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Poster for you

A biochemical basis for the hepatoprotective effects of REP 2139 during host mediated transaminase flares

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Introduction/Summary

- The nucleic acid polymers (NAP) REP 2139 blocks the assembly and secretion of HBV subviral particles. High rates of HBsAg loss are uniquely achieved with REP 2139 and when used in combination with TDF and pegIFN, lead to almost universal occurrence of host mediated transaminase flares and high rates of functional cure of HBV and cure of HDV. The universally well tolerated nature of these transaminase flares, regardless of magnitude and or duration suggest additional hepatoprotective effects with REP 2139.
- The hallmark protein interaction domain for NAPs is uncomplexed amphipathic alpha helical domains (Figure 1A). These domains have been previously shown to underly the broad-spectrum anti-infective activity of NAPs in a variety of viral infections (via type 1 viral glycoproteins), prion disease (via prion proteins), inhibition of HBV subviral particle assembly (via DNAJB12) and HDV replication (via HDAg). Analogous domains are involved in the dimerization (and activity) of a variety of cytokines and chemokines. NAP interaction with diverse cytokines and chemokines was evaluated.

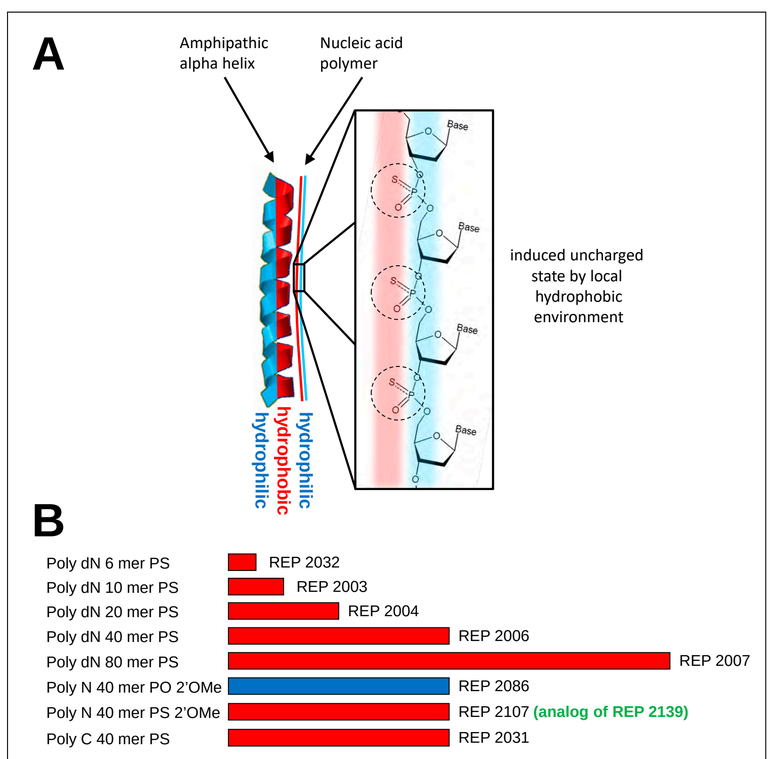

Figure 1. (A) Binding interface of NAPs showing hydrophilic (blue) and hydrophobic (red) features. (B) Fluorescent NAP analogues used in FP binding experiments. PS, phosphorothioate; PO, phosphodiester, 2'OMe; 2' O methyl ribose.

Methods

- Solution interaction of NAPs with purified recombinant human cytokines was evaluated by fluorescence polarization (FP) using a series of CY3 labelled prototypic NAP compounds (Figure 1B) used to identify size dependent amphipathic interactions. This FP interaction assay has been validated to predict biochemical activity of NAPs in a variety of in vivo animal models and in human HBV / HDV infection.

Results

- Interaction of the prototypic degenerate 40mer NAP REP 2006 with a variety of cytokines was observed with the strongest interactions measured for IL1β, IL4, IL6, IL8, IL13, IL23 and CCL2, 5 and 11 (Figure 2).
- These cytokine interactions were size dependent (Figures 3 and 4), with longer NAPs exhibiting stronger binding and sequence independent with similar interaction observed for degenerate (REP 2006) and poly C (REP 2031) NAPs (Figures 4 and 5). Interactions were dependent on the presence of phosphorothioation (required for induced hydrophobicity but were not altered by the presence of 2'O methyl ribose modification (present in REP 2139).

