Werewolf THERAPEUTICS

Shifting the Balance In Cytokine Therapeutics

AAI 2024

Abstract # 6850

Development of Conditionally Active IL-10 INDUKINE® Molecules for the Treatment of Inflammatory Bowel Disease

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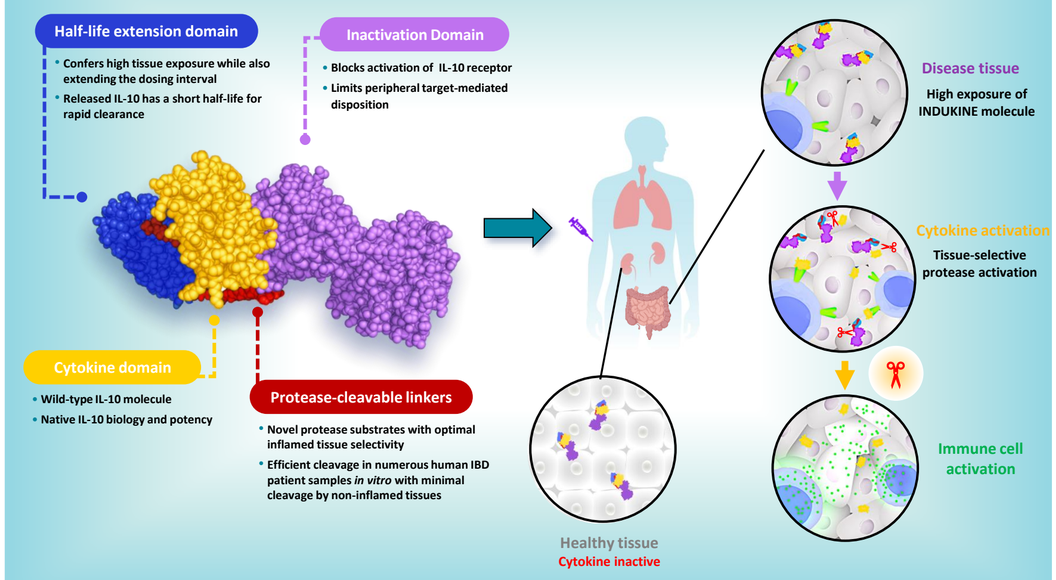
BACKGROUND

Development of IL-10 INDUKINEs for the Treatment of IBD

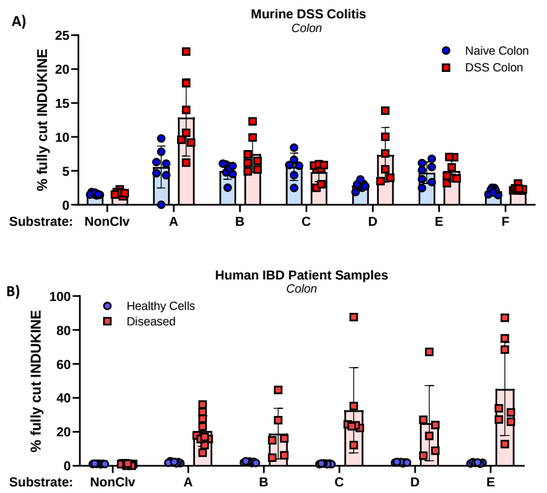
Inflammatory bowel disease (IBD) encompasses two chronic inflammatory diseases of the gastrointestinal tract, Ulcerative Colitis (UC) and Crohn's disease. Although great strides have been made in the treatment of IBD, subsets of patients do not respond or lose responsiveness to therapy, underlining a need for new treatments. Interleukin 10 (IL-10) is an immunoregulatory cytokine, known to suppress and inhibit inflammatory responses of a variety of immune cells including monocytes, macrophages, and T cells. Polymorphisms associated with reduced expression of IL-10 and IL-10R have been linked with IBD. Clinical trials using recombinant IL-10 in IBD have been unsuccessful, highlighting a need for further engineering to utilize IL-10 as a therapeutic agent. We have developed IL-10 INDUKINE molecules that are designed as prodrugs containing human IL-10, an inactivation domain, and a half-life extension domain tethered together by protease-sensitive linkers. IL-10 INDUKINE molecules are designed to be peripherally inactive until the linkers are cleaved in the inflamed colon due to the dysregulation of the protease milieu, releasing IL-10 locally. We have identified proprietary linkers which are cleaved by UC and Crohn's disease human colon samples. In mouse models of colitis, IL-10 INDUKINE molecules prevent weight loss, intestinal damage and inhibit inflammatory cytokine production within the colon. Together these data support the potential of an IL-10 INDUKINE molecule for the treatment of IBD.

