Research Article

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Circulating Soluble Cytokine Receptors and Colorectal Cancer Risk

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Abstract

Background: Soluble cytokine receptors and receptor antagonist of proinflammatory cytokines can modify cytokine signaling and may affect cancer risk.

Methods: In a case–cohort study nested within the Women's Health Initiative cohort of postmenopausal women, we assessed the associations of plasma levels of interleukin (IL)-1 receptor antagonist (IL-1Ra) and the soluble receptors of IL-1 (sIL-1R2), IL-6 (sIL-6R and sgp130), and TNF (sTNFR1 and sTNFR2) with risk of colorectal cancer in 433 cases and 821 subcohort subjects. Baseline levels of estradiol, insulin, leptin, IL-6, and TNF-α measured previously were also available for data analysis.

Results: After adjusting for significant covariates, including age, race, smoking, colonoscopy history, waist circumference, and levels of estrogen, insulin, and leptin, relatively high levels of sIL-6R and sIL-1R2 were associated with reduced colorectal cancer risk [HRs comparing extreme quartiles ($HR_{Q4\text{-}Q1}$) for sIL-6R, 0.56; 95% confidence interval (CI), 0.38–0.83; $HR_{Q4\text{-}Q1}$ for sIL-1R2, 0.44; 95% CI, 0.29–0.67]. The associations with IL-1Ra, sgp130, sTNFR1, and sTNFR2 were null. The inverse association of sIL-1R2 with colorectal cancer risk persisted in cases diagnosed ≤5 and >5 years from baseline blood draw; the association with sIL-6R, however, was not evident in the latter group, possibly indicating that relatively low levels of sIL-6R in cases might be due to undiagnosed cancer at the time of blood draw.

Conclusions: High circulating levels of sIL-1R2 may be protective against colorectal carcinogenesis and/or be a marker of reduced risk for the disease.

Impact: sIL-1R2 has potential to be a chemopreventive and/or immunotherapeutic agent in inflammation-related diseases. *Cancer Epidemiol Biomarkers Prev; 23(1); 179–88. ©2013 AACR.*

Introduction

Experimental studies have shown that potent proinflammatory cytokines, such as TNF-α, interleukin (IL)-6, and IL-1β, have oncogenic effects (1, 2). However, soluble cytokine receptors or receptor antagonists of these cytokines can modify cytokine signaling and may also affect cancer risk (3, 4). This case–cohort study nested within the Women's Health Initiative Observational Study (WHI-OS) assessed circulating levels of the soluble cytokine receptors and receptor antagonist of these three potent inflammatory cytokines for their associations with risk of colorectal cancer, an inflammation-associated disease (5–7). Specifically, IL-1 receptor antagonist (IL-1Ra) and the soluble receptors of IL-1 (sIL-1R2), IL-6 (sIL-6R and sgp130), and TNF (sTNFR1 and sTNFR2) were examined. With the exception of sTNFR2 (8), none of these analytes have ever been studied for their colorectal cancer associations.

Soluble cytokine receptors can be formed by proteolytic cleavage of cell-surface receptors or by alternative splicing of mRNA that deletes the transmembrane domain of membrane-associated receptors (3). Many soluble cytokine receptors (e.g., sIL-1R2, sTNFR1, and sTNFR2) may act as decoy receptors, block binding of the ligands to cognate functional membrane receptors, and hence inhibit cytokine signaling (4, 9–11). The biologic effects of IL-6 soluble receptors are more complex. IL-6 signaling requires the interaction of IL-6 with its receptor complex consisting of IL-6R and the signal-transducing protein gp130. Although gp130 is ubiquitously expressed, IL-6R is not present on all cells. Nevertheless, IL-6R has a soluble form sIL-6R that binds to IL-6 to form a soluble complex, which binds to cell-surface gp130 on cells that lack the membrane-bound IL-6R and initiates signaling. As such,

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sIL-6R is thought to be an IL-6 agonist enhancing IL-6 signaling (9). However, there is also a soluble form of gp130. IL-6 can be trapped in the soluble ternary complex with sIL-6R and sgp130, resulting in inhibition of IL-6 signaling (12). IL-1Ra is a naturally occurring receptor antagonist that binds to, without activating, the membrane-bound IL-1 receptors and hence competitively blocks IL-1 binding to its cell-surface receptors (4, 13).

The six analytes were chosen because of their biologic activities that they could modify signaling of the potent inflammatory cytokines. This study tested the hypothesis that the levels of IL-1Ra, sIL-1R2, sgp130, sTNFR1, and sTNFR2 would be inversely associated with colorectal cancer risk, whereas sIL-6R might have the opposite effect. Moreover, we had previously examined the associations of the ligands, IL-6 and TNF-α, with colorectal cancer risk on the same cases and subcohort subjects (7); this present study would have the opportunity to assess how the cytokine ligands and their soluble receptors might act together to affect colorectal cancer risk.

Materials and Methods

Study population

The WHI-OS is a prospective study of 93,676 postmenopausal women ages 50 to 79 years at recruitment between 1993 and 1998 in the United States (14). At baseline, participants completed epidemiologic questionnaires, and morning, fasting blood samples were collected, centrifuged, frozen on-site at −80°C, and later shipped to the central specimen repository. Diagnosis of colorectal cancer was ascertained by annual self-administered questionnaires and confirmed through centralized review of medical records.

