



Autoimmune diabetes mellitus and the leaky gut

Anneli Peters^a and Hartmut Wekerle^{a,b,1}

Over the past decade, our understanding of the immune reactivity and, in particular, of autoimmune disorders has witnessed a silent revolution. It has become clear that many, if not all autoimmune diseases entertain an intimate connection to the bacterial gut flora, a cosmos of trillions of different bacteria, forming diverse consortia distributed along the length of the intestinal tube. However, the microbiota's role seems to be remarkably ambiguous, depending on the particular autoimmune disease. Experimental studies of disease models indicate that, for example, in autoimmune diseases of the brain and the eye microbiota are essentially required to trigger and maintain autoimmunity (1, 2), while in stark contrast, in autoimmune type 1 diabetes (T1D), gut bacteria may protect from disease (3–6). Local interactions between luminal microbes with the surrounding gut-associated lymphoid tissue (GALT) drive the immune system into either direction. In PNAS, Sorini et al. (7) describe an alternative interactive mechanism. They observe that in 2 models of T1D changes in the luminal gut wall precede onset of clinical autoimmune diabetes. These changes are most evident in the mucus layer of the large intestine, where they allow translocation of bacteria from the lumen into extraluminal tissues and where they enable activated self-reactive T cells to home to the pancreatic islets and ultimately destroy the insulin-forming β cells (Fig. 1).

Sorini et al.'s (7) studies were inspired by the clinical observation that not only overt intestinal inflammation (8, 9), but also subtle subclinical intestinal irritation with increased barrier permeability can precede the onset of T1D in patients (10). Consequently, the investigators examined intestinal changes in nonobese diabetic (NOD) mice, a classical model of spontaneous T1D. In harmony with previous reports (11), they found that several weeks before onset of clinical disease, a fluorescent marker passed through the epithelium of the intestinal wall. The intestinal barrier consists of a double layer of mucus and a sheet of epithelial cells sealed by tight junctions, which, on the one hand, protects the organism from invading

pathogens, but at the same time allows the exchange of molecular signals (Fig. 1). While most previous studies focused on changes of the epithelial layer (11, 12), Sorini et al. (7) examine also structure and composition of the mucus layer, an essential element of intestinal barrier integrity. While transcription of tight junction proteins was only modestly reduced, the mucus layer in the colon was significantly changed. In particular, expression of anti-inflammatory membrane-associated proteins Muc1 and Muc3 was lowered, while that of the proinflammatory Muc4 was increased, creating a proinflammatory milieu within the colonic wall before and during development of T1D. Indeed mucus changes coincided with elevation of proinflammatory cytokine levels, and subsequently, starting around 10 to 12 wk of age proinflammatory Th17 cells became elevated at the expense of regulatory T (Treg) cells (7).

Are the mucosal changes causally related to the onset of T1D, or are they secondary consequences of a prodromal change? To address this question the authors used a different diabetes model, the transgenic BDC2.5XNOD mouse (13). These mice differ from regular NOD mice in several aspects. First, over 90% of CD4⁺ T cells use a transgenic T cell receptor (TCR) recognizing the β cell protein chromogranin A, whereas the NOD mouse contains an unknown but presumably very low frequency of autoantigen-specific T cells. As shown by transfer experiments, activated BDC2.5 T cells mediate acute diabetes in recipient animals. However, the most important difference from the NOD model is that BDC2.5XNOD mice do not develop spontaneous disease. Although TCR transgenic BDC2.5 T cells surround the pancreatic islets, invasion and destruction of islets and development of diabetes depend on additional activation stimuli, provided for example by a concomitant infection (14). To determine whether a compromised intestinal barrier can deliver these additional stimuli, Sorini et al. (7) targeted the mucus layer by repeated administration of low-dose dextran sodium sulfate (DSS), a chemical irritant that opens the gut barrier, allowing

^aInstitute of Clinical Neuroimmunology, Hospital and Biomedical Center of the Ludwig-Maximilians-University, 82152 Martinsried, Germany; and ^bHertie Senior Professor Group, Max-Planck-Institute of Neurobiology, 82152 Martinsried, Germany

Author contributions: A.P. and H.W. wrote the paper.

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¹To whom correspondence may be addressed. Email: hwekerle@neuro.mpg.de.

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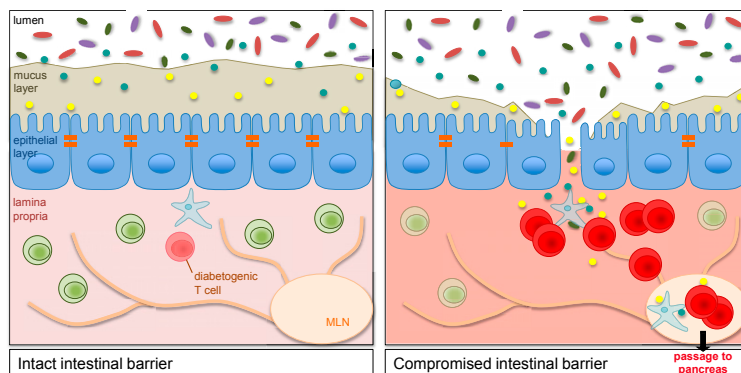


