Original Investigation

Isotretinoin Exposure and Risk of Inflammatory Bowel Disease

Shadi Rashtak, MD; Shahryar Khaleghi, PharmD; Mark R. Pittelkow, MD; Joseph J. Larson, BS; Brian D. Lahr, MS; Joseph A. Murray, MD

IMPORTANCE Isotretinoin is the standard treatment for refractory severe nodulocystic acne. A true association between prior isotretinoin use and development of inflammatory bowel disease (IBD) is uncertain. Addressing the reality of this association is important in decision making for both the clinician and the patient when isotretinoin treatment is indicated.

OBJECTIVE To assess the risk of IBD mainly in patients with acne with and without isotretinoin exposure.

DESIGN, SETTING, AND PARTICIPANTS In this retrospective, single-center study, the electronic medical records of patients who were primarily seeking acne treatment were reviewed for isotretinoin exposure. *International Classification of Diseases, Ninth Revision (ICD-9)* codes were used to search for IBD diagnosis. participants included 1078 patients from 1995 to 2011, with isotretinoin referenced in their medical records, and who had ongoing local medical care defined as having had a serum sample collected between 2006 to 2011 for any reason while an Olmsted County, Minnesota, resident at the time of serum sample collection.

EXPOSURES The exposed group included the patients with confirmed prior isotretinoin exposure (n = 576), and the nonexposed group were defined as patients who never received isotretinoin or received it after the diagnosis of IBD (n = 502).

MAIN OUTCOMES AND MEASURES Risk of IBD among isotretinoin-exposed vs nonexposed patients.

RESULTS Both groups were comparable by race, prior systemic antibiotic use, and systemic tetracycline use. Inflammatory bowel disease developed less frequently in the isotretinoin-exposed group vs the nonexposed group (0.9% vs 2.6%; $P = .03$; unadjusted odds ratio [OR], 0.33; 95% CI, 0.12-0.93; $P = .04$). The negative association between isotretinoin exposure and IBD remained after adjusting for sex (OR, 0.28; 95% CI, 0.10-0.80; $P = .02$) and for sex and nonacne indication (OR, 0.28; 95% CI, 0.10-0.79; $P = .02$).

CONCLUSIONS AND RELEVANCE Our study did not show an increased risk of IBD with prior isotretinoin use. If anything, the risk seemed to be decreased. Although these results may be due to chance given the small number of IBD cases, the anti-inflammatory and immune-modulating effects of isotretinoin may be worth exploring.

JAMA Dermatol. 2014;150(12):1322-1326. doi:10.1001/jamadermatol.2014.1540
Published online September 10, 2014.

Author Affiliations: Department of Dermatology, Mayo Clinic College of Medicine, Rochester, Minnesota (Rashtak); Department of Immunology, Mayo Clinic College of Medicine, Rochester, Minnesota (Khaleghi); Department of Dermatology, Mayo Clinic College of Medicine, Scottsdale, Arizona (Pittelkow); Department of Biomedical Statistics and Informatics, Mayo Clinic College of Medicine, Rochester, Minnesota (Larson, Lahr); Department of Gastroenterology, Mayo Clinic College of Medicine, Rochester, Minnesota (Murray).

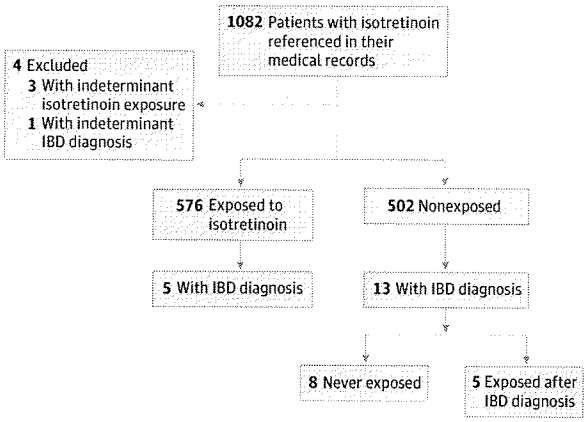
Corresponding Author: Joseph A. Murray, MD, Department of Gastroenterology, Mayo Clinic College of Medicine, 200 First St SW, Rochester, MN 55901 (murray.joseph@mayo.edu).

Isotretinoin (13-cis-retinoic acid) is a synthetic vitamin A derivate that is standard treatment for recalcitrant severe nodulocystic acne. Isotretinoin binds to and activates nuclear retinoic acid receptors, thereby regulating cell proliferation and differentiation. A possible association between isotretinoin exposure and inflammatory bowel disease (IBD) has been a subject of debate in the literature.

