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Oral Delivery of IL-27 Recombinant Bacteria Attenuates Immune Colitis in Mice

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BACKGROUND & AIMS: Treatment of inflammatory bowel disease would benefit from specific targeting of therapeutics to the intestine. We developed a strategy for localized delivery of the immunosuppressive cytokine interleukin (IL)-27, which is synthesized actively in situ by the food-grade bacterium *Lactococcus lactis* (LL-IL-27), and tested its ability to reduce colitis in mice. **METHODS:** The 2 genes encoding mouse IL-27 were synthesized with optimal codon use for *L lactis* and joined with a linker; a signal sequence was added to allow for product secretion. The construct was introduced into *L lactis*. Colitis was induced via transfer of $CD4^+CD45RB^{hi}$ T cells into $Rag^{-/-}$ mice to induce colitis; 7.5 weeks later, LL-IL-27 was administered to mice via gavage. Intestinal tissues were collected and analyzed. **RESULTS:** LL-IL-27 administration protected mice from T-cell transfer–induced enterocolitis and death. LL-IL-27 reduced disease activity scores, pathology features of large and small bowel, and levels of inflammatory cytokines in colonic tissue. LL-IL-27 also reduced the numbers of $CD4^+$ and $IL\text{-}17^+$ T cells in gut-associated lymphoid tissue. The effects of LL-IL-27 required production of IL-10 by the transferred T cells. LL-IL-27 was more effective than either LL-IL-10 or systemic administration of recombinant IL-27 in reducing colitis in mice. LL-IL-27 also reduced colitis in mice after administration of dextran sodium sulfate. **CONCLUSIONS:** LL-IL-27 reduces colitis in mice by increasing the production of IL-10. Mucosal delivery of LL-IL-27 could be a more effective and safer therapy for inflammatory bowel disease.

Keywords: Mouse Model; Crohn's Disease; Ulcerative Colitis; Immune Regulation.

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a significant public health problem in Western societies, affecting 1 in 1000 individuals, and is characterized by chronic, nonspecific inflammation in the large and/or small intestine.[1] IBD greatly predisposes to colorectal cancer, in that 20% of ulcerative colitis patients will develop it unless the colon is surgically removed.[2] It is currently thought that IBD represents an atypical inflammatory immune response to normal gut flora.[3,4]

The existing treatments for IBD include anti-inflammatory drugs, immunosuppressive drugs, and, in severe cases, partial or complete resection of the bowel. Use of therapeutics resulting in total immunosuppression risks compromising protection against pathogens such as viruses and bacteria. Selective delivery to the target organ would be desirable. Interleukin (IL)-10, for example, is an anti-inflammatory cytokine that has a protective role in both mouse[5] and human[6] IBD; however, systemic IL-10 treatment has yielded rather disappointing results in multicenter trials,[7,8] most likely owing to low final concentrations of IL-10 in the intestine.

IL-27, a pleiotropic cytokine belonging to the IL-12 family, is composed of IL-27p28 and Epstein–Barr virus–induced protein 3 (Ebi3).[9] It is expressed primarily by antigen-presenting cells and signals through a heterodimeric receptor (IL-27R) that contains a IL-27Rα (WSX-1, T-cell cytokine receptor) subunit and a gp130 subunit, which is shared by several cytokine receptors in the IL-6 family.[10]

IL-27 initially was described as an immune stimulator of T-helper cell (Th)1 responses[9]; however, recent studies have identified mechanisms in which IL-27 has an immunosuppressive role,[11,12] including its ability to antagonize Th17 development,[13–16] induce IL-10 production,[12,16–18] suppress IL-6–induced T-cell proliferation,[13] and promote T-regulatory cell generation.[19] Furthermore, a therapeutic effect in experimental allergic encephalomyelitis,[15] collagen-induced arthritis,[20] and colitis[21] was observed after IL-27 administration, and in a genome-wide association study, low expressing variants of the *IL-27* gene were found to be associated specifically with human early onset IBD.[22]

Abbreviations used in this paper: **DAI, disease activity index; DP, double positive; DSS, dextran sulfate sodium; Ebi3, Epstein–Barr virus–induced protein 3; GI, gastrointestinal; IBD, inflammatory bowel disease; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; LAL, limulus amebocyte lysate; LL, *Lactococcus lactis*; LPS, lipopolysaccharide; MLN, mesenteric lymph node; mRNA, messenger RNA; PBS, phosphate-buffered saline; rm, recombinant mouse; SI, small intestine; Th, T-helper cell.**

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In this study, we investigated mucosal delivery of IL-27 using a well-described delivery system that enables oral delivery of biopharmaceuticals to the gastrointestinal tract by genetically engineered *Lactococcus lactis* (LL).[23–25] We show that LL-IL-27 has a therapeutic benefit in T-cell–dependent chronic enterocolitis, suggesting it may offer a safer, more effective treatment option for IBD patients.

