A Superagonist Antibody to Human Interleukin-21 Increases HIV-Specific T-Cell Function

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Abstract

Interleukin-21 (IL-21) is a cytokine predominantly produced by activated CD4 T cells. It has a broad range of immuno-stimulatory properties that is required to maintain cytotoxic immunity and to prevent exhaustion during chronic infection. We took a novel approach to develop a unique agonistic antibody, 2P2 to human IL-21 (hIL-21), which enhances bioactivity of hIL-21 to 20-50 folds *in vitro* and significantly enhanced the half-life and bioactivity of hIL-21 *in vivo*.

Discovery of the mAb 2P2 that enhances hIL-21 bioactivity

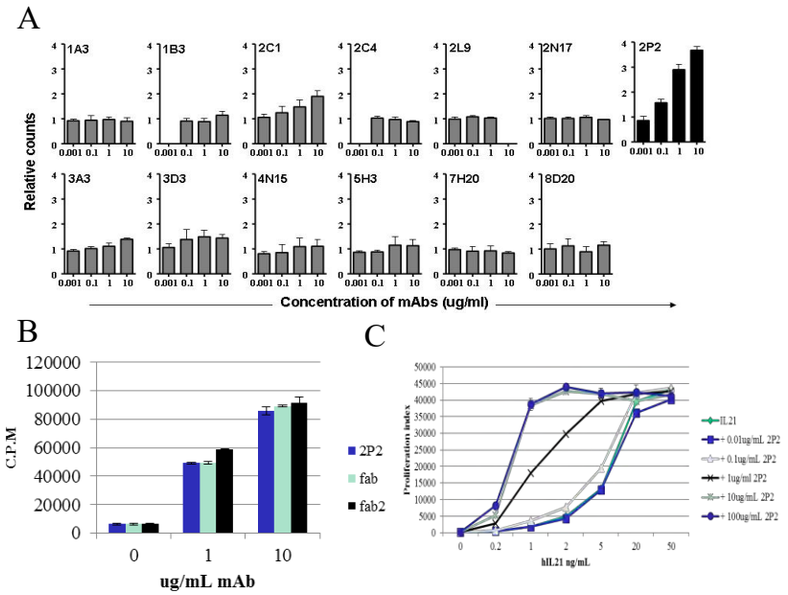


Figure 1. 2P2 enhanced hIL-21 bioactivity *in vitro*. (A) Screening of agonistic antibody from anti-hIL-21 antibody library. (B) Fab, Fab'2 and whole 2P2 antibody induced the same agonistic effect. (C) Increasing concentration of 2P2 further enhanced hIL-21 activity, up to 20-50 times.

Altered confirmation of hIL-21 upon 2P2 binding

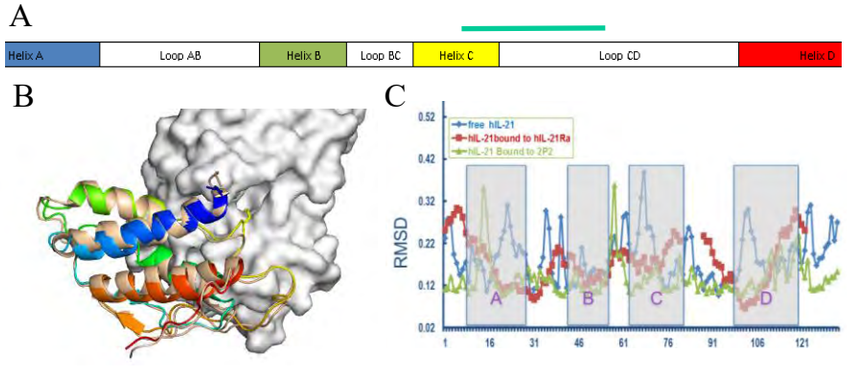


Figure 2. Proposed molecular mechanism of 2P2's agonistic effect. (A) Schematic structure of hIL-21. Cyan line represents epitope of 2P2. (B) Change of the hIL-21 conformation after binding with its agonist antibody 2P2 (in grey). Free form hIL-21 in golden cartoon was overlaid with the bound form in spectral color from blue (N-term) to red (C-term). (C) The flexibility of hIL-21 bound with 2P2 was altered to resemble with that of IL-21 bound with its alpha receptor, especially at part of helix A and D.