The original suspicion regarding a potential link between isotretinoin exposure and IBD came from sporadic case reports.[1-4] Later, 2 large case-control studies focused on addressing this issue. One of these studies[5] found an association between prior isotretinoin exposure and development of ulcerative colitis, whereas the other[6] showed no difference in isotretinoin use among the patients with IBD vs controls. Neither study controlled for potential confounding factors, such as acne or systemic antibiotic use. This is particularly important because a recent population-based cohort study suggested an association between IBD and acne and not with isotretinoin therapy per se.[7]

In our retrospective cohort study, we aimed to compare the risk of IBD among a community-based convenience sample of patients who were exposed to isotretinoin vs those for whom isotretinoin treatment was considered or discussed but who were not administered isotretinoin or took it after their diagnosis of IBD. Because the main indication for isotretinoin use is unresponsive severe acne, we used these criteria for patient selection to reduce differences between the exposed and nonexposed groups in terms of presence of acne or severe acne and receiving prior acne treatments, such as systemic antibiotics.

Figure. Study Flow Diagram of Patients



The number of patients who met the inclusion and exclusion criteria are shown. IBD indicates inflammatory bowel disease.

Methods

Patient Population

The study was approved by the Mayo Clinic institutional review board. The study population was selected from patients who had access to medical care defined as having had at least 1 blood test performed at the Mayo Clinic for any reason between June 2006 and June 2011 and who were resident of Olmsted County, Minnesota, at the time of serum sample collection. The electronic medical records of these patients were searched for the word "isotretinoin" and all its brand names (Accutane, Sotret, Claravis, and Amnesteem) using Data Discovery and Query Builder (DDQB) toolset, a Mayo/IBM Collaboration product.

DDQB is a unique text search engine that allows searching for specific words and phrases within all the electronic records, including clinical notes, hospital notes, diagnosis, medications, and so on. DDQB automatically excludes federal medical center patients; patients declining research authorization; patients flagged with a security, legal, or administration stop; patients who do not wish to be contacted; and nonvalid patients (eg, nonreal patients used for testing purposes).

Using the DDQB search toolset, we identified a total of 1082 patients who had a mention of isotretinoin or its brand names in their medical records between June 1, 1995, and June 1, 2011. The medical records of all the 1082 patients were then reviewed manually. Data on patients' demographics, isotretinoin exposure, number of isotretinoin courses, start and end dates of treatment, indication for treatment, and antibiotic use were abstracted from the medical records. Patients were considered to have exposure to systemic antibiotics or systemic tetracyclines (eg, tetracycline, doxycycline, minocycline) only if they used these medications for the purpose of acne treatment. Short-term antibiotic exposures owing to other reasons were not considered positive for systemic antibiotic or tetracycline use. Because the number of patients who did not take antibiotics for acne was very small, we grouped these patients and those who had missing information about antibiotic exposure together.

Routinely at the Mayo Clinic, patients who are treated with isotretinoin for their acne are placed on a cumulative dose of 120 to 150 mg/kg over a 5-month period. For patients who had a remote history of isotretinoin use and the exact dates of treatment were unknown for them, we considered a 5-month course of treatment starting on January 1 of the most probable year. After reviewing the medical records, 3 of 1082 patients were excluded because their prior exposure to isotretinoin was unclear.

To determine the presence of IBD in this population, we then searched *International Classification of Diseases, Ninth Revision, Clinical Modification* (*ICD-9-CM*) codes for Crohn disease and ulcerative colitis (*ICD-9-CM* codes containing 555 and 556) using the same DDQB search tool. Twenty-five patients were originally identified based on the presence of at least 1 of these codes, while the remaining 1054 patients lacking a code were assumed to not have IBD. After careful review of the medical records on those 25 patients, 18 patients were found to truly have IBD, 6 patients were determined to not have IBD, and for 1 patient the diagnosis of IBD was unclear and therefore this patient was excluded from the analysis. Of these 18 patients, 5 were receiving isotretinoin prior to their IBD diagnosis, 8 had never received isotretinoin, and 5 were administered isotretinoin after the diagnosis of IBD (**Figure**). To ensure that the cases captured using *ICD-9-CM* codes were inclusive of all IBD cases,