INDUKINE Molecules:

Targeting Cytokine Activity to Disease Tissue

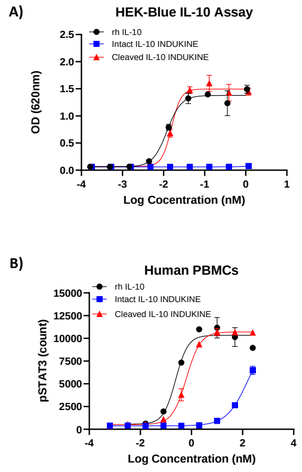


Proof of Concept Inflammatory Disease Linkers are Preferentially Cleaved by Inflamed Tissues



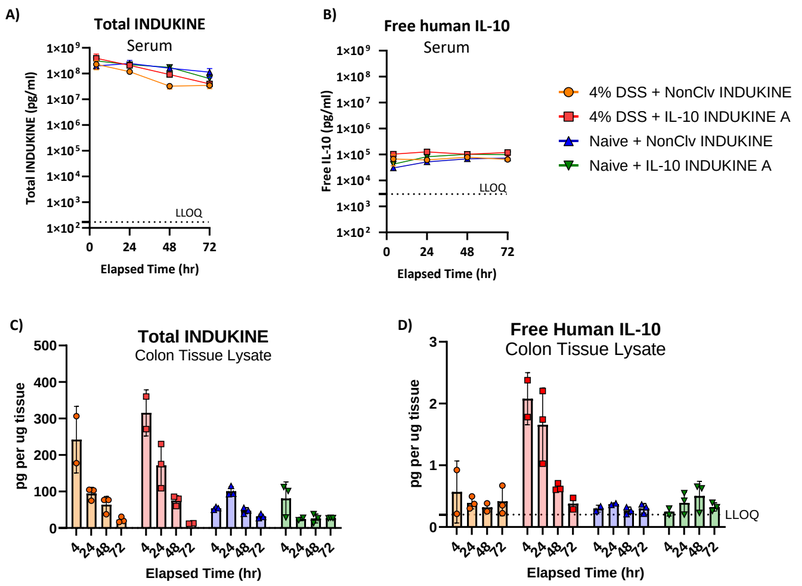
*Indicated tissues were dissociated into single cell suspensions and incubated for 72 hours with IL-10 INDUKINE molecules with different linker substrates. Cleavage of linkers assessed by release of free IL-10 measured by capillary western blot. Cleavage of linkers by **(A)** murine colons from naïve (blue) or 4% DSS treated mice at day 10 (red), **(B)** healthy human cells (blue) or human colon samples from patients with Ulcerative Colitis (UC) or Crohn's Disease (red).*

Inducibility of POC IL-10 INDUKINE Molecule



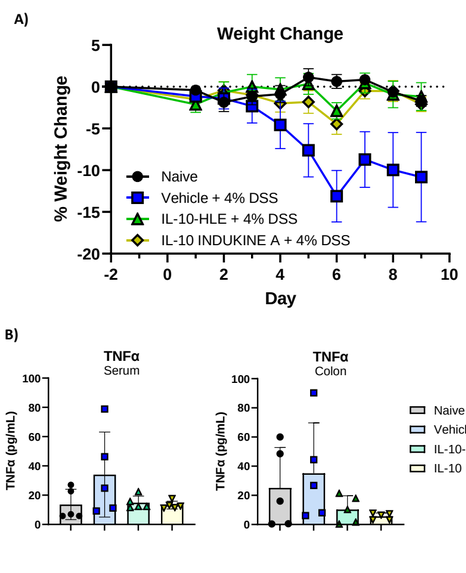
***(A)** In vitro activity of IL-10 INDUKINE molecule in the HEK-Blue IL-10 reporter assay or **(B)** measurement of pSTAT3 in human PBMCs after incubation comparing intact IL-10 INDUKINE (blue squares) and in vitro cleaved/activated IL-10 INDUKINE (red triangles) to recombinant IL-10 (black circles).*

