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An Innate Disposition for a Healthier Gut: GLP-1R Signaling in Intestinal Epithelial Lymphocytes

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The GLP-1 system affects multiple physiological processes, ranging from potentiation of glucose-stimulated insulin secretion to gut motility, satiety, and even hepatic glucose production (1–3), through distinct mechanisms at separate locations. While there is some debate about whether GLP-1 mediates these actions through the circulation as a hormone (4–6), GLP-1 signaling via its receptor (GLP-1R) on target tissues has been extensively studied and shown to support therapeutic application. As a result, GLP-1 receptor agonists and inhibitors of dipeptidyl peptidase-4 that delay GLP-1 inactivation are now widely used for the treatment of type 2 diabetes and, more recently, obesity (7–10). Thus, there is a substantial and incontrovertible body of evidence supporting a role for GLP-1 in metabolic control.

Notable among recent studies is a new direction—interaction between GLP-1 and pathways of inflammation. For example, the enteroendocrine L cells, which are thought to be the primary source of GLP-1, increase their secretion in response to interleukin-6 and lipopolysaccharide, as well as in other settings of systemic inflammation (11–13). At the level of GLP-1 action, satiety effects in the central nervous system seem to be mediated, at least in part, by cytokines (14). A role of GLP-1 in inflammation appears to have some clinical relevance as plasma concentrations are elevated in critically ill patients (12,15). Despite the compelling nature of these findings, the cellular and molecular connections between L cells, GLP-1R, and the immune system are not well understood. Thus, the article by Yusta et al. (16) in this issue of *Diabetes* is both timely and important as it traces at least one pathway connecting GLP-1 and immune function. Here, the authors show that GLP-1Rs regulate intestinal epithelial lymphocytes (IELs), a novel finding with broad implications that seems likely to advance the understanding in intestinal mucosal function and provide traction for research on the role of GLP-1 in inflammation.

The intestinal mucosa is highly developed to act as an environmental barrier, while also serving as the conduit of nutrient and water absorption. The lining of the gut contains structural components that contribute to the innate immunity against harmful microorganisms and inflammatory substances but also facilitate symbiosis with commensal intestinal microbiota (17–19). A key component are the IELs, a distinct subset of T cells that patrol the lamina propria and control host-bacteria interactions, mucosal growth and regeneration, wound healing, and inflammation (18). IELs are a component of the innate immune system and also play key roles in the skin and lung, other organs lined by epithelia with extensive exposure to the external environment. Within the T-cell population, IELs are unique in that they exist in a partially activated state at baseline, with engaged cytolytic capability, a phenomenon posited to be advantageous given their position on the front line of host defenses (17). Full IEL activation by internal and environmental stimuli leads to secretion of interferon γ, a proinflammatory cytokine and decreased release of interleukin-10, an inflammation-muting cytokine. IELs are capable of sensing mucosal cell damage via ligands for natural killer cell receptors released by endothelial cells, and this renders them fully active both in their cytotoxic program of cell-mediated immunity and in promoting tissue repair (17,20).

Yusta et al. (16) demonstrate the expression of GLP-1R mRNA in isolated mucosal lymphoid cells compared with control populations of T cells from the spleen, lymph nodes, and bone marrow. Fractionated cells expressing the major T-cell receptors characteristic of intestinal IEL subpopulations (Tαβ and Tγδ) and carrying IEL-specific markers such as integrin αE and CD3γ were especially enriched in the GLP-1R. This is the first evidence of GLP-1R expression in IELs, and it raises the possibility of a new target for GLP-1 action in the gut. In fact, this appears to be the case as the authors demonstrate that

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treatment of FACS-sorted IELs with the GLP-1R agonist exendin-4 (Ex-4) caused a dose-dependent increase in cAMP levels in both partially activated as well as in fully activated IELs, effects that were comparable to forskolin treatment. Ex-4 treatment also suppressed expression of proinflammatory genes in IELs, but not in splenocytes, an effect not present in GLP-1R knockout (GLP-1R$^{-/-}$) IELs. These findings identify a discrete subset of lymphocytes with cell markers characteristic of IELs localized in the intestinal submucosa and regulated by the GLP-1R.