Table 1. Patient Characteristics

Characteristic	Patients, No. (%)			
	Total (N = 1078)	Nonexposed (n = 502)	Isotretinoin-Exposed (n = 576)	*P* Value[a]
IBD diagnosis[b]	18 (1.7)	13 (2.6)	5 (0.9)	.03
Age at diagnosis, mean (SD), y	27.4 (8.7)	25.6 (8.5)	32.1 (8.5)	NA
Sex (female)	714 (66.2)	368 (73.3)	346 (60.1)	<.001
Race[b]				
White	957 (92.3)	444 (91.5)	513 (92.9)	.40
Nonwhite	80 (7.7)	41 (8.5)	39 (7.1)	
Indication				
Acne	1014 (94.1)	458 (91.2)	556 (96.5)	<.001
Nonacne	64 (5.9)	44 (8.8)	20 (3.5)	
Systemic				
Antibiotic use	795 (73.7)	375 (74.7)	420 (72.9)	.51
Tetracycline use	729 (67.6)	344 (68.5)	385 (66.8)	.56
Family history of IBD	21 (1.9)	9 (1.8)	12 (2.1)	.73

Abbreviation: IBD, inflammatory bowel disease.

[a] *P* values derived from χ^2 test.

[b] Missing patients not included in denominator (n = 17 in nonexposed group, n = 24 in exposed group).

we also searched the medical history sections of all notes for the phrases "ulcerative colitis," "Crohn," and "inflammatory bowel." No additional patients with IBD were identified. We also searched the family history sections of the notes for the aforementioned phrases to identify patients with a possible family history of IBD. We then manually reviewed the medical records on this subset of patients in order to verify a positive personal or family history of IBD and assess if such history affected the decision to administer isotretinoin.

Statistical Analysis

Patient characteristics were summarized using descriptive statistics. Since treatment was not randomly allocated, each patient variable was assessed for an association with isotretinoin exposure (ie, group difference between exposed and nonexposed patients) using a χ^2 test. The effect of isotretinoin exposure on the outcome of IBD was estimated via logistic regression, with adjustment of potential confounding in the model from those factors found to be associated with exposure. An adjusted odds ratio (OR) (with 95% CI) is reported to convey the direction and magnitude of association between isotretinoin exposure and a subsequent IBD diagnosis, independent of adjusting covariates in the model. $P < .05$ was considered statistically significant. All analyses were performed using the SAS statistical software package (version 9.3; SAS Institute Inc).

Results

Of a total of 1078 patients who had a mention of isotretinoin in their medical record notes, 576 patients had true isotretinoin exposure, and 502 patients were nonexposed (497 were never exposed, and 5 were exposed after IBD diagnosis). **Table 1** summarizes the characteristics of both groups. Both isotretinoin-exposed and nonexposed groups were comparable by race, systemic antibiotic use, and prior use of the tetracycline family of antibiotics. The proportion with a family history of IBD was also comparable between the exposed and nonexposed groups (2.1% and 1.8%, respectively; $P = .73$).There were more females in the nonexposed group compared with isotretinoin-exposed group (73.3% vs 60.1%; $P < .001$). In addition, 3.5% of isotretinoin-exposed patients had indications other than acne compared with 8.8% in the nonexposed group ($P < .001$). These included rosacea (2.1% vs 1.6%), folliculitis (0.5% vs 1.1%), or discussion of isotretinoin use in the setting of dermatologic procedures (0.0% vs 1.7%) in the exposed vs nonexposed groups, respectively. Interestingly, a subsequent diagnosis of IBD was found in only 5 of 576 exposed patients (1 with Crohn disease and 4 with ulcerative colitis) compared with 13 of 502 nonexposed patients (3 with Crohn disease and 10 with ulcerative colitis) (0.9% vs 2.6%; $P = .03$). The negative association between isotretinoin exposure and IBD remained significant when adjusting for sex and nonacne indication ($P = .02$) (**Table 2**). From medical chart review of the 21 patients with a positive family history of IBD, there was no suggestion in the notes that any of the 9 nonexposed patients were withheld isotretinoin on the basis of their positive family history.

Discussion

In this retrospective cohort study, we showed that in a community-based convenience sample of patients who were mainly seeking acne treatment, exposure to isotretinoin was associated with a lower risk of IBD. To our knowledge, this is the first cohort study that demonstrates a negative association between isotretinoin and IBD development.