Favorable Exposure and Activation of IL-10 INDUKINE Molecules in Inflamed Colons



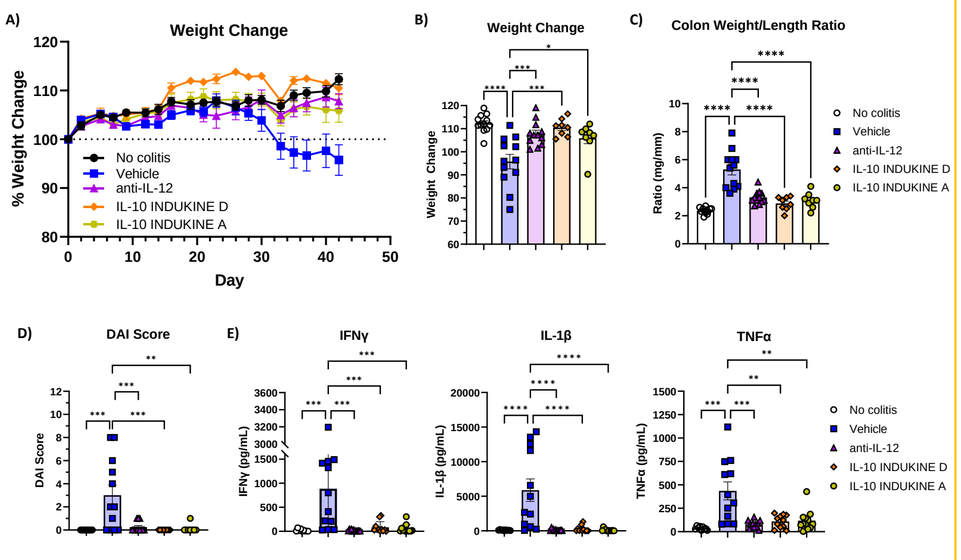
*C57BL/6 mice were randomized into treatment groups, and colitis was induced by providing mice with water containing 4% DSS for 6 days. Animals were dosed with equimolar non-cleavable IL-10 INDUKINE or POC IL-10 INDUKINE A for a single dose on day 10. Serum and colon samples were collected at indicated timepoints. Total INDUKINE from **(A)** serum or **(C)** colon lysate and free IL-10 from **(B)** serum or **(D)** colon lysate measured by custom ECLIA assay.*

POC IL-10 INDUKINE Molecule Prevents Damage in DSS Induced Colitis Model



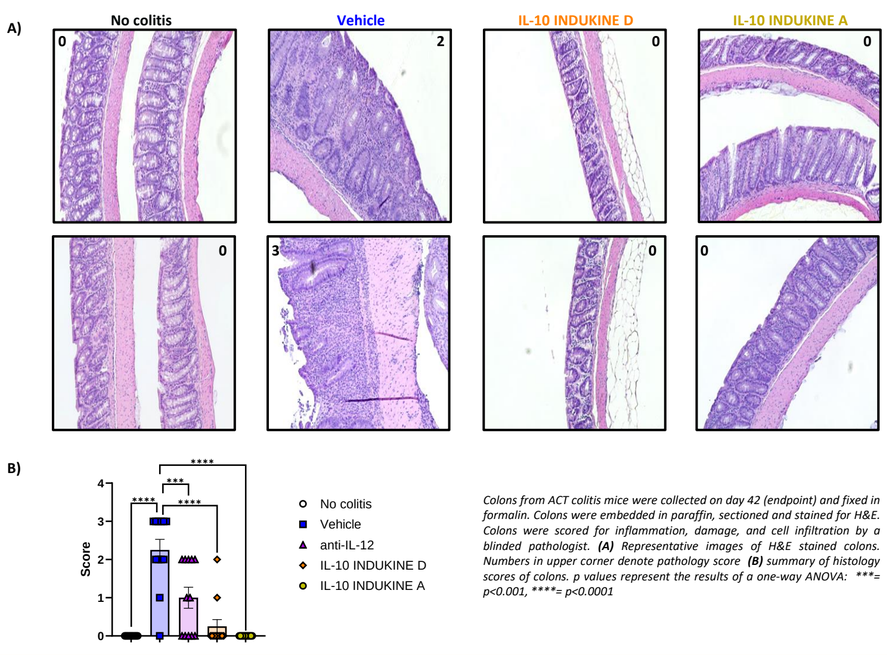
*IL-10KO Balb/c mice were given normal water as control (naïve) or water with 4% DSS for 6 days to induce colitis. Animals were dosed IP with half life extended IL-10 (IL10-HLE) or IL-10 INDIKINE A on day 1 and 6. Animals were monitored daily for body weight changes. Animals were sacrificed on day 10, serum and colons collected. **(A)** Body weight changes of animals **(B)** TNFα levels in the serum or colons of animals.*

