

Increased Th17-Related Cytokine Serum Levels in Patients With Multiple Polyps of Unexplained Origin

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OBJECTIVES: Most patients with multiple colonic polyps do not have a known genetic or hereditary origin. Our aim was to analyze the presence of inflammatory cytokines and levels of glucose, insulin, and C-reactive protein (CRP) in patients with multiple colonic polyps.

METHODS: Eighty-three patients with 10 or more adenomatous or serrated polyps and 53 control people with normal colonoscopy were included. Smoking habits were registered, and glucose, CRP, and basal insulin in the serum/blood were measured. Quantification of IL-2, IL-4, IL-6, IL-10, IL-11, IL-17A, and IL-23 cytokine levels in the serum was performed by a high-sensitivity enzyme-linked immunosorbent assay.

RESULTS: Smoking and diabetes were more prevalent in those with colonic polyps than in the control people (67% vs 16%, $P = 0.001$; 11% vs 2%, $P = 0.048$). In addition, the cytokine serum levels were higher, i.e., IL-2 ($P = 0.001$), IL-4 ($P = 0.001$), IL-6 ($P = 0.001$), IL-17A ($P = 0.001$), IL-23 ($P = 0.014$), and CRP ($P = 0.003$). Adjusting for sex, smoking, and diabetes in a multivariate analysis, IL-2, IL-4, IL-6, IL-17A, and IL-23 remained independently elevated in cases with multiple polyps.

DISCUSSION: These results indicate that immune responses mediated by Th17 cells may be involved in the pathogenesis of multiple colonic polyps.

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INTRODUCTION

A phenotype of multiple colonic polyps is a frequent finding in fecal immunochemical test–based colorectal cancer (CRC) screening programs. Some of these cases are due to germline mutations in either APC or MUTYH genes; such mutations are found in no more than 20%–30% of all cases (1–4). However, in most cases of attenuated polyposis, no genetic cause for this phenotype is found, and these multiple colonic polyps are of unknown origin. In these cases, an attenuated polyposis could be due to the involvement of other unknown high predisposition genes or potentially related to environmental factors (5).

The potential risk factors for this multiple colonic polyp phenotype may include those that have been previously described as

risk factors for the development of sporadic adenomas or serrated polyps, such as obesity, smoking, metabolic syndrome, or other factors related to increased inflammatory response. One of the most relevant immunological pathways sustaining chronic inflammation consist of the differentiation and expansion of the adaptive T helper (Th)17 response. An increase of Th17 cell sub-lineage and IL-17/IL-23 levels has been linked to obesity (6,7) and smoking (8). This proinflammatory route is triggered and stabilized after microenvironment upregulation of interleukin (IL)-6 and IL-23 concentrations, respectively. Although IL-23 and the Th17 main cytokine product IL-17 have been described to importantly contribute to colorectal tumorigenesis and inflammation-related cancer (9), these cytokine levels have not

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been evaluated so far in the serum of patients with multiple colonic polyps. Because these proinflammatory cytokines activate and perpetuate intracellular signaling through STAT3 and nuclear factor κ B (10), which are the 2 major factors linking inflammation to cancer, their serum concentration increment would suggest an explanation for tumor development through a polarized Th17 sustained response in these patients. In fact, patients with an attenuated polyposis are at increased risk of developing CRC compared with the general population (11). The aim of this study was to analyze the association between serum inflammatory cytokines and the presence of the phenotype of multiple colonic polyps of unknown origin.

MATERIALS AND METHODS

Study population

An attenuated polyposis was defined as the presence of 10–99 synchronous adenomatous or serrated polyps at endoscopy. Patients came from the EPIPOLIP registry, a nationwide multicenter registry that investigated the causes of multiple colonic polyps and incorporated patients from 24 Spanish hospitals (12). Patients diagnosed with inflammatory bowel disease or those with hyperplastic rectosigmoid polyps as the only finding were not included in the registry. For this particular substudy, patients were prospectively recruited when fulfilling the inclusion criteria, and a blood sample was obtained at the time of inclusion, after colonoscopy and polypectomy. Patients with APC or MutYH germline pathogenic variants were excluded, and inclusion of patients with variants of uncertain significance in APC and MUTYH was allowed. Healthy controls were recruited at the Endoscopy Unit of the Hospital General Universitario de Alicante from subjects undergoing a colonoscopy because of symptoms or a positive fecal immunochemical test with a normal result in this colonoscopy. The exclusion criteria for both cases and controls were a history of CRC, inherited CRC syndromes, such as the Lynch syndrome and familial adenomatous polyposis, previous intestinal resection, and concurrent chronic bowel disorders, such as inflammatory bowel disease.