There is a controversy in the literature as to whether isotretinoin is associated or has any causal effect for ensuing IBD. Sporadic case reports mostly describe what seems to be merely colitis or enteritis during or after isotretinoin treatment.[1,2,4] Other studies have reported cases of definite IBD a few months after isotretinoin treatment, but they have questioned a true association between the 2.[3,8] All these reports, combined with the 85 possibly isotretinoin-attributed IBD cases reported to the US Food and Drug Administration,[9] represent about 100 patients among the several million isotretinoin users and more than a million patients with IBD in the United States.[6,10,11]

Some of the speculated mechanisms for development of IBD or simply colitis in this setting of isotretinoin use include induction of apoptosis, similar to what happens in the sebaceous glands, and inhibition of cell growth. These mechanisms are effective for acne treatment but could potentially result in disruption of mucosal epithelial cells, leading to ulceration and inflammation.[2,3,12] Yet, there are a few potential factors that might explain a coincidental positive association between isotretinoin exposure and development of IBD rather than a true relationship or causal effect. For example, the peak age for IBD diagnosis is young adulthood, the same age range during which most patients receive isotretinoin for their acne treatment.[3,8] Moreover, a few conditions, such as hidradenitis suppurativa or acne inversa, which coexist with Crohn proctocolitis could, in fact, be the indication for isotretinoin treatment.[8,13] It is also possible that isotretinoin could simply unmask a preexisting subclinical IBD that will then resolve on discontinuation of the medication.[9] A similar phenomenon can occur even with acne as occasionally patients develop a flare-up of their acne on initiation of isotretinoin therapy.[14] In addition, patients with subclinical symptoms of IBD may be inclined to seek medical attention after they become aware of a potential association with isotretinoin exposure.[9]

Following the reports of the cases mentioned herein, Guslandi[15] assessed prior isotretinoin use among a total of 306 consecutive patients with an established IBD diagnosis in an IBD clinic in Italy. Only 1 patient recalled taking an anti-acne medication a few years previously, but she was not able to confirm if it was isotretinoin. None of the others recalled taking isotretinoin or any of its commercial brands. This study was not published until about 4 years later (in 2007) owing to its negative results. Subsequently, 2 large case-control studies were conducted to address this issue. While 1 study[5] found an association with ulcerative colitis and prior isotretinoin use, the other population-based, matched, case-control study[6] did not find any association between the 2. Neither of these studies had adjusted for potential confounders, such as acne or prior acne treatments (eg, tetracycline class of antibiotics). This is despite the fact that both acne and the tetracycline family of antibiotics are shown to be possibly associated with IBD.[7,16]

More recently, a meta-analysis of a pool of 5 published and unpublished studies by Etminan et al[17] showed that there was no evidence that isotretinoin exposure could be linked to IBD. Among these studies, a case-control study from the French national health insurance data showed that isotretinoin was not associated with any increased risk for ulcerative colitis and was associated with even a decreased risk of Crohn disease.[18] Etminan et al[17] also referred to their unpublished case-control study of 20 237 cases of IBD and 60 136 controls, in which they found an OR of 0.62 (95% CI, 0.43-0.89) for the use of isotretinoin and IBD, indicating a protective effect.[19]

Given the rarity of IBD cases among isotretinoin users and controls, no cohort study had been performed until very recently. A very large retrospective population-based cohort studyin 2013 by Alhusayen et al,[7] in British Columbia, Canada, showed that, compared with over 1.5 million untreated individuals, there was no significant association between isotretinoin use and IBD among 46 922 isotretinoin-treated patients and 184 824 patients who received topical acne medication. However, in prespecified secondary analyses, they[7] found some evidence of IBD association with both the isotretinoin and the topical acne medications, concluding a possible association between IBD and acne itself, rather than treatment of acne. Interestingly, in their cohort, the risk of hospitalization for exacerbation of IBD was lower in patients who received isotretinoin compared with those who were treated with topical acne medications. To our knowledge, this is the only cohort study other than ours that has assessed the association between isotretinoin use and IBD, and, similarly, they found some protective effect regarding the exacerbation of IBD in isotretinoin users who had known IBD.

