Diet Modifies Colonic Microbiota and CD4$^+$ T-Cell Repertoire to Induce Flares of Colitis in Mice With Myeloid-Cell Expression of Interleukin 23

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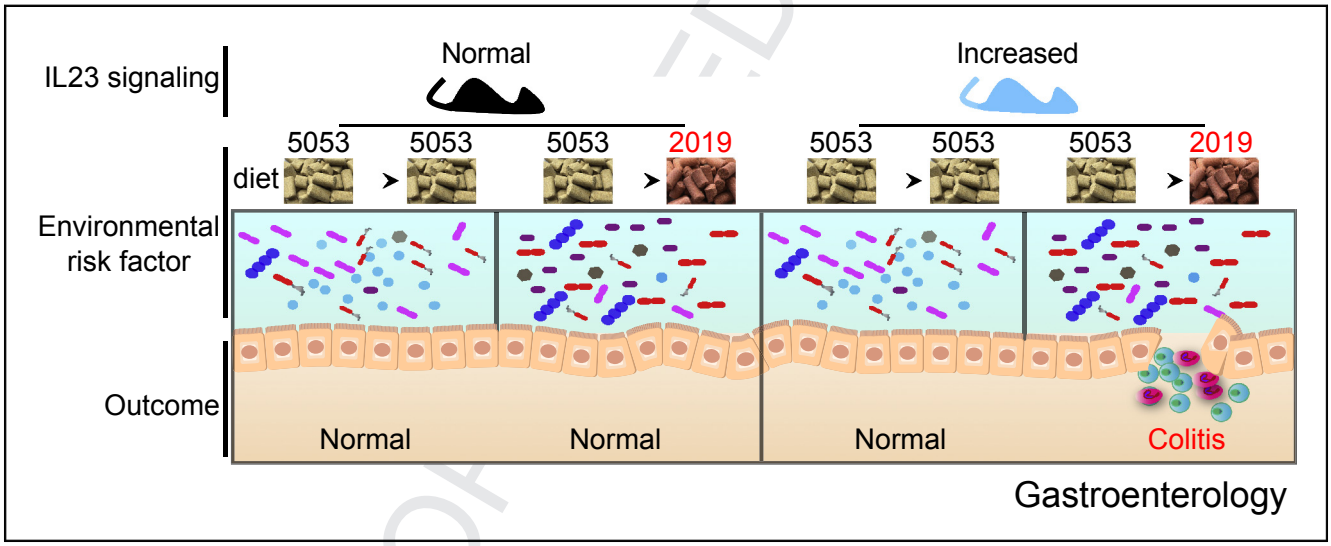

BACKGROUND & AIMS: Several studies have shown that signaling via the interleukin 23 (IL23) receptor is required for development of colitis. We studied the roles of IL23, dietary factors, alterations to the microbiota, and T cells in the development and progression of colitis in mice. **METHODS:** All mice were maintained on laboratory diet 5053, unless otherwise noted. We generated mice that express IL23 in CX3CR1-positive myeloid cells (*R23FR* mice) upon cyclic administration of tamoxifen dissolved in diet 2019. Diets 2019 and 5053 have minor differences in the overall composition of protein, fat, fiber, minerals, and vitamins. CX3CR1^{CreER} mice (*FR* mice) were used as controls. Some mice were given antibiotics, and others were raised in a germ-free environment. Intestinal tissues were collected and analyzed by histology and flow cytometry. Feces were collected and analyzed by 16S rDNA sequencing. Feces from C57/Bl6, *R23FR*, or *FR* mice were fed to *FR* and *R23FR* germ-free mice in microbiota transplant experiments. We also performed studies with *R23FR/Rag*$^{-/-}$, *R23FR/Mu*$^{-/-}$, and *R23FR/Tcrd*$^{-/-}$ mice. *R23FR* mice were given injections of antibodies against CD4 or CD8 to deplete T cells. Mesenteric lymph nodes and large intestine CD4$^+$ cells from *R23FR* or *FR* mice in remission from colitis were transferred into *Rag*$^{-/-}$ mice. CD4$^+$ cells were isolated from donor *R23FR* mice and recipient *Rag*$^{-/-}$ mice, and T-cell receptor sequences were determined. **RESULTS:** Expression of IL23 led to development of a relapsing–remitting colitis that was dependent on the microbiota and CD4$^+$ T cells. The relapses were caused by switching from the conventional diet used in our facility (diet 5053) to the diet 2019 and were not dependent on tamoxifen after the first cycle. The switch in the diet modified the microbiota but did not alter levels of IL23 in intestinal tissues compared with mice that remained on the conventional diet. Mesenteric lymph nodes and large intestine CD4$^+$ cells from *R23FR* mice in remission, but not from *FR* mice, induced colitis after transfer into *Rag*$^{-/-}$ mice, but only when these mice were placed on the diet 2019. The CD4$^+$ T-cell receptor repertoire of