Clinical and environmental risk factors

Information about age, sex, number, location, size and histological type of polyps, body mass index, smoking habit (active and former smokers), and diagnosis of diabetes mellitus (DM) was obtained at recruitment. In addition, at inclusion, fasting serum levels of glucose and insulin were collected in cases and controls. Glucose was measured by an enzymatic method by using hexokinase (Roche/Hitachi, Mannheim, Germany), Cobas 8000. A homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the formula $(\text{Glucose} \times \text{Insulin})/405$.

Polyps

Adenomatous polyps included tubular, tubulovillous, and villous adenomas. Serrated polyps included hyperplastic polyps, sessile serrated polyps, and traditional serrated adenomas (13). Serrated polyposis syndrome (SPS) was diagnosed when the WHO criteria for this disease were fulfilled (13). Patients with an attenuated polyposis were classified into 2 groups: adenomatous and serrated. Adenomatous polyposis was considered when $>50\%$ of the polyps were adenomatous. Serrated polyposis was considered when $>50\%$ of the polyps were serrated. SPS was considered if the patients met the WHO criteria for this disease (13).

Serum inflammatory markers

Blood samples were obtained in cases and controls for measurement of serum inflammatory markers. Serum levels of the human inflammatory markers IL-2 and TNF- α (as markers of early inflammation and lymphocyte activation, respectively), interferon gamma (IFN- γ) (as a Th1 marker), IL-4 (as a marker), IL-6, IL-23, and IL-17 (as Th17 markers), and regulatory IL-10 were determined by the commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits using standard protocols. The levels of IL-2, IL-4, IL-6, IL-10, IL-11, IL-23, and IFN- γ were determined by the high-sensitivity ELISA kits, following the manufacturer's protocol (Abcam, Cambridge, United Kingdom). Serum IL-17A was detected using a high-sensitivity ELISA (#BMS2017HS, eBioscience, Vienna, Austria) and TNF- α (BMS223HS, Bender MedSystems GmbH, Vienna, Austria). Serum C-reactive protein (CRP) levels were measured using an *in vitro* immunoturbidimetric method (Roche/Hitachi), Cobas 800.

Statistical analyses

Data analyses were performed with SPSS software (SPSS 19.0, Chicago, IL). Continuous variables were reported as the mean, SD, and interquartile range (P25–P75). Normal distribution of continuous variables was evaluated using the Kolmogorov–Smirnov test. Frequencies or percentages were used to report categorical variables. Differences between samples were determined with the Mann–Whitney *U* test for nonparametric quantitative data and the Student *t* test for parametric data. The χ^2 method for categorical data followed by the Yates correction or Fisher exact test was used to analyze statistical differences between the groups. We included univariate and multivariate linear regression models to determine the association between factors, such as smoking, DM, and belonging to the case or control group. Both analyses were performed after adjusting for the sex of patients. In addition, variables found to be significant in the univariate analysis were included in the multivariate analysis. Both univariate and multivariate models were considered statistically significant at $P < 0.05$.

RESULTS

Descriptive characteristics of the multiple colonic polyps of unknown origin phenotype in patients and controls

The study group included 83 patients with an endoscopic diagnosis of attenuated polyposis and 52 controls with normal colonoscopy. The descriptive data of case-control individuals are summarized in Table 1. We included 71 men (86%) and 12 women (14%) in the case group and 42 men (81%) and 10 women (19%) in the control group ($P = 0.481$). A total of 55 cases (66%) were smokers compared with 14 (27%) controls ($P = 0.001$). Moreover, 9 cases (11%) had DM, and only 1 control (2%) showed this condition ($P = 0.048$). There were no differences in mean HOMA-IR and mean body mass index between cases and controls (4.3 ± 5.2 vs 3.2 ± 1.8 , $P = 0.083$; and 27.6 ± 4.8 vs 28.7 ± 3.7 , $P = 0.255$, respectively).