Table 2. Association of Isotretinoin Use and Inflammatory Bowel Disease Outcome From Logistic Regression

Model	Odds Ratio (95% CI)	*P* Value
Unadjusted	0.33 (0.12-0.93)	.04
Adjusted for sex	0.28 (0.10-0.80)	.02
Adjusted for sex and nonacne indication	0.28 (0.10-0.79)	.02

The strengths of our study include the community-based convenience sample and the cohort design of the study. Moreover, the selection criteria in this study of included only those medical records in which there was some reference to isotretinoin because this likely reduced sources of potential confounding and group imbalance (eg, acne history and use of tetracyclines) between isotretinoin-exposed and nonexposed patients. In addition, we manually reviewed all the notes in which there was a mention of IBD, Crohn disease, or ulcerative colitis in the medical or family history sections of the notes to ensure that the negative association between isotretinoin exposure and IBD was not because the drug was simply avoided in patients with disqualifying conditions (eg, a personal or family history of IBD). Using the aforementioned criteria, we achieved comparable groups in terms of family history of IBD and history of systemic antibiotic, and, specifically, the tetracycline family of antimicrobial usage among the 2 groups. Furthermore, factors that statistically were found to be different between groups were controlled for via regression adjustment in measuring the exposure effect.

Limitations of our study include the retrospective design of the study and small number of IBD cases among both isotretinoin-exposed and nonexposed groups. Although one might suspect that these results could be due to chance given the small number of IBD cases, anti-inflammatory and immunoregulatory effects of isotretinoin could have a potential role explaining this inverse association.

In vitro studies suggest that retinoic acid may mediate its anti-inflammatory effects in the gut through a transforming growth factor β-dependent inhibition of proinflammatory T_H17 response.[20] It is also proposed that retinoic acid could potentially prevent autoimmunity in the gut through its key role in induction of regulatory T-cells.[21] The immunomodulatory effects of isotretinoin through regulation of T_H1/T_H2 balance[22,23] and normalization of exaggerated Toll-like receptor-2 (TLR-2) expression are other potential mechanisms that can explain

the negative association with IBD. Activation of TLR-2 has been implicated in the pathogenesis of IBD.[24,25] It is shown that microbial components have a role in IBD pathogenesis by binding to TLRs and activating innate immune responses.[26] Interestingly, TLR-2 is expressed at higher levels on monocytes from patients with acne and is induced at significantly greater levels after stimulation of those monocytes with *Propionibacterium acnes* compared with normal controls. It is shown that in patients with acne, isotretinoin treatment substantially decreases monocyte TLR-2 expression and production of inflammatory cytokine in response to *P acnes,* and such effects are sustained for at least 6 months after discontinuation of the therapy.[27] This sustained decrease in expression of TLR-2 following isotretinoin therapy may explain the decreased IBD risk in the patient with acne treated with isotretinoin.

Conclusions

Our study indicates that isotretinoin is associated with a decreased risk of IBD development. Based on our study and review of previous studies, there is little evidence that isotretinoin has any causal effect for development of IBD. We believe that clinicians should not unnecessarily avoid prescribing this effective acne therapy for largely unfounded or meager associations with IBD.

ARTICLE INFORMATION

Accepted for Publication: May 26, 2014.

Published Online: September 10, 2014. doi:10.1001/jamadermatol.2014.1540.

Author Contributions: Dr Rashtak and Mr Larson had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Rashtak, Khaleghi, Pittelkow, Murray.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Rashtak, Pittelkow.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Larson, Lahr.
Administrative, technical, or material support: Murray.
Study supervision: Rashtak, Pittelkow, Murray.

Conflict of Interest Disclosures: Dr Murray is on the advisory board for Alvine Pharmaceuticals Inc and acts as a consultant for AMAG Pharmaceuticals, Entera Health Inc, Sonomaceuticals LLC, and BioLineRx. He also reports receiving grant support from Alba Therapeutics and Alvine Pharmaceuticals Inc not relevant to this study. No other disclosures are reported.

REFERENCES

1. Martin P, Manley PN, Depew WT, Blakeman JM. Isotretinoin-associated proctosigmoiditis. *Gastroenterology*. 1987;93(3):606-609.

2. Reniers DE, Howard JM. Isotretinoin-induced inflammatory bowel disease in an adolescent. *Ann Pharmacother*. 2001;35(10):1214-1216.

3. Passier JL, Srivastava N, van Puijenbroek EP. Isotretinoin-induced inflammatory bowel disease. *Neth J Med*. 2006;64(2):52-54.

4. Spada C, Riccioni ME, Marchese M, Familiari P, Costamagna G. Isotretinoin-associated pan-enteritis. *J Clin Gastroenterol*. 2008;42(8):923-925.

5. Crockett SD, Porter CQ, Martin CF, Sandler RS, Kappelman MD. Isotretinoin use and the risk of inflammatory bowel disease: a case-control study. *Am J Gastroenterol*. 2010;105(9):1986-1993.

