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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

Mapping the Duodenal Crypt-Villus Transport Axis

The epithelial cells that line the intestines have a physiological imperative to absorb nutrients while forming a semipermeable barrier that prevents the incursion of undesirable luminal solutes.[1] They also both absorb and secrete electrolytes to drive the accompanying passive transfer of water, thereby maintaining appropriate levels of luminal fluidity to support the processes of digestion and absorption. It has been estimated that the gut is presented with 8–9 L of fluid per day, with only the minority coming from oral intake of food and beverages—the remainder derives from the various intestinal secretions that mediate digestive and other functions. To avoid dehydration, the fluid in these secretions must be reclaimed, and thus there is close regulation of the electrolyte transport processes that provide for this. The various segments of the bowel also may perform specialized transport functions suited to the conditions that they encounter. For example, the proximal duodenum actively secretes bicarbonate to neutralize acid coming from the stomach, and to protect the mucosa from injury that otherwise might result.[2] Indeed, patients suffering from duodenal ulcer disease show reduced levels of duodenal bicarbonate secretion both at baseline and in response to luminal acid.[3] In the current issue of *Cellular and Molecular Gastroenterology and Hepatology,* Yin et al[4] reported elegant studies that further elucidate how ion transport is regulated in the human duodenum, with findings that may challenge our existing models of transport processes in this important gut segment (and perhaps others).

Indeed, there has been a long-standing dogma pertaining to small intestinal physiology to the effect that "crypts secrete, villi absorb"[5] (Figure 1). This segregation of transport functions may provide, for example, for ongoing flushing of the crypts and protection of the crucial stem cell niche, and thereafter reclaiming the associated fluid when no longer needed for this housekeeping function. Nevertheless, the separation of transport functions has been challenged over the years, albeit usually based on indirect approaches such as immunohistochemistry, to define the census of transport proteins at a given point on the crypt-villus axis, rather than by directly measuring transport function. Techniques that can assess electrogenic transport function, such as the Ussing chamber, are applicable to segments of gut tissue overall, and hitherto there had been no way to isolate purified crypt vs villous cells in such a way that they could easily be used for functional studies. Furthermore, studies in cells of human origin are challenging and subject to the same limitations, and those in transformed cell lines are not necessarily reflective of the properties of the native gut.

The study of the intestinal epithelium, however, has been revolutionized in recent years by the introduction of enteroids, 3-dimensional culture models derived from human (or animal) intestinal epithelial stem cells that spontaneously generate all of the differentiated epithelial lineages of the native gut.[6] By providing or withdrawing specific growth stimuli, enteroids can be directed to continued proliferative activity and immaturity akin to the native crypt, or differentiated to resemble the properties of cells on the villi. Furthermore, these enteroid structures also can be broken apart and plated as 2-dimensional monolayers on permeable supports that then are amenable to electrophysiological studies of transport function, such as in Ussing chambers.

Yin et al[4] reported studies that deployed these enteroid-derived monolayers to ask questions about how transport functions may evolve along the crypt-villus axis of the human duodenum. They generated enteroids from several human donors undergoing endoscopic or surgical procedures. They then compared the expression of various transporters as well as adenosine 3′,5′-cyclic monophosphate (cAMP)–stimulated anion secretion in matched monolayers that were either maintained in an undifferentiated state (UD) with retention of stem cell and proliferative markers, or were induced to differentiate (DF). Their premise was that the UD monolayers reflect the properties of the crypts in the intact human duodenum, while the DF monolayers represent the villi. This was borne out by comparisons of their various transporters of interest between the monolayers and native tissue. The differentiation process resulted in up-regulation of some, but not all, transporters involved in absorption, such as the chloride/bicarbonate exchanger, down-regulated in adenoma (DRA), and loss of transporters involved in chloride secretion, including the cystic fibrosis transmembrane conductance regulator (CFTR), sodium/potassium/two chloride cotransporter (NKCC1), and Potassium voltage-gated channel, Isk-related family, member 3 (KCNE3). DF cells also had higher levels of two carbonic anhydrase isoforms that can generate intracellular bicarbonate for subsequent secretion. Importantly, however, none of the transporters studied were absolutely segregated to either UD or DF monolayer types. The true advance of this study, moreover, was the capacity for functional analyses. These showed that although cAMP–triggered anion secretion was reduced by differentiation, a meaningful component persisted even in DF monolayers. Moreover, by using both ion substitution and pharmacologic approaches, Yin et al[4] could show that the reduction in secretory capacity occurring upon differentiation into DF monolayers was accounted for by the loss of active chloride secretion, whereas electrogenic bicarbonate secretion persisted at levels that were comparable with those seen in the UD cultures.

Chloride secretion appeared to account for the majority of anion secretion in UD monolayers, but also a meaningful portion in DF cultures, in which the machinery for secretion