Materials and Methods

Induction of Enterocolitis by T-Cell Transfer, LL Administration

The T-cell transfer model was used to induce enterocolitis as reported by Ostanin et al.[26] Male $Rag^{-/-}$ mice were used for recipients, and female C57BL/6, IL-$10^{-/-}$, or IL-17A/F dual reporter mice were used for donors (see the Supplementary Materials and Methods section for details). Enterocolitis was induced 7–7.5 weeks after cell transfer. We determined that the onset of enterocolitis occurred when mice lost 5% or more of their original body weight and had pasty, semiformed stools. For experiments in which C57BL/6 or IL-$10^{-/-}$ mice were cell donors, *L lactis* administration began after enterocolitis induction and continued with 14 daily gavages (5 days/wk). Tissues were either harvested immediately after death (untreated, LL control) or at 1 or 7 days after gavage (LL-IL-27). For experiments in which IL-17A/F dual-reporter mice were cell donors, *L lactis* administration began at 4 weeks and continued with 14 daily gavages. Tissues were harvested 8 weeks after cell transfer. C57BL/6 and $Rag^{-/-}$ mice not receiving a T-cell transfer were serially gavaged every half hour for 5 hours on day 1 and had 1 gavage on day 2. Tissues were harvested an hour after gavage.

Systemic Treatment With Recombinant Mouse IL-27

Seven weeks after T-cell transfer, $Rag^{-/-}$ mice were injected intraperitoneally daily for 5 days with phosphate-buffered saline (PBS), 500 ng or 1 μg murine recombinant mouse (rm) IL-27 (R&D Systems, Minneapolis, MN). Mice were euthanized 3 days after the final injection and their colons were processed for histopathology analysis.

Histologic Analysis

Tissues (small and large intestine) from mice were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with H&E. H&E tissue sections were evaluated and graded in coded fashion by a veterinary pathologist (M.R.A.). The scoring criteria is listed in the Supplementary Materials and Methods section.

Statistics

Statistical analysis was performed using GraphPad Prism software (version 5.00; GraphPad, San Diego, CA). Data are expressed as ± SEM. The Student 2-tailed, unpaired, parametric *t* test was used to assess statistical differences between 2 experimental groups.

Results

Genetically Engineered L lactis *Express Bioactive IL-27*

Murine IL-27 was synthesized in *L lactis* by incorporating a linker sequence between its 2 chains, and using codons and a secretory signal sequence preferred by *L lactis* (LL-IL-27) (Supplementary Figure 1). Culture supernatants of LL-IL-27 were analyzed by Western blot, showing that LL-IL-27 expressed the Ebi3 (Figure 1*A*, left) and p28 (Figure 1*A*, right) subunits of IL-27 at the predicted molecular weight of the IL-27 hyperkine (48.2 kilodaltons).

LL-IL-27 induced phosphorylation of signal transducer and activator of transcription 1 and 3 (pSTAT1/3), albeit to a lesser degree than rmIL-27, at comparable concentrations (Figure 1*B*). Th1 transcription regulator T-box protein 21, or Tbet, was up-regulated by LL-IL-27 stimulation of naive $CD4^+$ T cells (Figure 1*C*). LL-IL-27 stimulated both IL-10 protein secretion (Figure 1*D*, left) and gene expression (Figure 1*D*, right) to comparable levels as rmIL-27 in $CD4^+$ cells. Neutralizing rmIL-27 and LL-IL-27 with IL-27 antibodies resulted in similar inhibition levels in all functional assays (Supplementary Figure 2), confirming that the bioactivity of LL-IL-27 is mediated by IL-27.

We investigated the localization and ability of LL-IL-27 to induce IL-10 in vivo. Healthy C57BL/6 mice were administered serial gavages of LL-IL-27 and gastrointestinal (GI) tract sections were assayed. The majority of *L lactis* was found in the intestinal lumen (Supplementary Figure 3*A*), more than 80% of gavaged *L lactis* was recovered (Supplementary Figure 3*B*), and increased IL-10 levels were found in intestinal luminal contents of LL-IL-27–treated mice compared with LL control–treated mice (Supplementary Figure 3*C*).

LL-IL-27 Treatment Improves Survival in Murine Enterocolitis

Transferring $CD4^+CD45Rb^{hi}$ T cells from healthy wild-type mice into $Rag^{-/-}$ mice induces a diffuse enterocolitis at 5–8 weeks after T-cell transfer.[27] Gavages of BM9 media[23] (untreated), LL control, or LL-IL-27 were begun 7.5 weeks after naive T-cell transfer and continued for 2 weeks. By week 8 after transfer, untreated and LL control–treated mice began to die or had to be euthanized because of the extent of disease, and by 10.5 weeks all had died of disease. In contrast, LL-IL-27–treated mice were protected from death (Figure 2*A*). A disease activity index (DAI) was used that reflects several parameters of IBD.[28] LL-IL-27–treated mice did not show occult/gross blood in stool and stool consistency was nearly normal, and weight loss was partially relieved, thus contributing to a decreased DAI (Figure 2*B*). Histopathologic analysis of distal colons showed that LL-IL-27–treated mice had normal morphology, whereas untreated and LL control–treated mice had extensive inflammatory infiltration and goblet cell loss (Figure 2*C*). LL-IL-27–treated mice also had less pathology in the small intestine compared with untreated and LL control–treated mice (Figure 2*D*).

To verify whether treatment with LL-IL-27 had a negative consequence on intestinal barrier function, we used the