This colorectal cancer study was a component of a case–cohort study in which three cancer outcomes (breast, colorectum, and endometrium) were previously examined for their associations with estradiol, insulin, IL-6, TNF-α, leptin, and other adipokines (7, 15–17). There were 496 cases that had developed an incident primary colorectal cancer by June 2004, after excluding those diagnosed during the first year of follow-up. A subcohort of 892 women was randomly sampled from the WHI-OS participants who had more than 12 months of follow-up and had no history of breast, colorectal, or endometrial cancer at 12 months after study enrollment, regardless of their cancer outcome thereafter. Of these, 433 cases and 821 subcohort members had baseline plasma samples available for the present study and did not have diabetes treatment at the time of blood draw (which would alter levels of insulin and leptin that were included in data analyses described later). There were 353 cases of colon cancer, 78 cases of rectosigmoid junction or rectal cancer, and two cases with unknown location of the colorectal cancer; 96% of the cases were diagnosed as adenocarcinoma.

Laboratory methods

The six analytes in EDTA plasma samples were assayed by the Millipore kits (EMD Millipore). The MILLIPLEX Human Cytokine/Chemokine Panel was used to measure IL-1Ra; sIL-6R, sgp130, sIL-1R2, sTNFR1, and sTNFR2 were measured in a multiplex assay using the MILLIPLEX Human Soluble Cytokine Receptor Panel. The MILLIPLEX assays used the Luminex's color-coded bead-based technology to achieve multiplexing (18).

The analytes were detectable in 99% to 100% of the samples (the lowest detectability was 99.2% for IL-1Ra). The interassay coefficients of variation (CV) in our laboratory, which were determined from four control samples inserted into each of the 42 assay plates run over a period of time by the same technician, were 4.5% for sTNFR2, 5.9% for sTNFR1, 5.5% for sIL-6R, 6.6% for sgp130, 7.1% for sIL-1R2, and 10.7% for IL-1Ra. The 3-year intraclass correlation coefficients (ICC) of the six analytes were estimated from two previous studies. On the basis of three independent plasma samples collected over a 3-year period (baseline, year 1, and year 3) from each of 17 healthy women (19), the ICCs were 0.52 for sIL-6R, 0.63 for sgp130, 0.65 for sTNFR1, and 0.78 for sIL-1R2 (20). In another study, plasma levels of sTNFR2 and IL-1Ra at baseline and year 3 were measured in 148 subjects randomized to the placebo group of the Aspirin/Folate Polyp Prevention Trial (21). The 3-year ICCs were 0.56 for IL-1Ra and 0.72 for sTNFR2 (unpublished data). These ICC data suggest that a single measurement of circulating levels of the analytes under study in the baseline blood sample reflects an individual's average levels over time.

Statistical analyses

From previous studies of this case–cohort study population, we found circulating levels of insulin, leptin, and estradiol to be significantly positively associated with colorectal cancer risk in multivariable analyses (7, 16). These variables were included in the data analyses described here. Although IL-6 and TNF-α were not significant risk factors in prior analyses, they were also included in data analyses, because they are ligands of the soluble receptors under study.

In univariable analyses, the prevalences of established colorectal cancer risk factors and analyte levels in quartiles between cases and subcohort subjects were compared using χ^2 test. In the subcohort, we evaluated whether various colorectal cancer risk factors were associated with the analyte levels using Kruskal–Wallis tests, and correlations among the analytes were estimated by Spearman rank correlation coefficients.

Multivariable analyses were conducted using Cox proportional hazard regression with robust variance estimation using the Self-Prentice method, which accounts for the case–cohort design in which cases may arise outside of or within the subcohort (22, 23). A base model was first developed to retain only the baseline covariates age (continuous), race (Whites vs. others), and colorectal cancer risk factors that were significant in multivariable analyses in our study population—smoking status (never, former, or current), ever had a colonoscopy, and estrogen level in four categories [serum estradiol in tertiles among women

Table 1. Baseline characteristics of colorectal cancer cases and subcohort subjects in the WHI

