Modulating gut immunity and neoplasia with oral cytokine adjuvants

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Keywords: cancer, immune therapy, inflammation, oral cytokine, gastrointestinal tract, bioerodible microparticles

Oral administration of particulate IL-10 suppressed polyposis, ameliorated systemic pathology and extended lifespan in APCmin/+ mice. Therapeutic effect was associated with selective activity of IL-10 on intestinal CD4+Foxp3+RORγt+IL-17+ pathogenic T-regulatory cells. Studies were recently extended to a bacterially-driven murine colon adenocarcinoma model with similar results. Clinical implications of these findings are discussed.

Chronic inflammation is a hallmark of gastrointestinal (GI) oncogenesis.1 Danger signals that are produced by hyperproliferative dysplastic enterocytes, and those released when commensal bacteria infiltrate the damaged epithelium, are thought to drive such inflammatory activity. This activity, in turn, can enhance tumor growth.1 Both the myeloid and the lymphoid immune cell subsets contribute to this process via secretion of pro-inflammatory cytokines. Among these, IL-17 and TNFα stand out in that elimination or blockade of these cytokines can result in suppression of tumor progression in pre-clinical models.1,2 The anti-inflammatory properties of IL-10 and its central role in maintaining immune homeostasis in the GI tract are well defined.3 In the context of inflammation-driven intestinal dysplasia, IL-10-production by T-regulatory cells is essential in the control of tumorigenesis.4 Similarly, recombinant IL-10 can ameliorate disease symptoms in murine models of inflammatory bowel disease (IBD).5 However, the results of clinical trials in IBD patients have been disappointing due to dose-limiting systemic toxicity.5 It has been suggested that targeting of IL-10 to the disease microenvironment could circumvent this drawback.

Oral bioerodible sustained-release microspheres have been used to deliver small molecule drugs and peptides to the intestine.6 This approach protects encapsulated biologicals from acid-induced hydrolysis and enzymatic digestion in the stomach, and provides efficient uptake in the intestinal mucosa. The therapeutic utility of this technology in targeting biological macromolecules to the intestinal immune structures, however, has yet to be determined.

We recently demonstrated that oral administration of IL-10-encapsulated polymeric microparticles can ameliorate both local and systemic pathologies in the APCmin/+ mouse model of intestinal polyposis.7 Initial studies revealed that the particles were taken up in the Peyer’s patches (PP) with subsequent localization to the mesenteric lymph nodes (MLN). Homing of the particles to the MLN appeared to involve both passive drainage and active transport by PP dendritic cells (DC) (Fig. 1A). A 4-week treatment regimen resulted in the suppression of polyposis growth and ameliorated anemia, splenomegaly, and cachexia in mice with established disease. Therapeutic effect was associated with the suppression of a TH17-like inflammatory profile. Surprisingly, at the cellular level, treatment correlated with a dramatic reduction in IL-17-producing CD4+Foxp3+RORγt+pathogenic T-regulatory cells but not in conventional TH17 cells. This finding was consistent with previous reports demonstrating a significant role for pgTreg in polyposis and colorectal cancer (CRC).8

APCmin/+ is not considered a bona fide model of CRC as polyps primarily occur in the small intestine and rarely progress to invasive adenocarcinoma.9 This limitation, combined with our failure to observe appreciable particle uptake in the colon, prompted the question whether the above approach would be effective in the treatment of CRC. Consequently, the strategy was tested in the APCmin/+ Bacteroides fragilis (B. fragilis) compound model in which inoculation of mice with an enterotoxic strain of B. fragilis (ETBF) induces the development of invasive colon adenocarcinoma.10 The results demonstrated that treatment not only reduced overall tumor burden in the colon but also blocked the adenoma to adenocarcinoma transition (Fig. 1B). These data also
suggested that direct delivery of the particles to the colonic mucosa was not necessary and that trafficking of IL-10-conditioned MLN cells to the colonic lamina propria (LP) was sufficient to achieve therapeutic effect (Fig. 1A).

Whereas our data established the efficacy of oral IL-10 in the treatment of GI dysplasia, they provided limited insight into the cellular mechanism(s) underlying its antitumor activity. As noted above, an unexpected finding was that treatment had a major impact on pgTreg prevalence but did not result in significant changes to the intestinal TH17 population. This observation, combined with the discovery that depletion of the entire Treg compartment itself reduced polyposis in synergy with IL-10, was interpreted as an evidence of a major role for the IL-10-pgTreg axis in polyp suppression. However, our findings do not exclude the possibility of additional effects on non-pathogenic Treg, myeloid subsets or non-hematopoietic stromal cells. Moreover, in view of the modest long-term survival benefit obtained with IL-10 alone, it is likely that subsets which poorly respond to IL-10 (i.e. TH17) are also involved in disease progression and may need to be targeted separately. Thus, it will be important to define the individual contributions of pgTreg, conventional Treg, TH17 cells, and the non-T-cell compartment to gut dysplasia, particularly in IL-10-treated animals. Finally, it will be of interest to delineate the molecular basis of the preferential susceptibility of pgTreg to IL-10.

Clinically, the technology utilized here is highly tractable in that a wide variety of biological macromolecules and combinations thereof can be simultaneously targeted to the gut-associated lymphoid tissue and the MLN in a controlled manner. For example, in the
context of polyposis and CRC, co-delivery of an IL17-blocking antibody with IL-10 could circumvent TH17 cell resistance to IL-10. Collectively, these studies provide initial proof-of-principle for the efficacy of the above approach in the treatment of GI-associated immune-based disorders; and possibly, of mucosal disorders in general.

Disclosure of Potential Conflicts of Interest
NKE has ownership interest in TheraPyX, Inc., which is developing cytokine-encapsulated microsphere technology for commercial use.

Funding
This work was supported by the NIH/NIAID grant AI092133 to N.K.E.