86
High							
Age-adjusted RR (95% CI)	1 (reference)	0.86 (0.66–1.12)	1 (reference)	0.75 (0.51–1.10)	1 (reference)	0.82 (0.66–1.02)	
Multivariable RR (95% CI)[b]	1 (reference)	0.83 (0.64–1.09)	1 (reference)	0.76 (0.52–1.12)	1 (reference)	0.81 (0.65–1.02)	

NOTE. Cut-off values for low and high tumor-infiltrating T-cell subset density (cells/mm^2) were as follows: 244.97 for $CD3^+$ cells, 236.65 for $CD8^+$ cells, 376.97 for $CD45RO^+$ cells, and 26.36 for $FOXP3^+$ cells.

[a]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to the density of T cells, using a trend test with 1 *df*, and the statistical significance of this test was presented as $P_{heterogeneity}$.

[b]Adjusted for the same set of covariates as in Table 2.

hence in more direct contact with the tumor cells containing somatic mutations. The degree of TIL, especially tumor-specific cytotoxic T cells, has been associated with a good prognosis in colorectal cancer.[47–50] As immunotherapy has emerged as an attractive strategy in the treatment of cancer, integrated analyses of tumor molecular characteristics, host factors (including dietary, lifestyle, and environmental exposures), and immune cells in the tumor microenvironment are increasingly important.[8] Our data strengthen the causal link between aspirin and the prevention of colorectal neoplasia by identifying a subgroup of colorectal cancer that may be sensitive to aspirin chemoprevention, and enhance our understanding of the mechanisms through which aspirin may exert its antineoplastic effects.

The strengths of our study include prospective and updated assessments of aspirin use during up to 30 years of follow-up evaluation. In addition, we collected detailed information on potential confounders and had high follow-up rates. Importantly, cancer immunity status, which rarely has been examined in epidemiologic studies, provides important information on interactions between tumor and host immune cells, which cannot be obtained from peripheral blood biomarkers.[51] In addition, our integrative molecular pathologic epidemiology approach enabled us to attribute the risk reduction to the tumor subgroup, refine effect estimates for the tumor subgroup, and provide evidence in support of causality.

Our study had limitations. First, the study was observational and subject to the influence of confounding. However, adjustment for a wide range of risk factors for colorectal cancer had minimal impact on our results. Second, because the majority of participants were non-Hispanic health professionals, generalizability to other ethnic or socioeconomic groups remains to be assessed. In addition, we were not able to retrieve tissue specimens from all incident cancers; however, the characteristics of those participants from whom we could collect tissue data were largely similar to those from whom we could not. Finally, replication of our findings is needed and studies that examine macrophages, myeloid-derived suppressor cells, natural killer cells, T-helper 2 cells, and other types of immune cells in tumor tissue may provide additional insights into the potential role of host immunity in mediating the chemopreventive effect of aspirin.

In conclusion, regular aspirin use, by dose and duration, is associated with a lower risk of colorectal cancer with low-level TILs, but not with risk of colorectal cancer with more intense patterns of TILs. This differential association appeared to be consistent across strata of tumor MSI, *BRAF* mutation, or PTGS2 expression status. Our findings highlight the potential importance of host immunity in mediating the activity of aspirin in colorectal cancer chemoprevention.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2016.07.030.

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The authors used HUGO (Human Genome Organisation)-approved official symbols for genes and gene products, including BRAF, CD3, CD8, FOXP3, PDCD1, PTGS2, PTPRC, and SELL, all of which are described at www.genenames.org. Gene names are italicized, and gene product names are nonitalicized.

Conflicts of interest
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Supplementary Table 1. Age-Standardized Characteristics Comparing Cases With and Without Immunity Data

