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T cell activation altersintestinal structure and function

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Commentary

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T cell activation alters intestinal structure and function

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Since the advent of immunosuppressive therapy with OKT3, a monoclonal antibody to CD3 on the surface of human T cells that first activates and then kills these cells, activated T cells have been recognized as a cause of profuse, albeit transient, diarrhea (1). Thinking that this may be germane to the intestinal pathophysiology produced in a number of intestinal inflammatory states, investigators have undertaken studies in which anti–murine CD3 monoclonal antibody is administered to wild-type and genetically modified mice (2–5). The resultant diarrhea (or intestinal fluid accumulation) was found to be short lived and coincident in time with temporary increases in the release of several cytokines (IFN-γ, TNF-α, IL-2, IL-3, and likely others). A diarrheagenic role for TNF-α in particular was recently demonstrated in this journal using TNF receptor–1$^{-/-}$ mice (*Tnfr1*$^{-/-}$ mice) (3) and is confirmed by Clayburgh et al. in this issue of the *JCI* (5) using both an antibody against TNF and an inhibitor of PKC-α, which mediates one of the epithelial transport–related effects of TNF.

Nonstandard abbreviations used: LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry mediator on T cells; MLC, myosin II light chain.

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Intestinal epithelial effects of TNF

Clayburgh et al. (5) identify 2 separate effects of TNF on mouse jejunal epithelium: (a) endocytosis of the epithelial brush border Na^+/H^+ exchanger, member 3 (NHE3), which thereby inhibits transepithelial Na^+ absorption; and (b) an increase in the permeability of the epithelium to proteins as well as smaller solutes and water (Figure 1). The permeability-enhancing effect of TNF (4, 6) and, more generally, intestinal inflammation (7–9), had previously been noted. Another member of the TNF superfamily, LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for HVEM on T cells), also causes this change in permeability, though, interestingly, it does not inhibit Na^+ transepithelial transport. In a prior study, Clayburgh et al. (4) had shown that the increase in permeability produced by anti-CD3 treatment is associated with tight junction disruption and an increase in epithelial myosin II light chain (MLC) phosphorylation and that both effects could be blocked by adding membrane-permeant inhibitor of MLC kinase (PIK) (10) to the luminal perfusate. In the present study (5), they show that the permeability-enhancing effects of both TNF and LIGHT are also blocked by PIK. With MLC phosphorylation, the actomyosin ring in the apical region of the epithelial cell, which is juxtaposed to the abutting tight junctions, contracts causing rearrangement of 2 of its critical proteins, ZO-1 and occludin, with an associated increase in pore size and decrease in sieving selectivity. Thus, events in these cells can regulate the permeability of the belt that surrounds them laterally at their apices, also known as zona occludens. For a recent review of this subject along with new data, see Shen et al. (11).

With regard to epithelial transport, the PKC-dependent endocytic process activated by TNF is probably not confined to NHE3. Musch et al. (3) noted anti-CD3 antibody–induced (anti-CD3–induced) decreases in electroneutral (i.e., NHE3-dependent) Na^+ absorption, Na^+-dependent glucose absorption; cAMP-dependent active anion secretion; and Na^+/K^+-ATPase activity (the last was also determined following TNF treatment). All of these effects may be due to endocytosis of the relevant transporters. (The observed modest decrease in Na^+/K^+-ATPase activity, since it was measured in a crude membrane fraction in the absence of detergent, may represent sequestration of the enzyme in an endocytic compartment inaccessible to substrate).

Relation of these effects to intestinal fluid secretion

Relating Clayburgh et al.'s data (5) to the clinical observation of profuse diarrhea seen in patients treated with OKT3 is not a simple matter. The rates of fluid secretion the authors observed in TNF-treated mice were rather small when compared with their rates of absorption in control mice. Two of the authors' methodological decisions may account for this: (a) their use of a hypotonic luminal perfusate (136 milliosmoles at the most), which must have contributed to substantial water absorption by osmosis, especially in the LIGHT- or TNF-treated mice; and (b) their use of a luminal perfusate containing 20 mM glucose, thereby engaging a major absorptive pathway, Na^+-glucose cotransport, which the authors did not quantitate. Had they

