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EDITORIAL COMMENT

From Colon to Aortic Aneurysm: Trek of the Treg*

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The regulatory T cell (Treg) constrains the immune response; it can differentiate from precursor T cells in either the thymus or peripheral organs like the colon. From mouse studies, we have known for ~12 years that the development and function of colonic Tregs are enhanced by signals originating from anaerobic colonic bacteria. These microbiota-derived, Treg-inducing signals were identified as the short-chain fatty acids acetate, propionate, and butyrate when it was discovered that the colonic contents of germ-free mice lack these compounds, which are normally produced when colonic bacteria ferment dietary fiber.[1] Short-chain fatty acids administered orally were initially used to rescue the defectively low number of Tregs in the colons of germ-free mice.[1] Subsequently, orally administered short-chain fatty acids were used to ameliorate a variety of inflammatory or inflammation-dependent disorders in mice, including atherosclerosis, myocardial infarction, kidney allograft rejection, and hypertension.[2,3]

Propionate augments the proliferation of Tregs[1] and the differentiation of naive T cells into Tregs,[4] principally by stimulating a G protein–coupled receptor known as free fatty acid receptor 2 (previously known as GPR43).[1] Interestingly, propionate appears to increase the subpopulation of Tregs that expresses the anti-inflammatory cytokine interleukin-10, rather than transforming growth factor-β (which is the other major Treg-derived suppressive cytokine).[1] Over the last several years, multiple groups have used similar approaches to demonstrate the relationship between Tregs and the anti-inflammatory efficacy of propionate and/or other short-chain fatty acids. Correlative approaches have documented that Treg populations in the spleen upregulate when mice are fed short-chain fatty acids.[2] Approaches seeking to elucidate a causal link among orally administered short-chain fatty acids, Tregs, and anti-inflammatory effects have used anti-CD25 immunoglobulin G (IgG) to reduce Treg numbers and thereby abolish the anti-inflammatory effects of short-chain fatty acids. Similarly, the colitis-suppressing effects of propionate in lymphopenic (*Rag2*$^{-/-}$) mice were shown to require adoptive transfer specifically of Tregs and not just naive T cells.[1]

In light of these interesting investigations, several important questions remain regarding the mechanisms by which short-chain fatty acids induce Tregs to suppress inflammation. First, are Tregs the only cells mediating the anti-inflammatory effects of orally administered short-chain fatty acids? Although previous studies with anti-CD25 IgG are consistent with an important role for Tregs in mediating anti-inflammatory effects of short-chain fatty acids, the traditional anti-CD25 IgG approach may be confounded by the expression of CD25 on CD8^{+} T cells. Furthermore, short-chain fatty acids acting via free fatty acid receptor 2 reduce monocyte secretion of proinflammatory cytokines and reduce neutrophil recruitment to foci of inflammation.[4] Second, how do short-chain fatty acid–stimulated Tregs in the colonic lamina propria mediate anti-inflammatory effects at distant sites, like the arteries? Does interleukin-10 secretion by Tregs suppress inflammation at a distance, or do Tregs migrate from the colon to distant sites of inflammation and subsequently act locally to suppress inflammation?

These questions have been addressed well in the context of murine abdominal aortic aneurysm (AAA) by Yang et al[5] in this issue of *JACC: Basic to*

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Translational Science. Using both the elastase- and $Ca_3(PO_4)_2$-induced models of AAA in C57BL/6J mice, Yang et al showed a salutary effect of orally administered propionate on AAA development. They demonstrated that Tregs are overwhelmingly responsible for propionate's anti-inflammatory effect. To do so, they used not only the customary anti-CD25 IgG-mediated Treg depletion but also an appealingly more specific intervention to deplete Tregs. They used mice that express the simian diphtheria toxin receptor under the control of the endogenous forkhead box P3 promoter/enhancer regions contained within the transgene. Because mouse cells lack diphtheria toxin receptors and because the forkhead box P3 transcription factor is specific for Tregs, diphtheria toxin receptor expression in these mice is restricted just to Tregs. Consequently, the administration of diphtheria toxin ablates Tregs in these transgenic mice. When Yang et al[5] depleted their mice of Tregs by injecting diphtheria toxin for 2 days before AAA induction (and again 7 days later), they significantly exacerbated AAA formation and simultaneously abolished the salutary effect of orally administered propionate on AAA formation. By providing congruent data from 2 complementary approaches for Treg depletion, Yang et al[5] add significantly to our understanding of the Treg dependency of propionate's anti-inflammatory effects on AAA pathogenesis.