Characteristic	NHS		HPFS	
	Cases with immunity data	Cases without immunity data	Cases with immunity data	Cases without immunity data
Cases, n	830	928	628	632
Age at diagnosis, *y*[a]	68.3 (61.4–73.5)	66.6 (60.0–73.6)	72.3 (65.4–77.6)	70.7 (64.0–77.6)
Family history of cancer, %	20	20	18	17
History of diabetes, %	10	10	10	13
BMI, kg/m^2	25.1 (22.7–28.6)	24.9 (22.5–28.2)	25.7 (24.0–27.6)	25.9 (24.2–28.2)
Postmenopause, %	91	92	-	-
Menopausal hormone therapy, %	23	18	-	-
History of colonoscopy/sigmoidoscopy, %	37	32	45	45
Current use of multivitamin, %	52	49	49	46
Regular use of NSAIDs, %	24	20	15	12
Physical activity, MET-h/wk	11.1 (5.4–20.1)	11.3 (4.6–20.9)	23.0 (10.1–41.2)	19.9 (8.8–37.1)
Pack-year among ever smokers	24 (10–43)	25 (11–45)	23 (13–40)	24 (13–43)
Total calorie, *kcal/d*	1673 (1394–1956)	1615 (1358–1936)	1878 (1584–2312)	1859 (1563–2287)
Alcohol intake, *g/d*	2.0 (0.4–7.8)	2.5 (0.3–8.7)	8.1 (1.8–21.0)	7.4 (1.3–17.4)
Red and processed meat, *servings/wk*	5.8 (3.9–7.9)	5.9 (4.1–8.3)	5.8 (3.6–9.1)	5.9 (3.2–8.9)
Calcium, *mg/d*	880 (681–1114)	850 (648–1110)	834 (679–1100)	831 (645–1102)
Folate, *μg/d*	386 (297–531)	376 (274–516)	467 (342–656)	478 (347–679)
AHEI,[b] 2010	46.4 (40.6–52.6)	46.7 (40.5–53.4)	47.6 (41.1–55.0)	47.8 (41.1–55.0)
Stage, %				
I	24	24	28	26
II	35	26	28	17
III	27	25	29	21
IV	14	25	14	37
Tumor differentiation, %				
Well or moderately differentiated	88	93	93	96
Poorly differentiated	12	7	7	4
Tumor location, %				
Rectum	21	23	23	28
Distal colon	28	31	31	39
Proximal colon	51	47	46	33

BMI, body mass index; MET, metabolic equivalent task.
[a]All values other than age have been standardized directly to age distribution (in 5-year age groups) of all the participants. Medians (25th–75th percentile) are presented for continuous variables.
[b]Without alcohol intake.

Supplementary Table 2. Regular Aspirin Use and Risk of Colorectal Cancer by Stage and Tumor-Infiltrating Lymphocytes

			Aspirin use	
			Nonregular users	Regular users
Stage	Stage I/II	Cases, n (n = 769)	459	310
		Multivariable RR (95% CI)[a]	1 (reference)	0.76 (0.66–0.89)
	Stage III/IV	Cases, n (n = 554)	333	221
		Multivariable RR (95% CI)[a]	1 (reference)	0.76 (0.64–0.91)
	Stage I/II	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n (n = 533)	333	200
		Multivariable RR (95% CI)[a]	1 (reference)	0.68 (0.57–0.82)
		Intermediate/high		
		Cases, n (n = 236)	126	110
		Multivariable RR (95% CI)[a]	1 (reference)	0.97 (0.75–1.26)
	Stage III/IV	Tumor-infiltrating lymphocytes		
		Low		
		Cases, n (n = 449)	277	172
		Multivariable RR (95% CI)[a]	1 (reference)	0.71 (0.59–0.87)
		Intermediate/high		
		Cases, n (n = 105)	56	49
		Multivariable RR (95% CI)[a]	1 (reference)	1.03 (0.70–1.52)

[a]Adjusted for the same set of covariates as in Table 2.

Supplementary Table 3. Regular Aspirin Use, Tumor-Infiltrating Lymphocytes, and Colorectal Cancer–Specific Mortality

	Nonregular users	Regular users
TILs		
Low		
Cases, n	665	422
Events, n	219	126
Univariate RR (95% CI)	1 (reference)	0.93 (0.74–1.15)
Multivariable RR (95% CI)[a]	1 (reference)	0.88 (0.70–1.10)
Intermediate/high		
Cases, n	220	147
Events, n	36	37
Univariate RR (95% CI)	0.51 (0.36–0.73)	0.66 (0.47–0.94)
Multivariable RR (95% CI)[a]	0.53 (0.36–0.76)	0.66 (0.46–0.96)
*P*interaction[b]		
Univariate model		.20
Multivariable model		.17

[a]The multivariable Cox regression model initially included sex, age at diagnosis, year of diagnosis, family history of colorectal cancer, prediagnosis body mass index, tumor differentiation, tumor location, microsatellite instability status, CpG island methylator phenotype-specific promoter status, long interspersed nucleotide element-1 hypomethylation level, PTGS2 expression, and *KRAS*, *BRAF*, *PIK3CA* mutations. A backward elimination with a threshold *P* of .05 was used to select variables for the final models.