Comparisons between GLP-1R$^{-/-}$ and littermate controls (GLP-1R$^{+/+}$), or wild-type mice treated chronically with the GLP-1R agonist liraglutide or saline, did not identify changes of intestinal IEL composition or density, demonstrating that GLP-1R signaling is not required for IEL development or recruitment to the gut. Therefore, GLP-1R effects on submucosal IELs would be expected to work on cells already present in the intestine. Yusta et al. (16) did show that GLP-1R is required to promote and maintain the integrity of the intestinal mucosa. Colitis induced with dextran sodium sulfate provoked significantly more weight loss in GLP-1R$^{-/-}$ mice compared with GLP-1R$^{+/+}$ mice, with greater epithelial damage and shortened colonic length, indicating a role for GLP-1R signaling in maintaining the intestine in response to inflammation. The authors show that even in the absence of dextran sodium sulfate–induced colitis, GLP-1R$^{-/-}$ mice show reduced expression of epithelial repair and protection genes, a profile worsened by induction of inflammation. Reconstitution of bone marrow cells from irradiated GLP-1R$^{-/-}$ mice using transplants from GLP-1R$^{+/+}$ mice corrected this dysregulated mucosal gene expression, with mRNA levels for epithelial repair genes returning to normal coincident with donor intestinal IEL colonization of the recipient gut. This variant of loss-of-function/gain-of-function experiment provides compelling support for the model of GLP-1–IEL interaction proposed by the authors.

Finally, treatment with the GLP-1R agonist Ex-4 caused a rapid, transient upregulation of antimicrobial and immunomodulatory genes in wild-type mice and reduced edema with a more normal gene expression pattern in mice with colitis. Of note, comparisons between GLP-1R$^{-/-}$ and wild-type controls demonstrated a significant change in the microbial flora, with a greater prevalence of *Bacteroidetes* and *Actinobacteria* species in the absence of GLP-1 action. While it would have been more definitive to see that this variation in gut microbia could be rescued by GLP-1R$^{+/+}$ IEL, this finding implicates GLP-1 signaling in host-bacterial communication. Thus, GLP-1R signaling seems to influence the full spectrum of IEL functions, including inflammation, epithelial homeostasis, and bacterial interactions.

In total, the findings of Yusta et al. are remarkable, demonstrating a completely novel target for GLP-1, one that at first glance seems distinct from the metabolic portfolio typically associated with the peptide. There are