Baseline characteristics	Cases ($n = 433$) *n* (%)	Subcohort ($n = 814$)[a] *n* (%)	*P*
Age (y)			
50–54	33 (7.6)	130 (16.0)	<0.001[b]
55–56	141 (32.6)	350 (43.0)	
65–74	201 (46.4)	283 (34.8)	
75–79	58 (13.4)	51 (6.3)	
Race-ethnicity			
White	375 (87.0)	695 (85.7)	0.127
Black	35 (8.1)	54 (6.7)	
Other	21 (4.9)	62 (7.6)	
Smoking status			
Never	194 (45.51)	432 (53.7)	0.009[b]
Former	200 (47.0)	323 (40.2)	
Current	32 (7.5)	49 (6.1)	
Used NSAID for ≥2 weeks	178 (41.1)	298 (36.6)	0.120
Ever used oral contraceptives	130 (30.0)	336 (41.3)	<0.001[b]
Hormone therapy use at baseline			
No	280 (64.7)	445 (54.7)	0.002[b]
Estrogen + progesterone	67 (15.5)	182 (22.4)	
Estrogen alone	86 (19.9)	186 (22.9)	
Family history of colorectal cancer			
No	315 (72.7)	627 (77.0)	0.246
Yes	81 (18.7)	128 (15.7)	
Don't know	37 (8.5)	59 (7.2)	
Ever had colonoscopy	209 (49.0)	456 (56.4)	0.012
Had polyps removed among those ever had colonoscopy	47 (23.0)	78 (17.6)	0.101
Alcohol servings per week			
0	178 (41.2)	319 (39.2)	0.291[b]
0.1–1. 56	125 (28.9)	224 (27.6)	
≥1.57	129 (29.9)	270 (33.2)	
Total folate intake (μg) per kcal per day			
<0.30	124 (28.7)	195 (24.0)	0.136[b]
0.30–0.40	105 (24.3)	204 (25.1)	
0.41–0.58	97 (22.5)	205 (25.2)	
≥0.59	106 (24.5)	210 (25.8)	
Red meat intake (medium serving) per kcal per day × 10^3			
<0.20	111 (25.7)	209 (25.7)	0.714[b]
0.20–0.34	111 (25.7)	204 (25.1)	
0.35–0.52	112 (25.9)	202 (24.8)	
≥0.53	98 (22.7)	199 (24.5)	
BMI (kg/m^2)			
<25	149 (35.0)	316 (39.8)	0.028[b]
25–29.9	151 (35.5)	288 (36.3)	
≥30	126 (29.6)	190 (23.9)	
Waist circumference (cm)			
<74.6	67 (15.5)	196 (24.2)	<0.001[b]
74.6–81.9	91 (21.0)	185 (22.8)	
82.0–91.4	124 (28.6)	219 (27.0)	
≥91.5	151 (34.9)	210 (25.9)	
Physical activity (MET/wk)			
<3.8	124 (28.9)	203 (25.2)	0.030[b]
3.8–9.9	110 (25.6)	200 (24.8)	

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Table 1. Baseline characteristics of colorectal cancer cases and subcohort subjects in the WHI (Cont'd)

Baseline characteristics	Cases ($n = 433$) *n* (%)	Subcohort ($n = 814$)[a] *n* (%)	*P*
10.0–19.9	113 (26.3)	201 (24.9)	
≥20	82 (19.2)	203 (25.2)	
Serum estradiol (pg/mL)[c]			
<8	72 (26.5)	146 (33.4)	
8–13.9	103 (37.8)	132 (30.2)	0.018
≥14	97 (35.7)	159 (36.4)	0.271
Serum insulin (μU/mL)			
<3.2	73 (17.2)	193 (24.2)	<0.001[b]
3.2–4.9	92 (21.7)	189 (23.7)	
5.0–8.2	107 (25.2)	202 (25.4)	
≥8.3	153 (36.0)	213 (26.7)	
Plasma leptin (ng/mL)			
<7.4	82 (19.0)	213 (26.2)	0.001[b]
7.4–15.0	120 (27.8)	209 (25.7)	
15.1–25.5	88 (20.4)	201 (24.7)	
≥25.6	142 (32.9)	191 (23.5)	
Plasma IL-6 (pg/mL)			
<0.93	87 (20.2)	205 (26.0)	0.001[b]
0.93–1.47	98 (22.8)	207 (26.3)	
1.48–2.31	121 (28.1)	200 (25.4)	
≥2.32	124 (28.8)	176 (22.3)	
Plasma TNF-α (pg/mL)			
<1.82	99 (23.1)	196 (24.8)	0.147[b]
1.82–2.62	103 (24.0)	205 (25.9)	
2.63–3.62	106 (24.7)	203 (25.6)	
≥3.63	121 (28.2)	188 (23.7)	
Plasma levels of six analytes under study			
sgp130 (ng/mL)			
<146.2	99 (22.9)	203 (24.9)	0.697[b]
146.2–172.1	110 (25.4)	208 (25.6)	
172.2–200.0	123 (28.4)	199 (24.5)	
≥200.1	101 (23.3)	204 (25.1)	
sIL-6R (ng/mL)			
<16.7	126 (29.1)	207 (25.4)	0.023[b]
16.7–21.1	116 (26.8)	198 (24.3)	
21.2–25.4	105 (24.3)	204 (25.1)	
≥25.5	86 (19.9)	205 (25.2)	
sIL-1R2 (ng/mL)			
<5.8	130 (30.0)	197 (24.2)	0.019[b]
5.8–7.3	101 (23.3)	209 (25.7)	
7.4–9.1	122 (28.2)	208 (25.6)	
≥9.2	80 (18.5)	200 (24.6)	
IL-1Ra (pg/mL)			
<15.5	112 (25.9)	204 (25.1)	0.911[b]
15.5–24.1	98 (22.6)	205 (25.2)	
24.2–38.8	118 (27.3)	206 (25.3)	
≥38.9	105 (24.3)	199 (24.5)	
sTNFR1 (ng/mL)			
<1.0	82 (18.9)	210 (25.8)	0.005[b]
1.0–1.2	108 (24.9)	204 (25.1)	