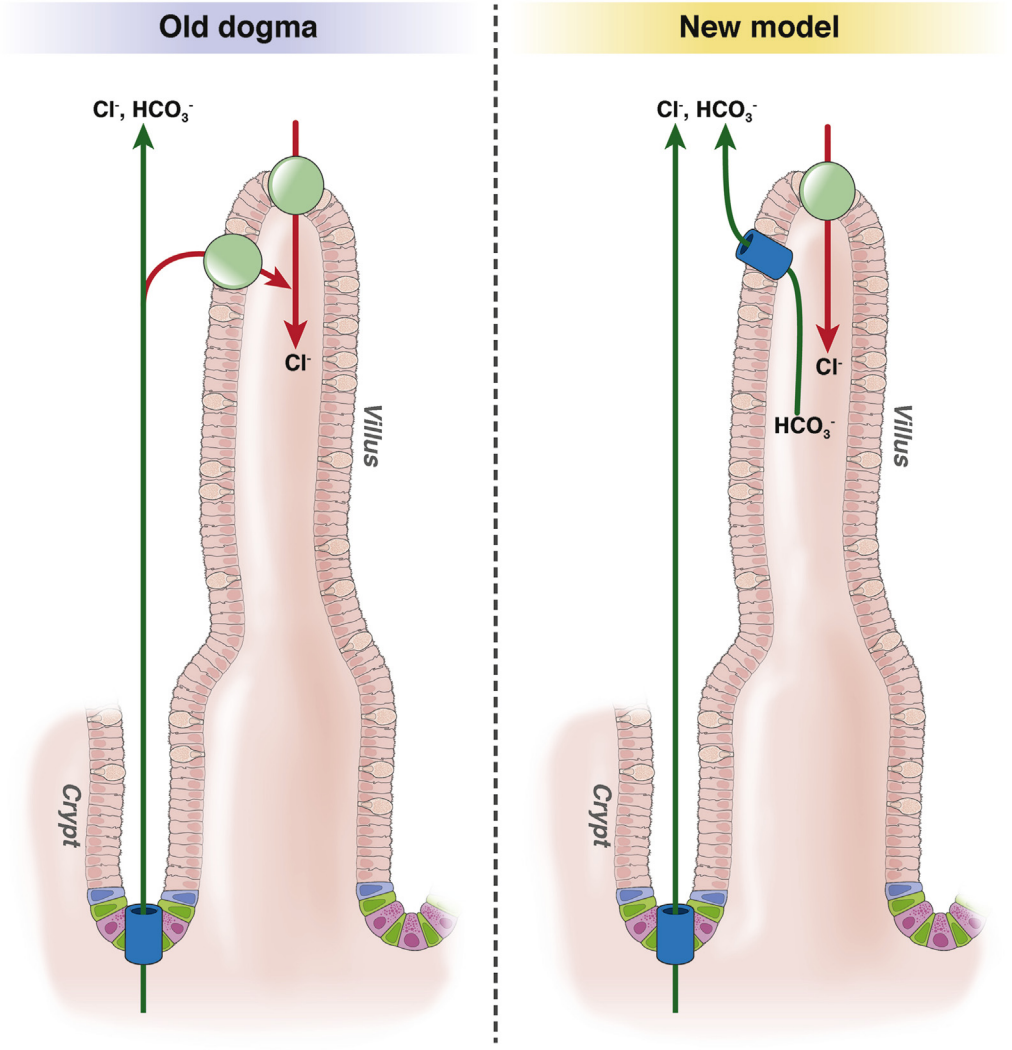

Figure 1. Previous model of anion transport across the duodenal crypt-villus unit and new insights provided by the studies of Yin et al.[4] In the old dogma (*left*), secretion of chloride and bicarbonate was considered to arise from the crypts (*green arrow*), with a portion of the chloride, and chloride otherwise derived from luminal contents, reabsorbed across the villous epithelium (*red arrows*). In the new model of Yin et al[4] (*right*), chloride and bicarbonate secretion still originate from the crypt (*straight green arrow*), but an equivalent level of bicarbonate secretion (with a minor amount of accompanying chloride, not shown) arises from the villous epithelium (*curved green arrow*). Absorption of chloride remains a villous function, likely via the significant up-regulation of DRA expression that occurs upon epithelial differentiation (*red arrow*).

is markedly down-regulated in both apical and basolateral membranes. Interestingly, bicarbonate secretion in both UD and DF monolayers was also wholly dependent on CFTR. This begs the question of how bicarbonate secretion persists unchanged after differentiation, when CFTR levels were reduced by approximately 50%. Chloride ions are transported more efficiently by CFTR than are bicarbonate ions; one can speculate that the relative lack of capacity for basolateral chloride loading via NKCC1, coupled with enhanced capacity for bicarbonate generation via carbonic anhydrase, tilts the balance between these anions to allow for sustained bicarbonate secretion, even if the apical exit pathway (CFTR) is rate-limiting.

There are some caveats to this study, well-recognized by Yin et al,[4] that doubtless will be addressed in future work. First, the investigators were not in a position to measure bicarbonate secretion per se using back-titration, but rather had to infer the relative contribution of bicarbonate and chloride to anion transport by studying the impact of ion substitutions and/or their transport inhibitors. Second, at least to some extent, the secretion of bicarbonate is dependent on that of chloride and vice versa, which could not be studied directly. Similarly, the Ussing chamber cannot be used to assess electroneutral transport processes that could contribute meaningfully to anion transport, and to bicarbonate secretion in particular. Finally, and perhaps most significantly, it is unlikely that transport functions along the crypt-villus axis exist at 2 extremes, but rather that they almost certainly evolve continuously as cells migrate and differentiate. Thus, transport at the base of the crypt may be different from that at the mouth; transport likely also changes along the length of the villi. It is unclear (and probably unknowable) precisely which points along this continuum the UD and DF monolayers represent, nor do the monolayers contain subepithelial cells types or a luminal microbiota that might further modulate transport function in vivo. Future studies could certainly examine the contributions of these latter factors.

Nevertheless, the work of Yin et al[4] represents an important step forward in dissecting the details of electrolyte transport function in the gut that are applicable to

human subjects. The study provides compelling evidence that villi secrete, and highlight this site as an important determinant of mucosal protection from acid-peptic injury. Future studies should continue to illuminate how the qualitative and quantitative aspects of electrolyte transport change along both the vertical and horizontal axes of the gut. This is a vital prerequisite if ultimately we wish to intervene in situations in which transport is abnormal, such as in peptic ulcer disease or in diarrheal conditions.

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Conflicts of interest
The author discloses no conflicts.

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