POC IL-10 INDUKINE Molecules Inhibit Colitis in Adoptive Cell Transfer Model



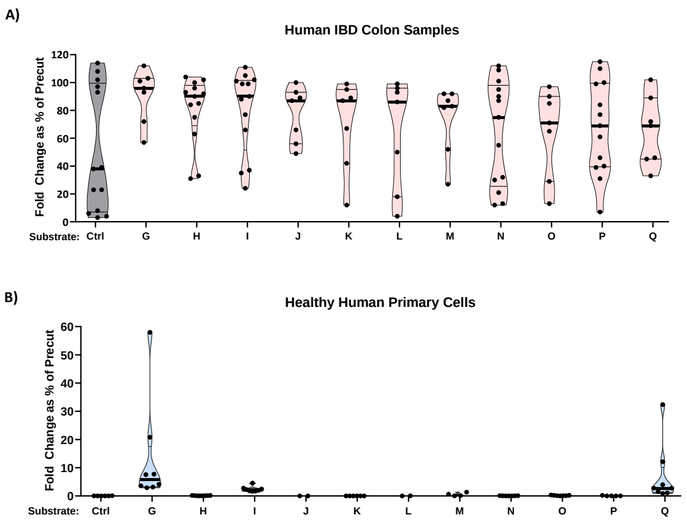
*ACT Colitis induced by transferring naive T cells (CD4+ CD25- CD62Lhi) into RAG$_2$KO mice. Mice were dosed once weekly with anti-IL-12 or with INDUKINE twice weekly starting on day 14 for 3 weeks. **(A)** Percent weight change over time **(B)** Percent weight change at study endpoint, day 42 **(C)** Colon weight/length ratio at day 42. **(D))** Disease Activity Index (DAI) Score at day 42. DAI score is a combination of weight loss and stool score at indicated timepoint. **(E)** Levels of inflammatory cytokines in colon tissue lysate at day 42. p values represent the results of a one-way ANOVA: *=p<0.05, ** = p<0.005, ***= p<0.001, ****= p<0.0001*

Reduced Colon Damage and Immune Infiltrates After IL-10 INDUKINE Treatment in ACT Colitis



*Colons from ACT colitis mice were collected on day 42 (endpoint) and fixed in formalin. Colons were embedded in paraffin, sectioned and stained for H&E. Colons were scored for inflammation, damage, and cell infiltration by a blinded pathologist. **(A)** Representative images of H&E stained colons. Numbers in upper corner denote pathology score **(B)** summary of histology scores of colons. p values represent the results of a one-way ANOVA: ***= p<0.001, ****= p<0.0001*

Novel IBD Specific Linkers Preferentially Cleaved by Human IBD Samples



*Dissociated colon samples from patients with IBD or healthy human primary cells were cultured with IL-10 INDUKINEs containing different linker substrates. Cleavage was measured by activity of free IL-10 in HEK-Blue IL-10 reporter assay. Data was normalized to pre-cute control. **(A)** Cleavage of linkers by colon samples from human IBD patient samples. **(B)** Cleavage of linkers by healthy human primary cells.*

SUMMARY and CONCLUSIONS

- POC IL-10 INDUKINE molecule demonstrates *in vitro* inducibility and activity
- Demonstrated differential cleavage of linker substrates between inflamed colon tissue and healthy cells with both mouse and human samples
- POC IL-10 INDUKINE molecule is inactive in the periphery and shows selective release of free IL-10 in inflamed colon
- POC IL-10 INDUKINE molecules inhibit inflammation in two murine models of IBD
- We have discovered novel linker substrates which are selectively cleaved by human colon samples from patients with IBD
- IL-10 INDUKINE molecule lead optimization ongoing for IBD

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