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Table 1. Baseline characteristics of colorectal cancer cases and subcohort subjects in the WHI (Cont'd)

Baseline characteristics	Cases ($n = 433$) n (%)	Subcohort ($n = 814$)[a] n (%)	P
1.3–1.5	117 (27.0)	203 (24.9)	
≥1.6	126 (29.1)	197 (24.2)	
sTNFR2 (ng/mL)			
<3.8	81 (18.7)	214 (26.3)	0.012[b]
3.8–4.4	112 (25.9)	207 (25.4)	
4.5–5.4	132 (30.5)	203 (24.9)	
≥5.5	108 (24.9)	190 (23.3)	

Abbreviation: MET, metabolic equivalent of task.
[a]Excluding seven cases in the subcohort.
[b]*P* value for trend.
[c]Among women not using hormone therapy at baseline.

who were not using hormone therapy (<8, 8–13.9, or ≥14 pg/mL) or women using hormone therapy at baseline], waist circumference (continuous), insulin level (continuous), and leptin level (in quartiles due to its nonlinear relationship with colorectal cancer risk). Colorectal cancer risk factors that were not statistically significant in multivariable modeling were excluded from the base model [e.g., use of nonsteroidal antiinflammatory drugs (NSAID) and alcohol, physical activity, family history of colorectal cancer, and folate and red meat intakes per day]. We then assessed the HR for the association of each of the six analytes with colorectal cancer risk adjusting for the base-model covariates. To be robust to any nonlinear effect, all six analytes were categorized and analyzed in quartiles. Trend tests were performed using Wald tests associated with fitting the quartile categories as continuous variables in the regression model.

Although colorectal cancer cases diagnosed within the first year of follow-up were already excluded, sensitivity analyses were conducted to further examine whether reverse-causality might explain the analyte–disease association. Cases were separated into two groups defined by number of years from baseline recruitment to case diagnosis (>1 to 5 years or >5 years). Subcohort members who had >1 year or >5 years of follow-up were included in the corresponding two groups, respectively.

Results

Table 1 shows the demographic factors and established risk factors for colorectal cancer in the cases and the subcohort. Briefly, as compared with the subcohort subjects, cases were older, more likely to be smokers, less physically active, and had higher body mass index (BMI) and waist circumference, with the latter having a stronger association with colorectal cancer than BMI. Cases were less likely to have had a colonoscopy, to have ever used oral contraceptives, and to be a hormone therapy user at baseline. Cases also had higher circulating levels of insulin and leptin and were more likely to have a moderately higher level of estradiol than the subcohort subjects. In terms of the six analytes under study, cases had lower plasma levels of sIL-6R and IL-1R2, but higher levels of sTNFR1 and sTNFR2 than the subcohort subjects. Table 1 also shows that the soluble receptors of IL-6 and TNF-α were circulating at a much higher concentration than their ligands (ng/mL vs. pg/mL).

Several of the demographic and lifestyle variables in Table 1 remained to be associated with colorectal cancer risk in multivariable analyses. Associations of the six analytes with these significant baseline risk factors for colorectal cancer are shown in Table 2. Sgp130, sTNFR1, and sTNFR2 increased with age. None was related to cigarette smoking. Use of hormone therapy was associated with reduced levels of all six analytes. Greater adiposity was associated with higher levels of IL-1Ra, sTNFR1, and sTNFR2. Concentrations of sIL-1R2, IL-1Ra, sTNFR1, and sTNFR2 increased with circulating levels of obesity-related factors (insulin and leptin).

Table 3 shows the correlations among the analytes and the ligands IL-6 and TNF-α. The highest correlations were between sTNFR1 and sTNFR2 ($r = 0.51$) and between TNF-α and sTNFR2 ($r = 0.43$). The ratios of soluble receptors to their ligands (e.g., sIL-6R/IL-6) were not meaningful indices, because they merely reflected the ligand levels and were highly correlated with them ($|r| > 0.80$).

The results of age-adjusted as well as multivariable adjusted analyses are shown in Table 4. After adjusting for the significant covariates in the base model (including age, race, smoking status, ever had colonoscopy, estrogen level, waist circumference, insulin level, and leptin level), two soluble cytokine receptors, sIL-6R and sIL-1R2, were inversely associated with colorectal cancer risk, with HRs comparing extreme quartiles ($HR_{Q4\text{-}Q1}$) of 0.56 for sIL-6R [95% confidence interval (CI), 0.38–0.83; $P_{trend} = 0.007$] and 0.44 for sIL-1R2 (95% CI, 0.29–0.67; $P_{trend} = 0.0004$); sTNFR1 and sTNRF2 were no longer significantly