Inflammatory markers

In the univariate analysis, several inflammatory markers were associated with the presence of multiple colonic polyps. Serum concentrations of IL-2 (2.5 vs 0.2 pg/mL; $P = 0.001$), IL-4 (4.2 vs 0.07 pg/mL; $P = 0.001$), IL-6 (3.5 vs 0.5 pg/mL; $P = 0.001$), IL-17A (1.5 vs 0.3 pg/mL; $P = 0.001$), IL-23 (6.4 vs 2.0 pg/mL; $P = 0.014$), and CRP (4.5 mg/L vs 0.2 mg/L; $P = 0.001$) were significantly increased in patients with polyposis compared with controls. None

Table 1. Descriptive characteristics of patients with multiple colonic polyps and controls

	Cases (n = 83)	Controls (n = 52)	P
Age, mean ± SD (yr)	59.98 ± 13.6	61.24 ± 6.67	0.443
Sex, % (n)			
Men	85.5% (71)	81% (42)	0.481
Women	14.5% (12)	19% (10)	
Smokers, % (n)	66% (55)	26.5% (14)	0.001 ^a
Diabetes mellitus, % (n)	11% (9)	2% (1)	0.048 ^a
HOMA-IR, mean ± SD	4.3 ± 5.2	3.2 ± 1.8	0.083
BMI, mean ± SD (kg/m ²)	27.6 ± 4.8	28.7 ± 3.7	0.255
BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance.			
^a Results are statistically significant.			

of the other factors (IL-10, IL-11, TNF- α , and IFN- γ) were related to the development of colon polyps in this study (Table 2).

In the multivariate analysis, after adjusting for sex, smoking, and history of DM, only having attenuated polyposis was independently associated with an increase in inflammatory markers (Table 3). All the factors that were significantly associated with polyp development in the univariate analysis were identified as independent predictors in the linear regression analysis: IL-2 ($P < 0.001$), IL-4 ($P < 0.001$), IL-17A ($P < 0.001$), IL-6 ($P < 0.001$), IL-23 ($P = 0.001$), and CRP ($P = 0.003$). The relationship between multiple colonic polyps and the Th17 pathway can be seen in Figure 1.

No correlation was found for IL-17A with IL-2 or for IL-4 and IL-23 with IL-2 and IL-4. By contrast, IL-17A and IL-23 did show a correlation ($\rho = 0.419$; $P < 0.001$).

Classification and characteristics of the polyps and their relationship with inflammatory markers

Adenomatous polyposis was diagnosed in 46 cases (55%) and serrated polyposis in 37 cases (45%). Eight patients met the criteria for SPS. We found a mean of 23 (SD 13) polyps per person, and the mean of both serrated and adenomatous polyps per person was 9 (SD 12). A total of 25 patients (30%) showed more than 10 adenomas. Characteristics of polyps can be seen in Table 4. Concentrations of the investigated cytokines were not different between adenomatous and serrated polyposis except for TNF- α , the concentration of which was 5-fold higher in the serrated group (0.5 vs 2.7, $P = 0.010$) (Table 5).

DISCUSSION

The main result of our study is that, when adjusted by smoking and presence of DM, several soluble inflammatory markers associated with different immune activities are independently related to the existence of an attenuated polyposis of unknown origin. Specifically, a Th17-related cytokine response is significantly increased in patients with multiple colonic polyps. As these patients remain “genetic orphans” because no constitutional mutation can be demonstrated (14), our findings may be hypothesis generating for future studies aimed at linking sustained inflammatory pathways to the development of multiple colonic polyps and open the possibility of investigating the potential immunomodulatory treatments

for this condition. This phenotype is uncommon in primary screening colonoscopy but increasingly found in FIT-based CRC screening individuals.

The relationship between inflammation and neoplasia has long been supported, and epidemiological, pharmacological, and genetic evidence provide solid support that inflammation can increase cancer risk and promote tumor progression (15). Thus, studies have shown that individuals with chronic inflammatory bowel disease have a higher risk of CRC than those without such a condition (16,17). Animal models with continuous inflammatory conditions are predisposed to CRC development (18). In the case of colitis-associated cancer, it was suggested that chronic inflammation and colonic injury might directly cause DNA alterations (19,20). Now, it is generally accepted that up to 25% of human malignancies are related to chronic inflammation, including viral and bacterial infections (21).