Fig. 1. A compromised intestinal barrier triggers activation of diabetogenic T cells in the GALT. An intact intestinal barrier (Left) is composed of a thick mucus layer and an epithelial layer with tight junctions (orange). The rare diabetogenic T cells in the underlying lamina propria remain quiescent. In contrast, a compromised intestinal barrier (Right) characterized by a thin and discontinuous mucus layer and a breached epithelial barrier allows invasion of microbiota into the lamina propria. This results in activation and expansion of diabetogenic T cells in the GALT, which then travel via mesenteric (MLN) and pancreatic lymph nodes to the pancreas and induce diabetes.

bacterial translocation to the mesenteric lymph nodes. In a streptozotocin-induced diabetes model intestinal bacteria traveled farther onto pancreatic lymph nodes to facilitate stimulation of diabetogenic T cells (15). However, in BDC2.5XNOD mice, activation of the TCR-transgenic T cells seems to occur in the GALT. Thus, gut-associated transgenic T cells displayed an activated and proinflammatory phenotype along with the gut-specific integrin $\alpha 4\beta 7$. This may reflect a local response to gut microbes, as in separate in vitro experiments TCR-transgenic T cells responded to stimulation with bacterial suspensions by producing more IFN- γ than polyclonal wild-type NOD T cells. This response could be partially blocked by anti-MHCII antibodies, arguing in favor of a TCR-dependent response against a microbial mimic. This particular marker profile was also noted in islet-infiltrating T cells, leading the investigators to conclude that diabetogenic T cells were activated directly in the GALT and then migrated to the pancreas to induce diabetes (7).

It should be kept in mind that the individual intestinal segments vary profoundly in terms of microbial composition and density. Also, the mucus layer is thicker and more structured in the colon than in the small intestine and thus provides a richer habitat for mucus-associated microbiota. The segmental differences are also reflected in the composition and function of GALT resident immune cells. Thus, the small intestine supports a higher frequency of Th17 cells, while Tregs are more enriched in the colonic lamina propria. In their study, Sorini et al. (7) show that targeting the mucus layer with DSS treatment affects colonic immune cells more profoundly than their counterparts in the small intestine, with an increase of Th1/Th17 phenotype effector cells preferentially in the colon. Along the same lines, in the spontaneous NOD model Treg frequency was significantly reduced in the colon but not in the small intestine (7). The importance of segmental differences has also been shown in the context of other diseases; for example, in experimental autoimmune encephalomyelitis pathogenic Th17 cells can be specifically recruited to the duodenum upon treatment with anti-CD3 antibodies, where they become suppressive, resulting in reduced disease severity (16).

What roles do the gut microbiota play in activation of diabetogenic T cells? Not surprisingly, DSS treatment also reduced diversity and changed the composition of the microbiota, such that a reduction in Bacteroidetes (*Prevotellaceae*, *Rikenellaceae*, *S24-7*) compensated by an increase in Firmicutes (*Erysipelotrichaceae*

and *Deferribacteres* preceded the onset of diabetes in DSS-treated BDC2.5XNOD mice (7). Importantly, a drop in alpha-diversity was also observed in human predisposed patients before onset of clinical T1D (17). Thus, preclinical detection of microbial changes could become a valuable addition to the present day's seroconversion diagnostics.

To substantiate the contribution of barrier integrity vs. microbial composition to the development of disease, Sorini et al. (7) combined fecal transfer with antibiotic treatment experiments. Remarkably, neither manipulation per se triggered disease. Fecal transfer from DSS-colitic mice into healthy BDC2.5XNOD mice treated previously with antibiotics to remove their own microbiota was not sufficient to induce T1D presumably due to an intact barrier in the host. Furthermore, treatment of TCR-transgenic mice with antibiotics before DSS treatment prevented the development of diabetes, suggesting that both loss of barrier integrity and presence of a changed microbiota are required for pathogenic activation of autoreactive T cells (7).

Following these pioneering observations, it will be of interest to identify the bacterial species responsible for activating diabetogenic T cells and driving them toward a pathogenic role or perhaps, as unrecognized here, toward a protective function similar to what was shown for *Akkermansia* in the NOD model (18). Which intestinal segments are sites of activation for autoreactive T cells in humans? Is it solely the colon or maybe also the ileum? Furthermore, which are the organisms that escape the gut lumen, and by which mechanism do they stimulate diabetogenic T cells to invade pancreatic islets and attack β cells? Finally, the findings reported here have clinical relevance. If indeed the change of the intestinal mucus lining is verified in other experimental models and especially in people with T1D, physicians might wish to correct this deficit, either by manipulating mucus production directly or, better, by eliminating the underlying disturbance. In addition, once translocation of gut bacteria has happened, elimination of the escaped organisms, for example by antibiotics, would be a valid therapeutic aim.

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