6. Bernstein CN, Nugent Z, Longobardi T, Blanchard JF. Isotretinoin is not associated with inflammatory bowel disease: a population-based case-control study. *Am J Gastroenterol*. 2009;104(11):2774-2778.

7. Alhusayen RO, Juurlink DN, Mamdani MM, Morrow RL, Shear NH, Dormuth CR; Canadian Drug Safety and Effectiveness Research Network. Isotretinoin use and the risk of inflammatory bowel disease: a population-based cohort study. *J Invest Dermatol*. 2013;133(4):907-912.

8. Rolanda C, Macedo G. Isotretinoin and inflammatory bowel disease. *Am J Gastroenterol*. 2007;102(6):1330.

9. Reddy D, Siegel CA, Sands BE, Kane S. Possible association between isotretinoin and inflammatory bowel disease. *Am J Gastroenterol*. 2006;101(7):1569-1573.

10. Kappelman MD, Rifas-Shiman SL, Kleinman K, et al. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin Gastroenterol Hepatol*. 2007;5(12):1424-1429.

11. Wysowski DK, Swann J, Vega A. Use of isotretinoin (Accutane) in the United States: rapid increase from 1992 through 2000. *J Am Acad Dermatol*. 2002;46(4):505-509.

12. Prokop LD. Isotretinoin: possible component cause of inflammatory bowel disease. *Am J Gastroenterol*. 1999;94(9):2568.

13. Church JM, Fazio VW, Lavery IC, Oakley JR, Milsom JW. The differential diagnosis and comorbidity of hidradenitis suppurativa and perianal Crohn's disease. *Int J Colorectal Dis*. 1993;8(3):117-119.

14. Borghi A, Mantovani L, Minghetti S, Virgili A, Bettoli V. Acute acne flare following isotretinoin administration: potential protective role of low starting dose. *Dermatology*. 2009;218(2):178-180.

15. Guslandi M. Isotretinoin and inflammatory bowel disease. *Am J Gastroenterol*. 2007;102(7):1546-1547.

16. Margolis DJ, Fanelli M, Hoffstad O, Lewis JD. Potential association between the oral tetracycline class of antimicrobials used to treat acne and inflammatory bowel disease. *Am J Gastroenterol*. 2010;105(12):2610-2616.

17. Etminan M, Bird ST, Delaney JA, Bressler B, Brophy JM. Isotretinoin and risk for inflammatory bowel disease: a nested case-control study and meta-analysis of published and unpublished data. *JAMA Dermatol*. 2013;149(2):216-220.

18. Racine A, Cuerq A, Bijon A, et al. Isotretinoin and risk of inflammatory bowel disease: a French nationwide study. *Am J Gastroenterol*. 2014;109(4):563-569.

19. Etminan M. Study website. http://www.broadmedical.org/asset/1406-finalprogressreport-etminan2.pdf. Accessed July 28, 2014.

20. Muci dA D, Park Y, Kim G, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science*. 2007;317(5835):256-260.

21. Coombs JL, Siddiqui KR, Arancibia-Cárcamo CV, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med*. 2007;204(8):1757-1764.

22. Iwata M, Eshima Y, Kagechika H. Retinoic acids exert direct effects on T cells to suppress Th1 development and enhance Th2 development via retinoic acid receptors. *Int Immunol*. 2003;15(8):1017-1025.

23. Zhu YF, Hu JZ, Zhao PN, Liu LX, Li Y. All-transretinoic acid regulates Th1/Th2 balance in CD4+ T cells when GATA-3 is deficient. *Biomed Environ Sci*. 2013;26(9):774-777.

24. Walsh D, McCarthy J, O'Driscoll C, Melgar S. Pattern recognition receptors: molecular orchestrators of inflammation in inflammatory bowel disease. *Cytokine Growth Factor Rev*. 2013;24(2):91-104.

25. Szebeni B, Veres G, Dezsõfi A, et al. Increased expression of Toll-like receptor (TLR) 2 and TLR4 in the colonic mucosa of children with inflammatory bowel disease. *Clin Exp Immunol*. 2008;151(1):34-41.

26. Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun*. 1998;66(11):5224-5231.

27. Dispenza MC, Wolpert EB, Gilliland KL, et al. Systemic isotretinoin therapy normalizes exaggerated TLR-2-mediated innate immune responses in acne patients. *J Invest Dermatol*. 2012;132(9):2198-2205.