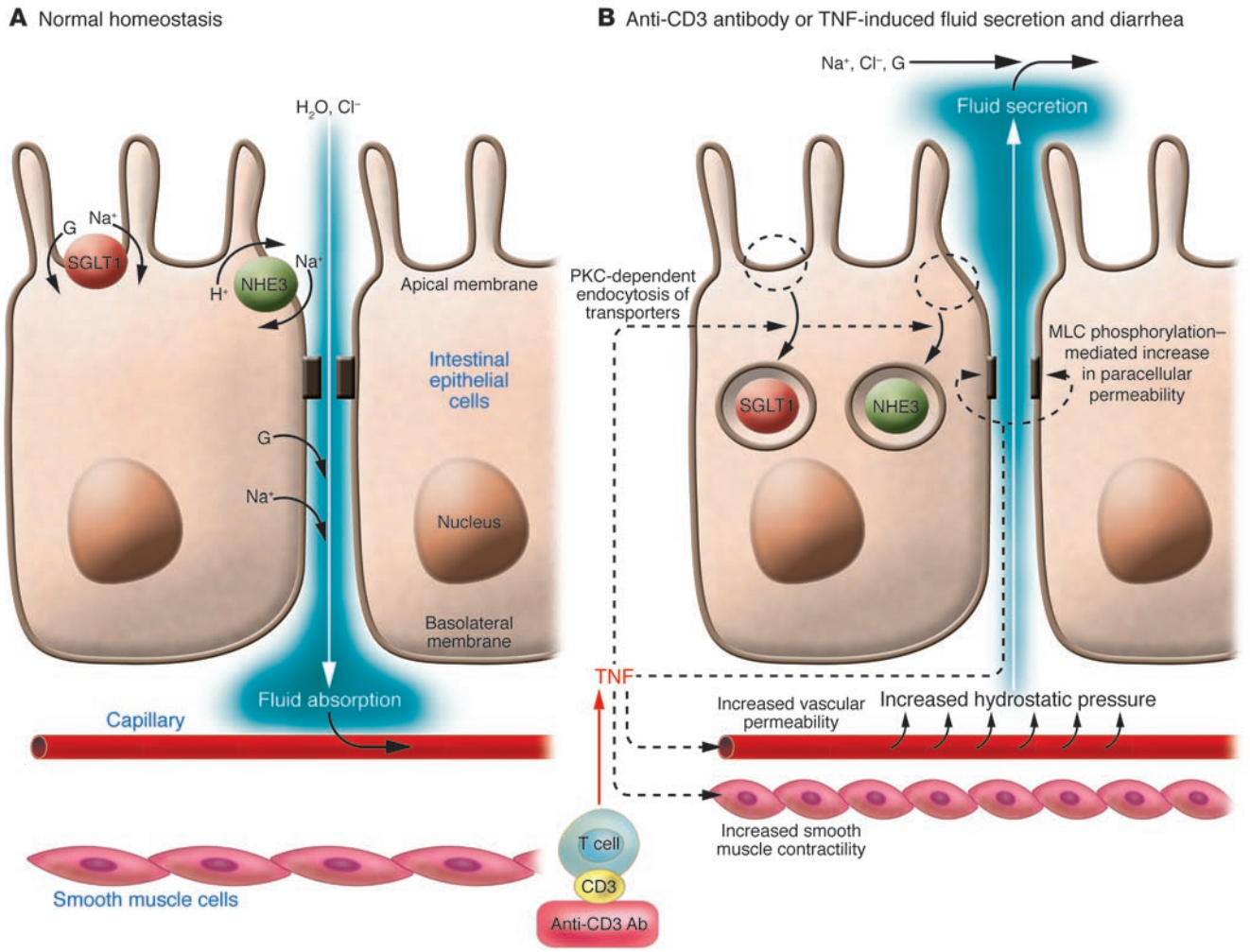

Figure 1
Influence of anti-CD3 or TNF on murine jejunal Na^+ absorption, paracellular permeability, and fluid homeostasis. Upon comparison with normal intestinal epithelial cell homeostasis (**A**), Clayburgh et al. (5) observed the following changes after injection into the jejunum of anti-CD3 or TNF: endocytosis of NHE3, Na^+/H^+ exchanger, member 3 (NHE3) with a resulting decrease in Na^+ absorption; increased paracellular permeability to protein, smaller solutes, and water; and a shift from fluid absorption to fluid secretion (**B**). Also suggested are endocytosis of sodium-glucose cotransporter 1 (SGLT1) with resulting decreases in sugar and Na^+ absorption (3), increased vascular permeability (15, 16), and increased smooth muscle contractility (2), the latter effects increasing subepithelial hydrostatic pressure, providing a driving force for fluid secretion. G, glucose.

used an isotonic perfusate not containing glucose, the net secretory response to TNF (i.e., the difference between the contributions of all absorptive processes and of all secretory processes) would undoubtedly have been much larger.

One fascinating aspect of these recent studies (3–5) is the apparently negligible contribution of active anion secretion to anti-CD3– and TNF-induced fluid secretion. This was demonstrated explicitly in TNF-treated CFTRΔF508 mice, which lack a functional apical anion channel in their intestine (curiously, secretion was actually greater in the CFTRΔF508 than in wild-type mice) (4).