Rag$^{-/-}$ mice with colitis (fed the 2019 diet) was less diverse than that from donor mice and *Rag*$^{-/-}$ mice without colitis (fed the 5053 diet) because of expansion of dominant T-cell clones. **CONCLUSIONS:** We developed mice that express IL23 in CX3CR1-positive myeloid cells (*R23FR* mice) and found that they are more susceptible to diet-induced colitis than mice that do not express IL23. The *R23FR* mice have a population of CD4$^+$ T cells that becomes activated in response to dietary changes and alterations to the intestinal microbiota. The results indicate that alterations in the diet, intestinal microbiota, and IL23 signaling can contribute to pathogenesis of inflammatory bowel disease.

Keywords: Cytokine; Immune Response; Inflammatory Bowel Disease Model; Microbiome.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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LIMITATIONS

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Crohn's disease (CD) and ulcerative colitis (UC) are 2 distinct phenotypic patterns of inflammatory bowel disease (IBD) affecting over 1.5 million people in Europe and almost 1 million people in North America.[1] At the turn of the 21st century, IBD has become a global disease, with newly industrialized countries now facing rising incidence, analogous to trends seen in the Western world during the latter part of the 20th century.[2] IBD is associated with morbidity, mortality, and substantial costs to the health care system. Both CD and UC are characterized by periods of asymptomatic remission interrupted by episodes of symptomatic disease flares or exacerbations. Although its exact cause is unknown, IBD seems to be due to a combination of genetic predisposition and environmental factors.[3–6] Genome-wide association studies identified polymorphisms in several genes, including in the interleukin (IL) 23 receptor (IL23R).[7,8] IL23 is a heterodimeric cytokine formed by the IL23-specific p19 subunit and the IL12p40 subunit. IL23 interacts with cells that co-express the IL23R subunit and the shared IL12R β1 chain.[9] Several experimental models support a role for IL23 in colitis. Loss-of-function studies show that IL23p19 is essential for chronic colitis development in *IL10*$^{-/-}$ spontaneous colitis model,[10] CD45RB^{hi}CD4$^+$ T-cell transfer models,[11] *Helicobacter hepaticus*–driven colitis,[12] anti-CD40–induced acute innate colitis model,[13,14] and chemically induced colitis.[15] Other studies show that IL23R expression by CD4$^+$ T cells is required for disease development in a murine T-cell transfer colitis model[16] and that IL23R expression by innate lymphoid cells is important for colitis development in an anti-CD40 antibody–induced acute innate colitis model.[14] Although mounting evidence suggests that IL23 is relevant in IBD pathogenesis, to our knowledge, no direct demonstration that increased IL23 signaling causes colitis exists to date.

Despite many advances, it is still not clear what environmental factors trigger development of IBD, nor it is known what factors cause flares of UC and CD. Besides genetic factors, smoking, diet, microbiota, and stress, appear to contribute to IBD development or aggravation.[17] Dietary changes have been proposed as a key factor in the increasing incidence of IBD in developing nations. Several large longitudinal studies have pointed to a lower risk of IBD among people who consume more fruits and vegetables and a higher risk in people who consume less of these and more animal fats and sugar.[18] Consumption of specific foods has also been associated with CD and UC flares.[18] In mice, the contribution of specific genes and the microbiota to colitis has been extensively analyzed. Mice bearing specific gene alterations develop colitis, but a significant number of them do not develop colitis when raised in germ-free (GF) conditions, suggesting a critical role for genes and the microbiota in promoting disease.[4,19] Other animal studies suggest a critical role of dietary components in the onset and severity of colitis.[20–22] However, the development of relapsing–remitting disease models has not been reported. Because of this limitation, it has been difficult to evaluate the contribution of diet and microbiota changes to disease initiation and progression.

To examine the contribution of IL23, the microbiota, and diet to the development of colitis, we created a novel mouse model in which IL23 is conditionally expressed by fractalkine chemokine receptor–positive (CX3CR1$^+$) cells. CX3CR1$^+$ macrophages and dendritic cells are the main cells expressing IL23 in the gut upon exposure to bacterial antigens.[23,24] Our results show that CX3CR1$^+$-derived IL23

***Authors share co-first authorship.**

Abbreviations used in this paper: **CD, Crohn's disease; CX3CR1, fractalkine chemokine receptor 1; GF, germ-free; IBD, inflammatory bowel diseases; H&E, hematoxylin and eosin; IL, interleukin; IL23R, interleukin 23 receptor; LI, large intestine; mLN, mesenteric lymph node; mRNA, messenger RNA; SPF, specific pathogen–free; TAM, tamoxifen; TCR, T-cell receptor; UC, ulcerative colitis.** Q7

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