Table 2. Associations of soluble cytokine receptors and receptor antagonist with significant risk factors of colorectal cancer among subcohort subjects in the WHI

		sgp130 (ng/mL)			sIL-6R (ng/mL)			sIL-1R2 (ng/mL)			IL-1Ra (pg/mL)			sTNFR1 (ng/mL)			sTNFR2 (ng/mL)		
	N	Median	IQR		Median	IQR		Median	IQR		Median	IQR		Median	IQR		Median	IQR	
Age																			
<55	131	163	134	193	20.4	15.8	25.8	7.2	5.3	9.0	24.0	14.7	45.1	1.1	1.0	1.4	4.1	3.4	4.9
55–64	351	171	146	195	21.1	16.5	25.0	7.2	5.8	9.2	24.1	15.6	36.7	1.2	1.0	1.5	4.3	3.6	5.2
65–74	287	176	150	205	21.5	17.7	26.1	7.5	5.9	9.2	24.3	15.5	39.4	1.4	1.1	1.7	4.8	4.1	5.8
≥75	52	180	160	201	22.4	16.8	27.4	7.6	6.2	9.4	24.5	15.7	38.5	1.4	1.2	1.8	5.2	4.5	6.5
P		0.010			0.391			0.448			0.975			<0.0001			<0.0001		
Smoking																			
Never	437	175	149	202	21.5	17.1	25.1	7.2	5.8	9.0	24.1	15.9	39.7	1.3	1.0	1.6	4.6	3.8	5.5
Former	323	169	143	195	20.8	16.7	25.6	7.6	5.9	9.4	25.3	15.4	39.2	1.2	1.0	1.6	4.4	3.7	5.3
Current	50	173	150	195	20.8	14.9	27.7	8.4	6.0	10.0	19.8	11.6	29.8	1.2	1.0	1.4	4.3	3.8	5.4
P		0.262			0.916			0.049			0.122			0.685			0.173		
Hormone therapy at baseline																			
No	450	179	153	207	21.6	17.2	26.3	7.8	6.1	9.4	25.6	16.5	41.7	1.3	1.1	1.6	4.7	3.8	5.5
Yes	370	166	141	186	20.4	16.1	24.6	7.0	5.5	8.7	22.8	14.3	35.0	1.2	1.0	1.5	4.3	3.6	5.2
P		<0.0001			0.009			<0.0001			0.006			0.001			0.002		
Colonoscopy ever																			
No	355	171	144	200	21.2	16.5	26.2	7.7	5.9	9.4	24.1	15.3	38.5	1.2	1.0	1.6	4.4	3.6	5.3
Yes	460	174	149	201	21.3	16.9	25.1	7.2	5.7	9.0	24.1	15.4	38.5	1.3	1.0	1.6	4.6	3.8	5.5
P		0.148			0.828			0.151			0.631			0.156			0.086		
Waist circumference																			
<74.6	199	175	146	203	20.5	15.9	25.4	7.3	5.8	9.0	19.6	12.6	33.0	1.2	0.9	1.4	4.2	3.6	4.9
74.6–81.9	186	168	141	189	20.8	16.5	25.6	6.9	5.4	8.4	24.2	15.1	36.4	1.2	1.0	1.5	4.3	3.6	5.2
82–91.4	220	175	155	205	22.2	17.8	25.9	7.8	6.1	9.5	25.6	15.6	38.2	1.3	1.1	1.5	4.5	3.9	5.5
≥91.5	212	168	141	203	20.6	17.0	25.2	7.8	5.9	9.5	27.9	18.2	44.5	1.4	1.1	1.7	5.0	4.2	5.9
P		0.011			0.187			0.000			<0.0001			<0.0001			<0.0001		
Estradiol (pg/mL)[a]																			
<8	147	186	150	213	23.1	19.1	27.3	8.3	6.4	9.8	25.7	17.7	46.8	1.3	1.1	1.5	4.6	4.0	5.6
8–13.9	134	173	152	201	21.7	16.5	26.0	7.9	6.2	9.4	24.7	15.6	36.7	1.3	1.1	1.7	4.6	3.7	5.3
≥14	161	179	153	209	20.4	16.8	25.2	7.3	5.7	9.2	26.7	16.5	43.3	1.3	1.0	1.7	4.8	3.8	5.7
P		0.230			0.014			0.045			0.439			0.903			0.279		
Insulin (μU/mL)																			
<3.2	196	175	146	202	20.3	16.2	25.2	7.0	5.6	8.6	21.7	12.9	35.8	1.1	0.9	1.4	4.1	3.5	4.9
3.2–4.9	191	172	146	199	21.1	15.8	25.1	7.2	5.8	8.6	22.0	14.0	32.0	1.3	1.1	1.6	4.4	3.8	5.4
5.0–8.2	203	172	141	203	22.0	17.2	26.1	7.7	6.1	9.1	22.2	14.7	35.2	1.3	1.1	1.5	4.5	3.7	5.3
≥8.3	214	172	150	196	21.5	17.3	26.0	7.9	6.1	9.8	30.9	19.7	51.2	1.4	1.1	1.7	5.0	4.1	6.1
P		0.988			0.163			0.003			<0.0001			<0.0001			<0.0001		
Leptin (ng/mL)																			
<7.4	216	171	142	193	20.5	15.8	25.4	7.0	5.5	8.4	22.2	13.6	35.7	1.2	1.0	1.4	4.3	3.6	5.0
7.4–15.0	210	171	149	204	21.4	16.9	25.6	7.2	5.7	9.1	23.5	15.3	34.5	1.2	1.0	1.5	4.3	3.6	5.2
15.1–25.5	203	172	147	201	21.3	17.2	26.1	7.4	6.1	9.1	23.4	15.1	40.2	1.3	1.1	1.6	4.3	3.7	5.3
≥25.6	192	174	149	202	21.7	16.6	25.5	8.2	6.0	9.7	27.9	18.2	47.9	1.4	1.1	1.7	5.1	4.3	5.9
P		0.665			0.538			0.001			0.001			<0.0001			<0.0001		

Abbreviation: IQR, interquartile range.