[b]$P_{interaction}$ (2-sided) was calculated using the Wald test for the cross-product of regular aspirin use (regular vs nonregular) and TILs (intermediate/high vs low) in the Cox regression model.

Supplementary Table 4. Regular Use of NSAIDs and Risk of Colorectal Cancer Overall and by Components of Lymphocytic Reaction

	NHS		HPFS		Combined		
	Nonregular users[a]	Regular users[a]	Nonregular users[a]	Regular users[a]	Nonregular users[a]	Regular users[a]	$P_{heterogeneity}$
Total colorectal cancer							
Person-years	1,237,311	1,184,473	453,472	522,072	1,690,783	1,706,545	
Cases, n (n = 1458)	426	404	286	342	712	746	
Age-adjusted RR (95% CI)	1 (reference)	0.82 (0.71–0.94)	1 (reference)	0.89 (0.75–1.04)	1 (reference)	0.85 (0.76–0.94)	
Multivariable RR (95% CI)[b]	1 (reference)	0.82 (0.71–0.94)	1 (reference)	0.99 (0.79–1.25)	1 (reference)	0.85 (0.76–0.94)	
TILs							
Low							
Cases, n (n = 1087)	311	280	234	262	545	542	
Age-adjusted RR (95% CI)	1 (reference)	0.78 (0.67–0.92)	1 (reference)	0.82 (0.69–0.99)	1 (reference)	0.80 (0.71–0.90)	.03
Multivariable RR (95% CI)[b]	1 (reference)	0.78 (0.66–0.92)	1 (reference)	0.83 (0.69–1.00)	1 (reference)	0.80 (0.71–0.91)	.04
Intermediate							
Cases, n (n = 221)	70	72	36	43	106	115	
Age-adjusted RR (95% CI)	1 (reference)	0.86 (0.62–1.20)	1 (reference)	0.94 (0.60–1.50)	1 (reference)	0.89 (0.68–1.16)	
Multivariable RR (95% CI)[b]	1 (reference)	0.87 (0.62–1.21)	1 (reference)	0.95 (0.60–1.50)	1 (reference)	0.89 (0.68–1.17)	
High							
Cases, n (n = 146)	44	51	15	36	59	87	
Age-adjusted RR (95% CI)	1 (reference)	0.99 (0.66–1.49)	1 (reference)	1.73 (0.94–3.19)	1 (reference)	1.18 (0.85–1.66)	
Multivariable RR (95% CI)[b]	1 (reference)	1.00 (0.66–1.50)	1 (reference)	1.70 (0.92–3.14)	1 (reference)	1.18 (0.85–1.65)	
Intratumoral periglandular reaction							
Low							
Cases, n (n = 191)	61	52	34	44	95	96	.08
Age-adjusted RR (95% CI)	1 (reference)	0.73 (0.50–1.06)	1 (reference)	0.74 (0.47–1.16)	1 (reference)	0.73 (0.55–0.98)	.09
Multivariable RR (95% CI)[b]	1 (reference)	0.72 (0.50–1.05)	1 (reference)	0.74 (0.47–1.17)	1 (reference)	0.73 (0.55–0.97)	
Intermediate							
Cases, n (n = 1077)	307	295	227	248	534	543	
Age-adjusted RR (95% CI)	1 (reference)	0.84 (0.71–0.98)	1 (reference)	0.85 (0.71–1.03)	1 (reference)	0.84 (0.75–0.95)	
Multivariable RR (95% CI)[b]	1 (reference)	0.84 (0.71–0.98)	1 (reference)	0.86 (0.72–1.04)	1 (reference)	0.85 (0.75–0.96)	
High							
Cases, n (n = 186)	54	57	25	50	79	107	
Age-adjusted RR (95% CI)	1 (reference)	0.89 (0.61–1.29)	1 (reference)	1.40 (0.86–2.30)	1 (reference)	1.05 (0.78–1.41)	
Multivariable RR (95% CI)[b]	1 (reference)	0.89 (0.61–1.30)	1 (reference)	1.36 (0.83–2.23)	1 (reference)	1.05 (0.78–1.41)	
Peritumoral lymphocytic reaction							
Low							
Cases, n (n = 205)	60	55	39	51	99	106	
Age-adjusted RR (95% CI)	1 (reference)	0.79 (0.55–1.14)	1 (reference)	0.76 (0.50–1.17)	1 (reference)	0.78 (0.59–1.03)	.06
Multivariable RR (95% CI)[b]	1 (reference)	0.79 (0.54–1.14)	1 (reference)	0.77 (0.50–1.18)	1 (reference)	0.78 (0.59–1.03)	.07
Intermediate							
Cases, n (n = 1012)	297	279	216	220	513	499	
Age-adjusted RR (95% CI)	1 (reference)	0.82 (0.69–0.97)	1 (reference)	0.82 (0.67–0.99)	1 (reference)	0.82 (0.72–0.93)	
Multivariable RR (95% CI)[b]	1 (reference)	0.82 (0.69–0.97)	1 (reference)	0.83 (0.68–1.01)	1 (reference)	0.82 (0.72–0.93)	