If active anion secretion is not a driving force in anti-CD3–induced diarrhea, what is?

Until these recent studies, profuse diarrhea in intestinal inflammation had been almost invariably attributed to active anion secretion stimulated by bacterial enterotoxins; neural transmitters such as vasoactive intestinal polypeptide; or inflammatory mediators such as prostaglandins. Cholera toxin–induced secretion is considered the prototype (12). (TNF, in particular, was shown to stimulate active Cl^- and K^+ secretion in distal colon, effects prevented by adding an inhibitor of prostaglandin synthesis [ref. 13]; thus, under some circumstances, TNF does stimulate active secretion.) But almost all of these studies employed in vitro preparations in which only the active ion transport processes of the epithelium could produce secretion. In vivo, extraepithelial forces — osmotic and hydrostatic — contribute to the absorption and secretion of water and solutes. Perhaps TNF, in addition to its epithelial effects, increases hydrostatic pressure beneath the epithelium through effects on intestinal capillaries and/or smooth muscle. The result might be the "weeping" of fluid from lamina propria into gut lumen. Thirty years ago, in an elegant study also published in this journal, Yablonski and

Lifson (14) showed in dogs that elevations of venous pressure as low as 4–6 cm H_2O caused "secretory filtration" by both providing a driving force for secretion and increasing the hydraulic permeability of the epithelium, *without which the driving force would be ineffective*. The resulting pores, which are reminiscent of those induced by TNF and LIGHT, are large enough for proteins to pass.

How might anti-CD3, or TNF in particular, affect the hydrostatic pressure difference between lamina propria and gut lumen? TNF is known to increase vascular permeability (15, 16). The resulting extrusion of fluid from small blood vessels into lamina propria would likely increase pressure there, since the latter is a relatively confined space limited by the epithelium in one plane and smooth muscle in the other. The contractile smooth muscle response to cholinergic stimulation has been shown to increase following anti-CD3 administration (2). In conjunction with increased vascular permeability, this increase in muscle tension may further increase interstitial pressure.

In summary, since the phenotypic changes in the intestinal epithelium of mice resulting from their treatment with anti-CD3 or TNF do not, in themselves, explain concomitant fluid secretion and diarrhea, a secretory driving force other than active anion secretion must be postulated. The likely candidate is an increase in interstitial pressure, the influence of which is facilitated by the associated increase in epithelial permeability. The postulated pressure increase could result from the effects of TNF and perhaps other cytokines on vascular permeability and smooth muscle action. The role of interstitial pressure in cytokine-induced intestinal secretion needs to be explored. Such secretion, unlike that activated by cAMP, should be especially sensitive to reversal by an increase in intraluminal pressure (assuming that pressure on the bulk luminal solution is transmitted to intestinal crypts, where much of the fluid leakage is likely to occur).

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Pili prove pertinent to enterococcal endocarditis

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***Enterococcus faecalis* is an important agent of endocarditis and urinary tract infections, which occur frequently in hospitals. Antimicrobial therapy is complicated by the emergence of drug-resistant strains, which contribute significantly to mortality associated with *E. faecalis* infection. In this issue of the *JCI*, Nallapareddy and colleagues report that *E. faecalis* produces pili on its surface and that these proteinaceous fibers are used for bacterial adherence to host tissues and for the establishment of biofilms and endocarditis (see the related article beginning on page 2799). This information may enable new vaccine strategies for the prevention of *E. faecalis* infections.**

Nonstandard abbreviations used: Ace, adhesion to collagen from *E. faecalis*; AS, aggregation substance; *ebp*, *endocarditis and biofilm-associated pili*; Esp, enterococcal surface protein; *fsr*, *E. faecalis regulator*; SrtC, sortase C.

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Enterococcus faecalis — snapshots of the pathogen

Enterococcus faecalis, a commensal bacterium of human biliary and gastrointestinal tracts, is a leading cause of surgical site, bloodstream, and urinary tract infections (1). Furthermore, *E. faecalis* is also a causal agent of bacterial endocarditis, whose complications — including congestive heart failure, septic emboli, and glomerulonephritis —result in mortality (2). Current management of enterococcal endocarditis involves administration of a combination of antimicrobials; however, the emergence of strains with multiple antibiotic resistance, including vancomycin resistance (e.g., vancomycin-resistant enterococci), present continuously increasing problems (3). Epidemiological studies show that more than 20% of enterococci isolated from infections of intensive care unit patients display vancomycin resistance (4). Thus, research that has the potential to unveil new therapies or preventive measures that disrupt the