[a]Among women not using hormone therapy at baseline.

associated with colorectal cancer risk. When all the six analytes and the ligands IL-6 and TNF-α were analyzed simultaneously, sIL-6R and sIL-1R2 remained significant (Table 4). In a saturated model, we adjusted for all the established risk factors, by including covariates that were not statistically associated with colorectal cancer into the base model (e.g., use of NSAIDs and alcohol, physical activity, family history of colorectal cancer, and folate and red meat intakes per day), and similar results were obtained (data not shown).

Table 3. Spearman rank correlations among soluble cytokine receptors, receptor antagonist, their ligands, and ratios of receptor to ligand (*P* values shown in parentheses)

	sIL-6R	sIL-1R2	IL-1Ra	sTNFR1	sTNFR2	IL-6	TNF-α	sgp130/IL-6	sIL6R/IL-6	sTNFR1/TNF	sTNFR2/TNF
sgp130	0.17 (<0.0001)	0.26 (<0.0001)	0.03 (0.331)	0.17 (<0.0001)	0.19 (<0.0001)	−0.01 (0.875)	0.06 (0.085)	0.32 (<0.0001)	0.08 (0.032)	0.03 (0.363)	0.03 (0.436)
sIL-6R		0.18 (<0.0001)	0.06 (0.098)	0.19 (<0.0001)	0.20 (<0.0001)	0.06 (0.099)	0.13 (0.0002)	0.01 (0.836)	0.33 (<0.0001)	−0.03 (0.392)	−0.04 (0.321)
sIL-1R2			0.10 (0.004)	0.12 (0.001)	0.10 (0.006)	0.03 (0.331)	0.06 (0.104)	0.05 (0.186)	0.03 (0.334)	0.03 (0.448)	0.01 (0.866)
IL1RA				0.18 (<0.0001)	0.18 (<0.0001)	0.14 (0.0001)	0.10 (0.006)	−0.12 (0.001)	−0.10 (0.004)	−0.02 (0.670)	−0.02 (0.535)
sTNFR1					**0.51** (<0.0001)	0.24 (<0.0001)	0.26 (<0.0001)	−0.17 (<0.0001)	−0.14 (<0.0001)	0.28 (<0.0001)	0.00 (0.978)
sTNFR2						0.30 (<0.0001)	**0.43** (<0.0001)	−0.23 (<0.0001)	−0.19 (<0.0001)	−0.14 (0.0001)	0.07 (0.052)
IL-6							0.23 (<0.0001)	**−0.93** (<0.0001)	**−0.90** (<0.0001)	−0.08 (0.025)	−0.09 (0.010)
TNF-α								−0.21 (<0.0001)	−0.17 (<0.0001)	**−0.80** (<0.0001)	**−0.84** (<0.0001)
sgp130/IL-6									**0.87** (<0.0001)	0.09 (0.009)	0.10 (0.005)
sIL6R/IL-6										0.07 (0.055)	0.07 (0.035)
sTNFR1/TNF											**0.82** (<0.0001)

NOTE: Correlations >0.4 were bolded.

There were no significant interactions among the soluble cytokine receptors, receptor antagonist, IL-6, and TNF-α (data not shown), or between the study analytes and colorectal cancer risk factors (e.g., waist circumference, insulin, and hormone therapy, etc.). Results were similar when data were stratified by NSAID status at baseline (used regularly for ≥ 2 weeks or not) or when the 78 rectosigmoid junction or rectal cancer cases were excluded (data not shown).

When colorectal cancer cases were stratified by the number of years from baseline blood collection to case diagnosis in a sensitivity analysis (Table 5), sIL-6R was inversely associated with colorectal cancer risk only in those diagnosed between >1 to 5 years of baseline, but not in the cases diagnosed >5 years after baseline. In contrast, the inverse association between sIL-1R2 and colorectal cancer risk persisted regardless of the year of diagnosis (the last case was diagnosed 8.2 years after baseline in the study population reported here).

Discussion

In this study, we found high levels of sIL-1R2, but not IL-1Ra, to be associated with a reduced risk of colorectal cancer. IL-1 signaling can be inhibited by its receptor antagonist (IL-1Ra) and soluble type I and type II IL-1 receptors (sIL-1R1 and sIL-1R2; refs. 4, 13). We measured sIL-1R2 instead of sIL-1R1, because sIL-1R2 is the dominant soluble receptor and has greater affinity for IL-1 than sIL-R1 (4, 24). sIL-1R2 functions as a molecular decoy that prevents interaction of IL-1 with the signal-transducing type I receptor. Our finding of the inverse association between colorectal cancer risk and sIL-1R2 is consistent with the results of a study on Crohn's disease, an inflammatory bowel disease associated with high risk of colorectal cancer, in which both circulating and mucosal sIL-1R2 levels were significantly higher in healthy controls than patients and treatment with corticosteroids induced a significant increase in sIL-1R2 (25). In accord with our present findings, in our previous study of patients with a history of colorectal adenoma, we did not observe any effects of IL-1Ra on adenoma recurrence (21).