Conclusions

- **NAPs (including REP 2139) interact with a variety of pro-inflammatory cytokines with similar hydrophobic interactions as observed previously for other target proteins (HDAg and DNAJB12).**
- **These cytokine interactions likely block receptor activation by these cytokines. The demonstrated liver accumulation of REP 2139 during therapy may establish a hepatoprotective buffer protecting the liver from pro-inflammatory activity which can accompany host mediated transaminase flares.**
- **These potential hepatoprotective effects many also play a role in the safety of NAP therapy and associated pegIFN therapy in compensated and decompensated cirrhosis.**

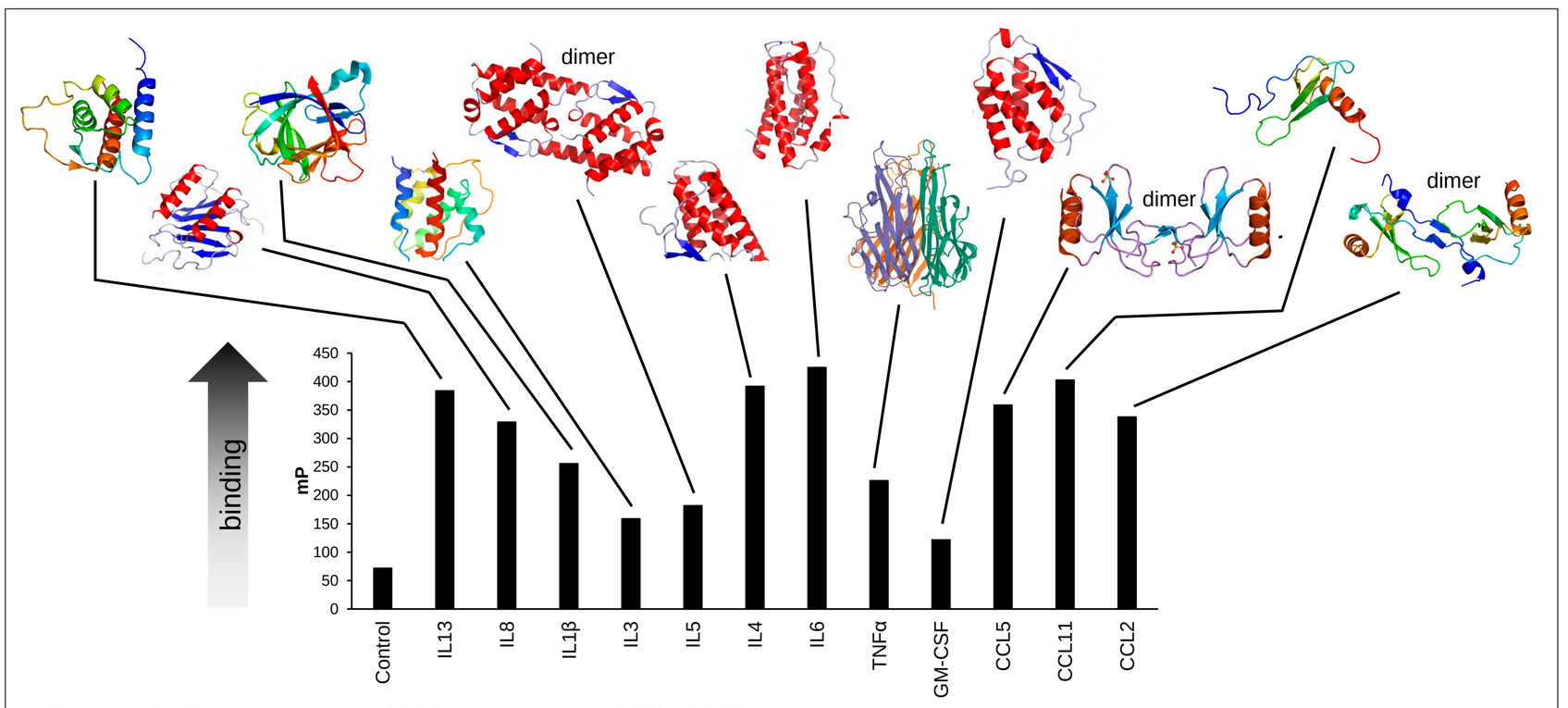

Figure 2. Surveillance of NAP interaction (REP 2006) with a diverse array of human recombinant cytokines and chemokines via fluorescence polarization. Crystal structures of these cytokines are indicated at the top.

