Calcium, vitamin D, and immunity in the colon

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Epidemiologic observations have linked an increased risk of colorectal cancer (CRC) and inflammatory bowel disease with distance from the equator (1–3). Notably, inflammatory bowel disease is a risk factor for developing CRC because of compromised epithelial membrane function and chronic inflammation (1). Vitamin D is produced in skin exposed to adequate UV-B light and heat, and low exposure to solar UV-B due to lifestyle and/or greater zenith angle has led to the hypothesis that lower vitamin D concentrations may be the cause for these conditions (3). Hepatic hydroxylation by cytochrome P450 2R1 (CYP2R1), followed by cytochrome P450 27B1 (CYP27B1)-catalyzed 1α-hydroxylation in peripheral tissues generate circulating 25(OH)D3 and hormonal 1,25-dihydroxyvitamin D3 [1,25(OH)2D3; calcitriol] forms of vitamin D, respectively. These are catalyzed by 1,25(OH)2D3-induced cytochrome P450 24A1 (CYP24A1) in a negative feedback loop (3). 1,25(OH)2D3 binds the vitamin D receptor (VDR) to induce nongenomic and transcriptional (or genomic) responses (3). One of the major functions of 1,25(OH)2D3 is to increase serum calcium (Ca2+) concentrations by promoting intestinal absorption (3). Observational, mechanistic, and clinical studies have shown that calcium inhibits colonocyte hyperproliferation, increases apoptotic potential, contributes to epithelial barrier function, and is inversely correlated with inflammation and CRC risk (1, 4, 5). Further evidence suggests that 1,25(OH)2D3 acts similarly (1). Because calcitriol upregulates calcium absorption, it has proved difficult to differentiate its direct cellular effects from those arising from higher intracellular calcium concentrations. This conundrum is further complicated by 1,25(OH)2D3-induced upregulation of the calcium sensing receptor (CaSR) and by the regulation of 1,25(OH)2D3 signaling by calcium, implying crosstalk between the 2 systems (4–6).

In this issue of the Journal, Protiva et al. (7) address this matter by designing a double-blind clinical trial assessing the effects of calcium in low- and high-1,25(OH)2D3 conditions in high-CRC-risk individuals who consumed a Western-style diet (WD). They specifically focused on the rectosigmoidal portion of the colon, where a high level of VDR expression has been shown (8). In crossover study A, 10 patients consumed a high-fat, low-calcium WD supplemented with low 1,25(OH)2D3 for 4 wk. After a 4-wk washout period, subjects consumed the same diet supplemented with high doses of calcium. Study B differed from study A in that the diet was supplemented with 0.5 μg (20 IU) 1,25(OH)2D3 daily. Rectosigmoid biopsy samples were obtained at the beginning of each study, before and after the washout period, and at the end of the study, followed by genomewide expression profiling analyses. Hormonal 1,25(OH)2D3 rather than vitamin D was used because of the short duration of the trials (4 wk)—a period that may not be sufficient to substantially elevate calcitriol concentrations had vitamin D supplements been used. In addition, vitamin D supplementation could also produce greater interpatient variability.

It has been proposed that the colon is more sensitive to luminal than circulating 1,25(OH)2D3 (9). However, one caveat that should be taken into account in the study by Protiva et al. (7) is that orally administered 1,25(OH)2D3 is mainly absorbed in the small intestine, suggesting that the bioavailability of 1,25(OH)2D3 in the rectosigmoidal portion of the colon in study B may have been limited. It would have been interesting to assess the effects of calcitriol by intrarectal administration or by using 1,25(OH)2D3-glucuronide, an analog that luminal bacteria digest to release active 1,25(OH)2D3 in the colon (10). Both of these methods would ensure elevated local concentrations of calcitriol. In addition, a noncalcemic 1,25(OH)2D3 analog could have been used to better differentiate between effects on the distal colon produced directly by calcitriol from those mediated by calcium.