There are studies supporting the relationship of CRP with CRC (22–24), and a positive association between CRP and the prevalence of large adenomas has been reported. Although CRP constitutes a solid soluble marker of inflammation, CRP is an acute-phase inflammatory marker and its utility in identifying inflammatory pathways is very limited. On the contrary, different cytokines associated with specific inflammatory responses may be of help in this regard. Several studies have shown that colonic adenomas exhibit upregulation of IL-23 and IL-17 expression relative to adjacent nontumor tissue (25,26). Furthermore, serum IL-17A levels are elevated in patients with CRC compared with healthy individuals (27), and increased IL-6 has been involved in the development of sporadic CRC and colitis-associated cancer (28) and associated with increased CRC risk (29).

In the present study, although CRP is also elevated, Th17-associated IL-6, IL-23, and IL-17 cytokine levels are independently related to the existence of an attenuated polyposis of unknown origin. These results point to the differentiation and proliferation of an adaptive Th17 cell response in this phenotype. In fact, IL-2 increased levels, evaluated as a marker of

Table 2. Cytokines and inflammatory marker values in cases and controls

	Cases (n = 83)	Controls (n = 52)	P
IL-17A	1.5 ± 1.4 (0–2)	0.3 ± 0.9 (0–0)	<0.001 ^a
IL-23	6.4 ± 4.9 (2.4–9.4)	2.0 ± 5.9 (0–2.6)	<0.001 ^a
IL-11	8.3 ± 15.0 (0–7.7)	12.6 ± 24.4 (0–17.4)	0.441
IL-10	4.3 ± 5.9 (0–7)	4.3 ± 7.7 (1.5–3.0)	0.409
IL-2	2.5 ± 3.9 (0–4.1)	0.2 ± 0.9 (0–0)	<0.001 ^a
IL-4	4.2 ± 6.4 (0–7.8)	0.07 ± 0.3 (0–0)	<0.001 ^a
IL-6	3.5 ± 5.1 (0–5.4)	0.5 ± 1.0 (0–0)	<0.001 ^a
CRP	4.5 ± 8.3 (0–8.4)	0.2 ± 0.2 (0–0)	<0.001 ^a
TNF- α	0.9 ± 3.3 (0–0)	1 ± 4.6 (0–0)	0.509
IFN- γ	1.3 ± 3.6 (0–0)	1.3 ± 5.6 (0.0)	0.792

All cytokine and inflammatory marker levels are presented as mean ± SD. Interquartile ranges are expressed between parentheses.

^aResults are statistically significant using the Mann–Whitney U test.

CRP, C-reactive protein; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Table 3. Linear regression analysis of inflammatory markers (IL-2, IL-4, IL-6, IL-17A, IL-23, and CRP) and their association with the case-control group adjusted by sex, smoking, and presence of diabetes mellitus

Variables in multivariate analysis		P-value	Coefficient β	95% Confidence interval
IL-17A	Attenuated polyposis	<0.001	1.213	0.693 to 1.733
	Sex	0.616	0.168	-0.494 to 0.830
	Smoking	0.400	-0.214	-0.715 to 0.288
	Diabetes mellitus	0.471	0.315	-0.548 to 1.179
IL-23	Attenuated polyposis	0.001	3.666	1.569 to 5.764
	Sex	0.850	0.254	-2.410 to 2.919
	Smoking	0.066	-1.916	-3.957 to 0.125
	Diabetes mellitus	0.517	1.167	-2.393 to 4.727
IL-2	Attenuated polyposis	<0.001	2.529	1.242 to 3.815
	Sex	0.418	0.668	-0.960 to 2.295
	Smoking	0.969	0.025	-1.224 to 1.273
	Diabetes mellitus	0.511	0.723	-1.446 to 2.892
IL-4	Attenuated polyposis	<0.001	4.254	2.260 to 6.249
	Sex	0.141	1.887	-0.636 to 4.411
	Smoking	0.493	0.673	-1.262 to 2.608
	Diabetes mellitus	0.992	0.017	-3.346 to 3.380
IL-6	Attenuated polyposis	<0.001	3.542	1.877 to 5.208
	Sex	0.760	-0.333	-2.481 to 1.816
	Smoking	0.236	0.970	-0.642 to 2.582
	Diabetes mellitus	0.532	0.888	-1.917 to 3.694
CRP	Attenuated polyposis	0.003	4.042	1.358 to 6.725
	Sex	0.803	-0.429	-3.838 to 3.979
	Smoking	0.268	-1.468	-4.079 to 1.144
	Diabetes mellitus	0.489	1.597	-2.957 to 6.151

CRP, C-reactive protein; IL, interleukin.