The most remarkably novel insight provided by Yang et al[5] pertains to the trafficking of Tregs that mediate the propionate-induced mitigation of AAA. After demonstrating that orally administered propionate augments Treg populations in the AAA tissue itself, Yang et al showed that Tregs originating from the colonic lamina propria migrate to the AAA and that this migration is required for the salutary effect of propionate on AAA development.[5] To do so, Yang et al used an elegant approach with transgenic mice expressing the Kikume Green-Red (KikGR) fluorescent protein. The KikGR protein fluoresces green until it is excited by 405 nm light, after which it fluoresces red. Therefore, Kik-red$^+$ fluorescence serves to identify cells originating from an organ illuminated with 405 nm light. Yang et al illuminated just the colon of KikGR transgenic mice with 405 nm light and 36 hours later quantitated Kik-red$^+$ Tregs in various locations. Although propionate-treated mice had fewer Kik-red$^+$ (ie, colon-derived) Tregs in the colonic lamina propria, they had greater numbers of colon-derived Tregs in the draining lymph nodes of the colon, blood, and AAA tissue. If Tregs migrate from the colonic lamina propria to the AAA via the colon's draining lymph nodes, as these Kik-red$^+$ Treg data strongly suggest, then the propionate-induced migration of Tregs into the AAA should be abrogated by surgical excision of the colon's draining lymph nodes. Indeed, that is what Yang et al found when they performed colonic lymphadenectomy at the time they induced AAA formation. Furthermore, by preventing Treg migration to the AAA, Yang et al also prevented propionate therapy from exerting beneficial effects on AAA formation, even though propionate amplified Treg populations in the colonic lamina propria.

What chemotactic cues drive the Treg colon-to-AAA migration that is promoted by propionate? This issue remains obscure, but Yang et al[5] have advanced our understanding in this dimension of Treg biology also. Other investigators previously found that the oral administration of propionate to mice upregulated the expression of the colonic Treg homing receptor G protein–coupled receptor 15, which could conceivably support Treg migration to the AAA if AAA cells express the ligand for G protein–coupled receptor 15 ligand—a possibility as yet untested. Yang et al expand possibilities for propionate-promoted Treg trafficking by demonstrating that propionate downregulates Treg expression of CD69, a transmembrane protein that inhibits Treg migration by inhibiting the function of the sphingosine-1-phosphate receptor-1. However, whether the pathogenesis of AAA involves the generation of sphingosine-1-phosphate remains to be determined.

In contemplating the translational potential of the AAA pathophysiology illuminated by Yang et al,[5] it is important to note that no mouse model of AAA faithfully recapitulates human AAA, which derives almost exclusively from exposure to cigarette smoking.[6] Specifically, rupture of the AAA occurs in no mouse model unless mice are given anti-interleukin-6 IgG, anti-transforming growth factor-β IgG, or the collagen cross-linking inhibitor 3-aminopropionitrile.[6] Thus, whether enteral propionate will be useful in mitigating human AAA progression is uncertain at best. Furthermore, the data supporting the potential for short-chain fatty acids to mitigate inflammation in any organ derive almost entirely from mouse studies. The multiple receptors activated by short-chain fatty acids may differ with regard to distribution and/or signaling bias between mouse and human. Moreover, randomized trials of short-chain fatty acids to treat inflammatory disorders in humans have been thus far too small to facilitate robust conclusions regarding efficacy.[4]

Yang et al[5] have provided fascinating insights into the Treg proliferation and trafficking promoted by propionate therapy in mice harboring experimental

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AAA. Nonetheless, we still await persuasive clinical trials to determine whether short-chain fatty acids can reduce any inflammatory pathology in humans. While we await these trials, it is important to note that randomized trials have demonstrated cardiovascular benefits in humans treated with dietary fiber,[2] the substrate from which colonic bacteria produce short-chain fatty acids. Perhaps, then, it might still be best to follow our parents' advice—and eat our vegetables.

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REFERENCES

1. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341:569–573.

2. Kaye DM, Shihata WA, Jama HA, et al. Deficiency of prebiotic fiber and insufficient signaling through gut metabolite-sensing receptors leads to cardiovascular disease. *Circulation*. 2020;141:1393–1403.

3. Wu H, Singer J, Kwan TK, et al. Gut microbial metabolites induce donor-specific tolerance of kidney allografts through induction of T regulatory cells by short-chain fatty acids. *J Am Soc Nephrol*. 2020;31:1445–1461.

4. Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M. Free fatty acid receptors in health and disease. *Physiol Rev*. 2020;100:171–210.

5. Yang F, Xia N, Guo S, et al. Propionate alleviates abdominal aortic aneurysm by modulating colonic regulatory T cell expansion and recirculation. *J Am Coll Cardiol Basic Trans Science*. 2022;7(9):934–947.

6. Lindeman JH, Matsumura JS. Pharmacologic management of aneurysms. *Circ Res*. 2019;124:631–646.

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