The authors adopted a systems approach whereby they examined perturbations in signaling pathways by performing Gene Set Enrichment Analysis rather than changes in single genes only. A comparison of results from study A with those of study B is complicated by the use of a more heterogeneous group in study B (3 African Americans out of 10 patients compared with 1 out of 10 in study A), and above all the use of different platforms for gene expression profiling. However, Gene Set Enrichment Analysis takes as input gene lists ranked by differential expression and outputs enrichment scores for Gene Ontology categories and pathways, allowing for indirect comparison between 2 unrelated studies. A WD in a low-1,25(OH)2D3 background (study A) showed limited enrichment for pathways implicated in inflammation and immune response, which is consistent with other

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Abbreviations used: AMP, antimicrobial peptide; CaSR, calcium sensing receptor; CRC, colorectal cancer; CYP2R1, cytochrome P450 2R1; CYP24A1, cytochrome P450 24A1; CYP27B1, cytochrome P450 27B1; VDR, vitamin D receptor; WD, Western-style diet; 1,25(OH)2D3, 1,25-dihydroxyvitamin D3.

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results (1, 5). Calcium reversed these effects, which is not surprising because high amounts of calcium neutralize harmful bile and ionized fatty acids that compromise epithelial barrier function and cause inflammation (1, 4, 5). They are also consistent with many reports that highlighted the beneficial effects of calcium in inflammation and CRC, which appear to be mediated by CaSR, whose expression correlates with CRC prognosis (1, 4–6, 8). What is surprising is that calcium largely abrogated the effects of 1,25(OH)2D3 in WD conditions, as seen by the reversal of gene enrichment in categories related to cell cycle control, cell adhesion, inflammation, and immune responses (study B). Calcitriol can upregulate innate immune and inflammatory processes [IL-1β induction, pattern recognition receptors (nucleotide-binding oligomerization domain and Toll-like receptor) pathways, triggering receptors expressed on myeloid cells (TREM) signaling, and antimicrobial peptide (AMP) production] in innate immune cells and in the epithelium (3, 11). It also regulates adaptive immune responses [induction of the T cell chemotactic molecule RANTES (regulated upon activation, normal T cell expressed and secreted) and suppression of IL-17] in innate immune cells and in the epithelium (3, 11). At the same time, it reduces inflammatory cytokines (e.g., TNF-α, IFN-γ) and increases the production of anti-inflammatory IL-10; thus, the enrichment of Gene Ontology categories such as inflammation, interferon signaling, and immune responses would be expected (1, 3, 10, 12). 1,25(OH)2D3 also acts directly on colonocytes to reduce proliferation and increase adhesion, differentiation, and apoptosis—effects similar to those of calcium (1, 4–6, 12). Most studies in mice suggest that 1,25(OH)2D3 and calcium act synergistically (1, 8), contrary to what is suggested by Protiva et al. (7), although it is important to note that some of the actions of 1,25(OH)2D3 in mice may not be conserved in primates (2). Certain clinical trials suggest that calcium and vitamin D act independently, whereas others showed evidence of positive interactions, with CuSR, whose expression is directly upregulated by 1,25(OH)2D3 in colonocytes, playing a critical role (1, 4–6). It would be interesting to assess CASR mRNA levels in the biopsy samples obtained after each treatment. Another player that may account for the results is the 1,25(OH)2D3 catabolic enzyme CYP24A1 (5). It was shown that low calcium significantly enhanced CYP24A1 expression, thus limiting 1,25(OH)2D3 availability, in the ascending, but not the descending, colon (5, 10).

The study by Protiva et al. (7) represents an important step toward elucidating the effects of calcium and 1,25(OH)2D3 alone and in combination in the distal colon of high-CRC-risk individuals. Double-blind, placebo-controlled trials in a greater number of participants, as well as noncalcemic 1,25(OH)2D3 analogs introduced locally, would greatly aid in clarifying the roles of calcitriol and calcium in CRC treatment and prevention.

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REFERENCES