Laboratory studies have shown that a very high concentration of sIL-1R2 relative to IL-1 is required to block IL-1 biologic activity, as affinity of the soluble receptor is generally weaker than that of the membrane-bound receptor (4). We did not measure IL-1β in this study, because of its low circulating level. In a pilot study of 25 EDTA plasma samples from postmenopausal women, 36% of the samples had IL-1β levels below the assay limit of detection of 0.06 pg/mL (MILLIPLEX High Sensitivity Human Cytokine Panel), and the median level among samples with a detectable level was 1.2 pg/mL. On the other hand, in the subcohort of this study, the median sIL-1R2 level was 7,385 pg/mL. It then seems that the circulating concentration of sIL-1R2 greatly exceeds that of IL-1β. As such, the ratio of sIL-1R2 to IL-1β levels, even

Table 4. HRs for the associations of soluble cytokine receptors and receptor antagonist with colorectal cancer risk

	Q1	Q2	Q3	Q4	*P* for trend
sgp130 (ng/mL)	<146.2	146.2–172.1	172.2–200.0	≥200.1	
# cases/# subcohort	99/204	110/209	123/199	101/209	
Age	1	1.04 (0.74–1.47)	1.17 (0.83–1.65)	0.90 (0.63–1.27)	0.694
Base model[a]	1	1.05 (0.72–1.53)	1.05 (0.71–1.54)	0.94 (0.64–1.37)	0.754
Base + other receptors + ligands[b]	1	1.19 (0.80–1.76)	1.14 (0.74–1.75)	1.12 (0.72–1.75)	0.654
sIL-6R (ng/mL)	<16.7	16.7–21.1	21.2–25.4	≥25.5	
# cases/# subcohort	126/207	116/202	105/205	86/207	
Age	1	0.86 (0.62–1.20)	0.78 (0.55–1.09)	0.58 (0.41–0.83)	0.002
Base model[a]	1	0.82 (0.56–1.21)	0.82 (0.55–1.21)	0.56 (0.38–0.83)	0.007
Base + other receptors + ligands[b]	1	0.82 (0.55–1.23)	0.82 (0.54–1.26)	0.59 (0.38–0.90)	0.022
sIL-1R2 (ng/mL)	<5.8	5.8–7.3	7.4–9.1	≥9.2	
# cases/# subcohort	130/202	101/209	122/210	80/200	
Age	1	0.73 (0.52–1.02)	0.85 (0.62–1.18)	0.59 (0.41–0.84)	0.014
Base model[a]	1	0.69 (0.47–1.01)	0.79 (0.55–1.13)	0.44 (0.29–0.67)	<0.001
Base + other receptors + ligands[b]	1	0.68 (0.45–1.03)	0.79 (0.53–1.16)	0.44 (0.28–0.71)	0.003
IL-1Ra (pg/mL)	<15.5	15.5–24.1	24.2–38.8	≥38.9	
# cases/# subcohort	112/206	98/207	118/207	105/201	
Age	1	0.86 (0.61–1.22)	1.05 (0.75–1.46)	0.93 (0.66–1.30)	0.942
Base model[a]	1	0.72 (0.48–1.06)	0.79 (0.54–1.16)	0.73 (0.49–1.10)	0.227
Base + other receptors + ligands[b]	1	0.80 (0.52–1.23)	0.78 (0.52–1.16)	0.84 (0.54–1.31)	0.420
sTNFR1 (ng/mL)	<1.0	1.0–1.2	1.3–1.5	≥1.6	
# cases/# subcohort	82/211	108/206	117/204	126/200	
Age	1	1.23 (0.86–1.75)	1.33 (0.94–1.90)	1.42 (0.99–2.03)	0.054
Base model[a]	1	1.11 (0.74–1.66)	1.31 (0.88–1.95)	1.03 (0.68–1.56)	0.766
Base + other receptors + ligands[b]	1	1.16 (0.74–1.81)	1.56 (0.98–2.49)	1.29 (0.80–2.10)	0.191
sTNFR2 (ng/mL)	<3.8	3.8–4.4	4.5–5.4	≥5.5	
# cases/# subcohort	81/214	112/208	132/206	108/193	
Age	1	1.26 (0.88–1.80)	1.42 (1.00–2.02)	1.14 (0.79–1.64)	0.428
Base model[a]	1	1.10 (0.74–1.62)	1.20 (0.81–1.79)	0.82 (0.53–1.28)	0.441
Base + other receptors + ligands[b]	1	1.05 (0.69–1.59)	1.27 (0.79–2.04)	0.90 (0.52–1.56)	0.893

[a]Base model included the following baseline covariates: age, race, smoking status, ever had colonoscopy, estrogen level, waist circumference, insulin level, and leptin level.