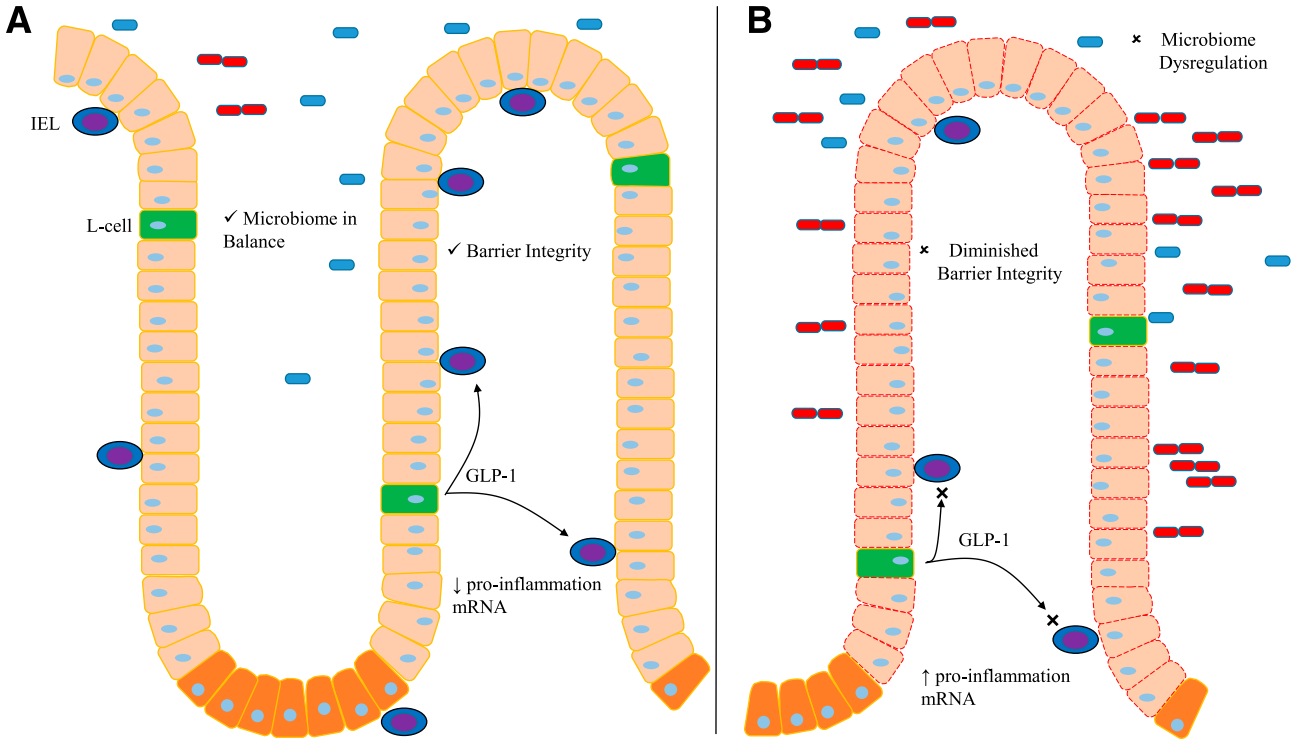

Figure 1—Schematic of the intestinal mucosa showing the normal state with intact GLP-1 signaling (*A*) and the consequences of impaired GLP-1 action on IELs (depicted as blue cells with purple nuclei) (*B*). GLP-1 stimulation of IELs promotes barrier function and normal microbial interaction and mutes inflammation. Loss of this activity has the potential to cause abnormalities in mucosal function.

several previous reports indicating that GLP-1 has a role in intestinal mucosal growth (10,21), an action in keeping with the recent findings. IELs are essential for the normal rapid turnover of gastrointestinal epithelial cells (18), and stimulation of these cells by intestinal GLP-1 could provide a link between nutrient absorption and ongoing rejuvenation of the mucosa, an effect complementary to the established actions of GLP-2 in intestinal growth (7). In this context, L cells can be seen as an integral part of the gut barrier function by communicating specific information from the environment (e.g., the availability and absorption of carbohydrate or lipid) to contribute to the balance of critical functions incumbent upon the intestinal mucosa (Fig. 1).

This work raises a number of interesting questions and is likely to drive research in the area of intestinal mucosal function and gastrointestinal regulatory peptides. The finding that GLP-1R signaling has a measurable impact on the nature of the microbiome provides a new tool by which to investigate host-bacterial interactions in the gut and is timely given the intense level of attention this area of biology is currently receiving. Similarly, the effects of GLP-1R signaling on IELs provide a path toward further insights into the role of GLP-1 in inflammation. The integration of L cells into essential epithelial functions provides a whole new perspective on enteroendocrine cells, which were previously conceptualized as a distinct gastrointestinal suborgan only tangentially related to the rest of the gut because of the systemic nature of many of their hormonal products. It is interesting to speculate that a local role in mucosal function might be the earliest physiological role for L cells, and perhaps other enteroendocrine cells as well; it will be important to know whether other enteroendocrine cells communicate with IELs or other parts of the intestinal barrier system. Finally, connecting the mucosal and metabolic roles of GLP-1 may lead to an important new understanding of physiology. It is conceivable that these roles share a mechanistic basis, as GLP-1 signaling in metabolic control has also been proposed to originate in the submucosa (4), and serve common global adaptive responses, such as to fasting/feeding or toxin exposure.

In summary, the article by Yusta et al. (16) may be one of the most important articles in the area of GLP-1 physiology in some time. If these findings are confirmed, they will define a new layer of L-cell function in the gut, adding paracrine signaling to endocrine and neural (22) mechanisms of action. This would extend the role of this multifaceted peptide to yet another area of biological regulation and provide a foundation for new advances in clinical science.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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