T-cell activation, would support this assumption. However, and despite the solid data on 3 different cytokines of the Th17 pathway, the study design does not allow us to prospectively evaluate patients' antigen-presenting cells in coculture with

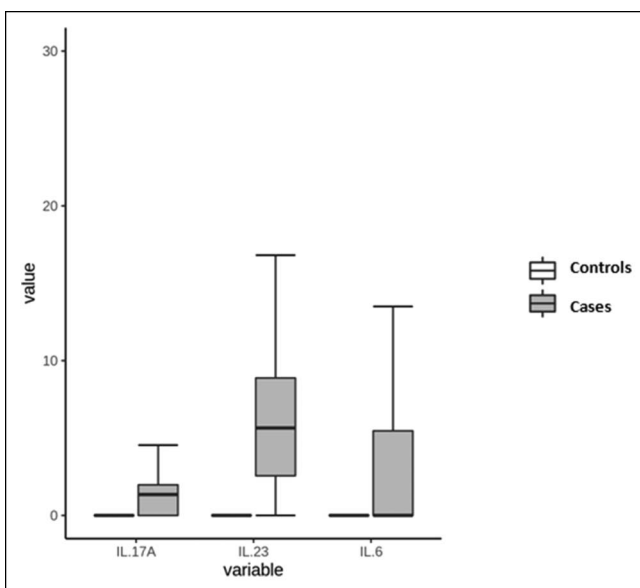


Figure 1. Differences in the serum concentrations of the Th17 pathway interleukins between cases and controls: IL-17A (1.6 pg/mL vs 0.3 pg/mL), IL-23 (22.1 pg/mL vs 17.7 pg/mL), and IL-6 (3.5 pg/mL vs 0.5 pg/mL).

naive T cells for functional characterization and immunophenotyping. This limitation, along with the increment in serum levels of IL-4, an indirect marker of a Th2 profile, prevents us from discarding the differentiation of other inflammatory pathways and their coexistence with the Th17 cascade. Supporting this, IL-4 is overexpressed in early events of CRC development, including hyperplastic polyps, adenomas, and serrated adenomas (30), and several experimental studies indicate an IL-4 protumorigenic effect on CRC (31–33).

These results describe for the first time the adaptive Th17-associated cytokine upregulation in serum of patients with multiple colonic polyps. Although further studies on *in vitro* Th differentiation are needed, implications in immunomodulatory strategies may be experimentally considered from this start point. Whether biological therapies targeting IL-23 and related immune checkpoints may be worth testing in preclinical models.

However, several environmental factors that could lead to a proinflammatory state have been associated with colorectal adenoma or cancer. In patients with type 2 diabetes, epidemiological studies show an increased risk of CRC (34). Research suggests that cigarette smoking promotes the development of polyps in the colon, especially serrated polyps (35). In an analysis of 42 studies, researchers found that current smokers were twice as likely as nonsmokers to develop colon polyps (36). Obesity is also an established risk factor for colorectal neoplasia and has been postulated to promote cancer development through inflammation-related mechanisms (37,38). Studies show that patients with DM (39) and smokers (40,41) have high CRP levels, and these factors may contribute jointly to the increased risk of adenoma. Moreover,

Table 4. Polyp characteristics

Polyp characteristics	
Mean no. of polyps	23
Polyp number, mean \pm SD	
Proximal colon	7.7 \pm 8.2
Distal colon	11.7 \pm 10.5
Location, %	
Proximal colon	40
Distal colon	60%
Adenomatous polyps	
Predominant type of polyps, % (n)	55% (46)
Mean no. of adenomatous polyps	9
Adenomatous polyps, %	
Rectosigmoid	29.6
Proximal to rectosigmoid	70.4
Patients with >10 adenomas, % (n)	30.1% (25)
Serrated polyps	
Predominant type of polyps, % (n)	45% (37)
Mean no. of serrated polyps	9
Serrated polyps, %	
Rectosigmoid	50.5
Proximal to rectosigmoid	49.5
Serrated polyposis syndrome	21.6% (8)

recent data also suggest that most of these environmental risk factors are shared by both adenomas and serrated polyps (35). In our study, we found that the increase in proinflammatory cytokines is independent of the highest proportion of DM and smokers found