[b]Base model covariates + sgp130 + sIL-6R + sIL-1R2 + IL-1Ra + sTNFR1 + sTNFR2 + IL-6 + TNF-α.

if data on IL-1β were available in our study, would not be a useful indicator for the level of free IL-1β in circulation.

Although we found that a low level of sIL-6R was associated with increased colorectal cancer risk, this inverse association was only seen in the cases diagnosed in the first 5 years, suggesting the relatively low levels of sIL-6R in the baseline blood samples of cases might have arisen as a result of undiagnosed colorectal neoplasia at the time of the blood draw. A previous study of colorectal tumor tissue also indicated that sIL-6R level could be a marker for tumor growth (26). Specifically, this study showed that a low level of sIL-6R expression in tumor correlated with increased IL-6 expression and with disease progression, inferring consumption of sIL-6R by increased binding with IL-6 in the cancer stroma may favor tumor growth (26).

We did not find any associations of sTNFR1 and sTNFR2 with colorectal cancer. Similarly, there were no effects of sTNFR2 on adenoma recurrence in our previous study of patients with a history of colorectal adenoma (21). Contrarily, the Nurses' Health Study found that increased sTNFR2 levels were associated with colorectal cancer risk (8). One possible explanation for this discrepancy is the fact that the assays used for sTNFR2 were different between this study and those used in the Nurses' Health Study.

Similar to the situation of sIL-1R2 and IL-1β, the circulating concentrations of soluble receptors of IL-6 and TNF-α were several thousand-folds greater than those of the ligands (ng vs. pg). As such, their ratios (e.g., sIL-6R/IL-6 or sTNFR1/TNF-α) were not meaningful indices to make any biologic inferences. Although one of the study

Table 5. HRs for the associations of sIL-6R and sIL-1R2 with colorectal cancer risk stratified by the number of years from baseline recruitment to case diagnosis

	Q1	Q2	Q3	Q4	*P* for trend
sIL-6R (ng/mL)	<16.7	16.7–21.1	21.2–25.4	≥25.5	
Cases diagnosed >1 to 5 years after baseline					
# cases/# subcohort	96/196	88/195	73/188	52/198	
Base + other receptors + ligands[a]	1	0.87 (0.57–1.34)	0.77 (0.49–1.21)	0.50 (0.31–0.79)	0.003
Cases diagnosed >5 years after baseline					
# cases/# subcohort	21/159	19/163	26/155	29/173	
Base + other receptors + ligands[a]	1	0.59 (0.24–1.44)	1.01 (0.44–2.31)	0.90 (0.41–1.98)	0.835
sIL-1R2 (ng/mL)	<5.8	5.8–7.3	7.4–9.1	≥9.2	
Cases diagnosed >1 to 5 years after baseline					
# cases/# subcohort	94/188	69/197	89/201	57/191	
Base + other receptors + ligands[a]	1	0.72 (0.46–1.12)	0.91 (0.60–1.37)	0.49 (0.30–0.80)	0.019
Cases diagnosed >5 years after baseline					
# cases/# subcohort	30/157	22/165	24/173	19/155	
Base + other receptors + ligands[a]	1	0.55 (0.24–1.23)	0.36 (0.14–0.91)	0.26 (0.09–0.77)	0.013

[a]Base model covariates (age, race, smoking status, ever had colonoscopy, estrogen level, waist circumference, insulin level, and leptin level) + sgp130 + sIL-6R + sIL-1R2 + IL-1Ra + sTNFR1 + sTNFR2 + IL-6 + TNF-α.

goals was to examine how the cytokine ligands and their soluble receptors might act together to affect colorectal cancer risk, we could not assess this effectively. Nevertheless, we did not observe any interactive effects between the soluble receptors and their ligands on colorectal cancer risk.

Our study has other limitations. The mechanisms of regulation of circulating sIL-1R2 are unclear. The inverse association between sIL-1R2 and colorectal cancer risk might have been confounded by unmeasured protective factors for colorectal cancer that stimulate the release of sIL-1R2. Moreover, circulating levels of the soluble cytokine receptors and receptor antagonist may not reflect tissue levels. Finally, our results are not necessarily generalizable to men or to premenopausal women.

It is unlikely that our results were confounded by NSAID use, although close to 40% of the study population had used an NSAID regularly for 2 weeks or more at baseline. NSAID use was not associated with colorectal cancer risk in multivariate analyses in our study population. Even when NSAID use was added into the regression model, similar results were obtained. Moreover, there is no evidence in the literature to indicate that NSAID use affects the levels of soluble cytokine receptors and receptor antagonists. In fact, our previous data from an aspirin clinical trial demonstrated that low-dose aspirin had no effects on the circulating levels of sTNFR2 and IL-1Ra (21).

In summary, our data suggest that high circulating levels of sIL-1R2 may be protective against colorectal carcinogenesis or be a marker for reduced colorectal cancer risk. Further investigations of this soluble cytokine receptor are warranted for its potential as a risk-prediction marker or as an immunologic agent for chemoprevention and therapy of colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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