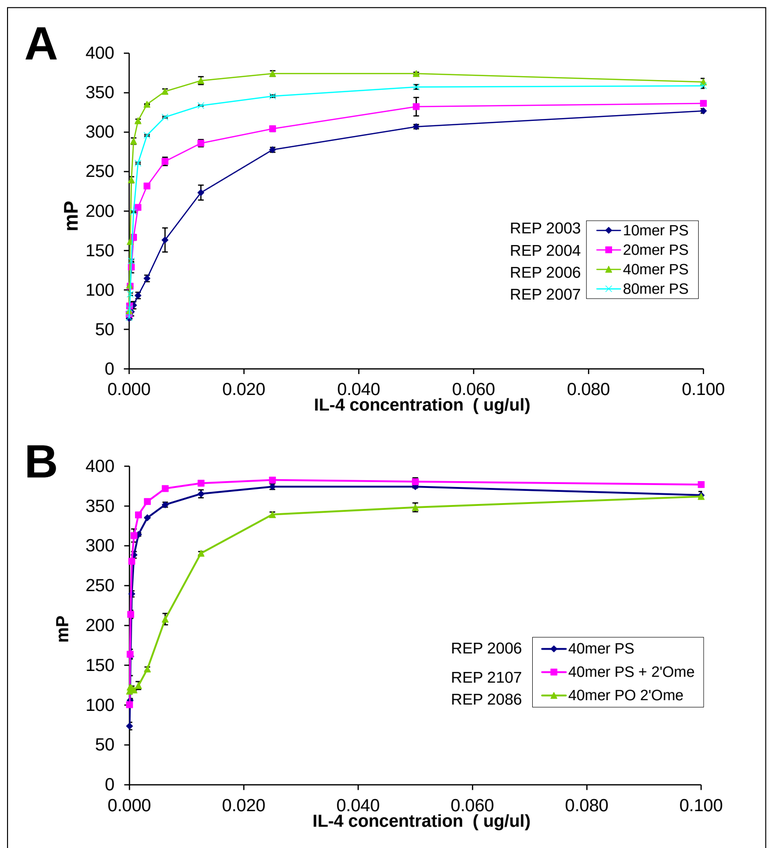

Figure 3. NAP interaction with IL4 showing size dependent (A) and hydrophobic dependent (B) binding properties.

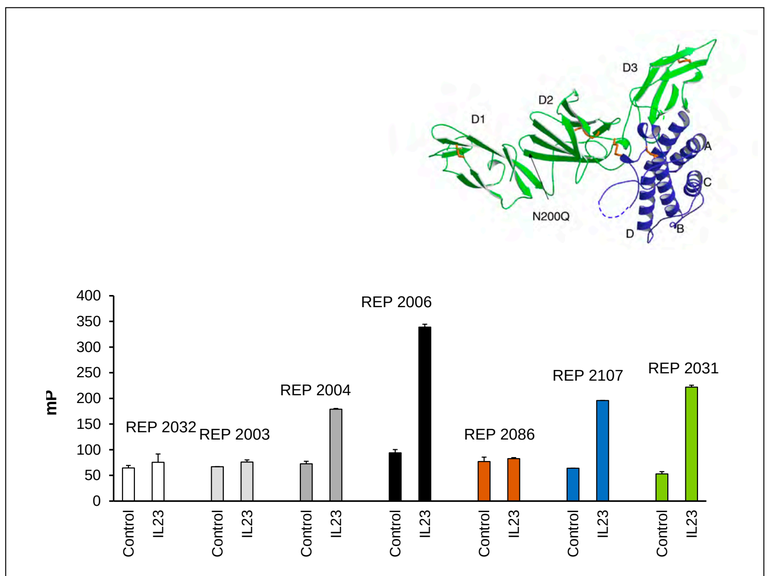

Figure 4. NAP interaction with IL23 showing size dependent (A) and hydrophobic dependent binding properties. The crystal structure for IL23 is indicated at the top.

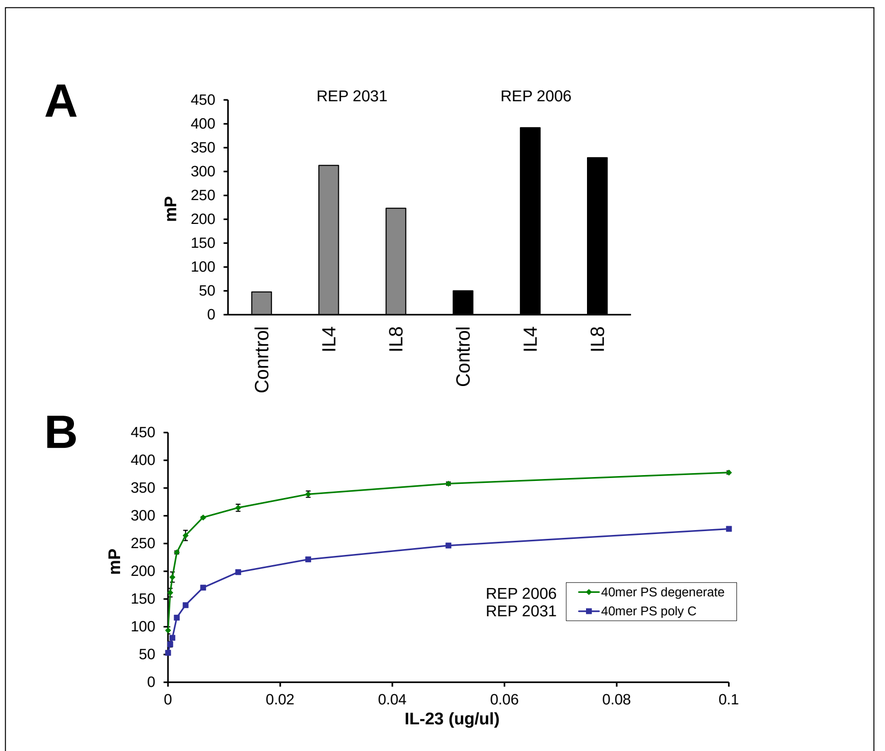

Figure 5. Sequence independent NAP interaction with IL4 and IL8 (A) and IL23 (B) demonstrated with REP 2006 (degenerate) and REP 2031 (poly C) NAP species