Supplementary Table 4. Continued

	NHS		HPFS		Combined		
	Nonregular users[a]	Regular users[a]	Nonregular users[a]	Regular users[a]	Nonregular users[a]	Regular users[a]	$P_{heterogeneity}$
High							
Cases, n (n = 231)	63	70	30	68	93	138	
Age-adjusted RR (95% CI)	1 (reference)	0.93 (0.66–1.30)	1 (reference)	1.50 (0.96–2.33)	1 (reference)	1.12 (0.86–1.46)	
Multivariable RR (95% CI)[b]	1 (reference)	0.93 (0.66–1.31)	1 (reference)	1.46 (0.94–2.27)	1 (reference)	1.11 (0.85–1.45)	
Crohn's-like lymphoid reaction							
Low							
Cases, n (n = 899)	271	254	177	197	448	451	
Age-adjusted RR (95% CI)	1 (reference)	0.81 (0.68–0.96)	1 (reference)	0.80 (0.65–0.98)	1 (reference)	0.80 (0.70–0.92)	.24
Multivariable RR (95% CI)[b]	1 (reference)	0.81 (0.68–0.96)	1 (reference)	0.80 (0.65–0.99)	1 (reference)	0.80 (0.70–0.92)	.30
Intermediate							
Cases, n (n = 205)	58	53	39	55	97	108	
Age-adjusted RR (95% CI)	1 (reference)	0.79 (0.54–1.15)	1 (reference)	1.14 (0.74–1.74)	1 (reference)	0.93 (0.70–1.23)	
Multivariable RR (95% CI)[b]	1 (reference)	0.79 (0.54–1.15)	1 (reference)	1.10 (0.72–1.68)	1 (reference)	0.91 (0.69–1.21)	
High							
Cases, n (n = 86)	26	27	12	21	38	48	
Age-adjusted RR (95% CI)	1 (reference)	0.87 (0.51–1.50)	1 (reference)	1.21 (0.59–2.49)	1 (reference)	0.98 (0.64–1.51)	
Multivariable RR (95% CI)[b]	1 (reference)	0.87 (0.51–1.50)	1 (reference)	1.15 (0.56–2.39)	1 (reference)	0.96 (0.63–1.48)	

[a]The reference group included individuals who regularly used neither aspirin nor other NSAIDs, and the comparison group included individuals who used aspirin and/or other NSAIDs regularly.

[b]Adjusted for family history of colorectal cancer (yes/no), history of diabetes (yes/no), body mass index (quartile), history of colonoscopy/sigmoidoscopy (yes/no), smoking in pack-years (never, 0.1–4.9, 5–19.9, 20–39.9, and ≥40), physical activity (quartile), alcohol intake (0, 0.1–4.9, 5–14.9, 15–29.9, and ≥30 g/d), current multivitamin use (yes/no), total energy intake (quartile), folate (quartile), calcium (quartile), red and processed meat intake (quartile), and Alternate Healthy Eating Index-2010 without alcohol (quartile). For women, we additionally adjusted for menopause status/MHT (premenopausal, postmenopausal and never use of MHT, postmenopausal and past use of MHT, and postmenopausal and current use of MHT). The Cox models also were conditioned on age in months, calendar year of the questionnaire cycle, and sex/cohort (in the combined cohort analysis).