Table 5. Association between polyposis type (adenomatous or serrated) and cytokine concentrations (IL-2, IL-4, IL-6, IL-17A, IL-23, IL-11, IL-10, TNF- α , IFN- γ , and CRP)

Polyposis type	Adenomatous	Serrated	P
IL-17A	1.5 \pm 1.5	1.5 \pm 0.9	0.879
IL-23	6.1 \pm 4.6	7.3 \pm 6.1	0.466
IL-6	3.4 \pm 4.6	4 \pm 6.7	0.741
IL-11	8.8 \pm 16	6.5 \pm 11.1	0.502
IL-10	3.8 \pm 4.4	6 \pm 10	0.409
IL-2	2.2 \pm 3.2	4 \pm 5.8	0.237
IL-4	3.7 \pm 5.4	6.2 \pm 9.1	0.299
TNF- α	0.5 \pm 1.3	2.3 \pm 4.5	0.010 ^a
IFN- γ	1.3 \pm 3.7	1.3 \pm 3.7	0.954
CRP	4.3 \pm 8	5.1 \pm 9.6	0.768

All cytokine and inflammatory marker levels are presented as mean \pm SD. CRP, C-reactive protein; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

^aResults are statistically significant.

in our cohort. This finding does not rule out a causal relationship between these environmental factors and the increase of inflammatory cytokines, but it suggests that the highest frequency of these environmental factors is not the only explanation for these findings. Our results highlight the need for more research to understand the cross talk between environmental factors, inflammation, and development of colonic polyps and CRC. We have also to remark the high proportion of men found in our cases with multiple colonic polyps who could be related to the highest prevalence of CRC and colonic polyps in men, but it is also possible that men could be more affected by this cytokine dysregulation found in our study.

Our study has some limitations. It is important to state the heterogeneity of multiple colonic polyps in our study, with patients with both adenomatous and serrated polyps indistinctly included. This study was unable to define whether there are phenotypical differences in interleukin activation. In addition, we have not made any longitudinal analysis in our patients aimed to support ongoing stimuli of cytokine activation in the genesis of attenuated polyposis phenotype. However, our results are plausible and robust, with cases that were prospectively recruited and controls with confirmed normal colonoscopy. Moreover, we cannot rule out the potential role of unknown genetic factors in the genesis of this phenotype, with potential germline mutations not yet found or potential genetic predisposition to producing T cells that elaborate different patterns of cytokines.

In summary, aside from the known genetic role of approximately 20%–30% of cases of attenuated polyposis, environmental factors may play a role in this phenotype, leading to immunological responses and bowel inflammation. An increased Th17-related immune response associated with smoking, DM, or metabolic syndrome could jointly contribute to the increased risk for the development of multiple colonic polyps. New studies should be developed that aim to validate these results in different cohorts and more clearly define the role of inflammation and the effect of modulation of the immune response on the development of this phenotype.

CONFLICTS OF INTEREST

Guarantor of the article: Rodrigo Jover.

Specific author contributions: Conception and design: M.A., R.J., and R.F.; development of methodology: M.A., E.H.-I., R.F., and R.J.; acquisition of data: M.A., E.H.-I., C.M., P.G., F.R.-M., J.C., L.d.-C., J.-C.M.G., A.H.-T., D.N.-P., and R.J.; analysis and interpretation of data: R.F., P.G., C.M.-C., M.A., M.G.-C., M.J., E.H.-I., O.M., A.M.-R., and R.J.; writing, review, and/or revision of the manuscript: all authors; and study supervision: R.J.

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Study Highlights

WHAT IS KNOWN

- ✓ Multiple colonic polyps are frequently found, especially in fecal immunochemical test–based colorectal cancer screening programs. In most cases, there are no genetic causes for this phenotype, and these cases are of unknown origin.
- ✓ Environmental factors, such as obesity, insulin resistance, or smoking, can activate proinflammatory cytokines, resulting in chronic inflammation and neoplasia development.

WHAT IS NEW HERE

- ✓ Proinflammatory Th17-related cytokines are significantly increased in serum of patients with multiple colonic polyps of unknown origin.
- ✓ Our findings can be hypothesis generating for future studies that aim to link the differentiation of this proinflammatory adaptive immune response to the development of multiple colonic polyps and open the possibility of investigating the potential immunomodulatory treatments for this condition.

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