2P2 boosted hIL-21 to promote HIV-1 specific immunity

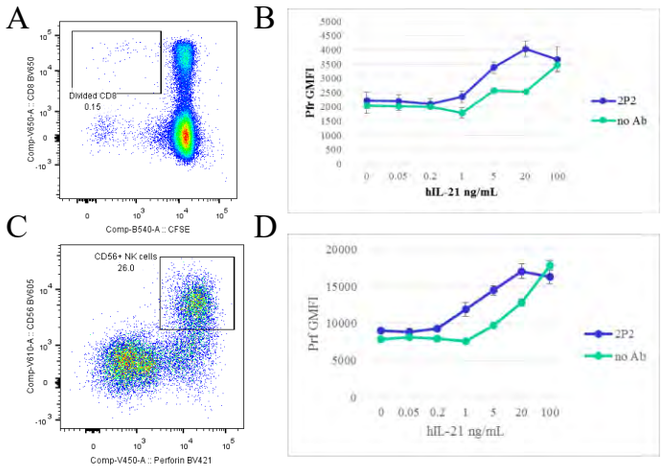


Figure 3. 2P2 increased HIV-specific CD8 and NK cells' perforin expression. PBMCs from cART treated patiences labelled with CFSE followed by 5 days cultured in the presence of MHCI-restricted GAG peptides pool. (A) Gating of proliferated CD8 T cells. (B) Mean fluorescence intensity (MFI) of perforin was enhanced by 2P2 in proliferated CD8 T cells. (C) Gating of CD56+ NK cells. (D) MFI of perforin was enhanced by 2P2 in NK cells.

Anti-viral activity of hIL-21/2P2 complex *in vivo*

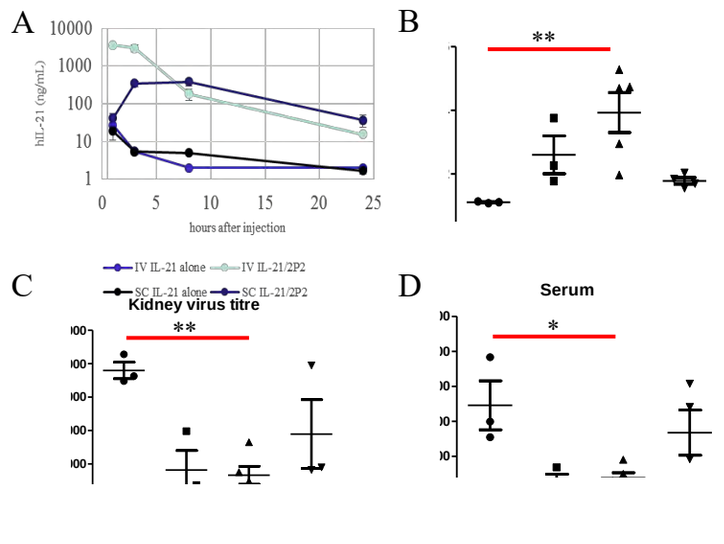


Figure 4. *In vivo* agonistic activity of 2P2. (A) Mice were injected with hIL-21 alone or hIL-21/2P2 complex via I.V. or S.C. followed by measuring hIL-21 in serum. Mice were infected with chronic lymphocytic choriomeningitis virus (LCMV) followed by treatment with hIL-21, hIL-21/2P2 or hIL-21/3A3 complexes. IFNg expression on virus specific CD8 T cells (B), and kidney (C) or serum (D) virus titre were measured on day 42 post-infection. *P < 0.05, **P < 0.01.

Conclusion

- 2P2 increased activity of hIL-21 up to 20-50 times *in vitro*, cross-linking of hIL-21R is not required.
- 2P2 may stabilize the tertiary structure of hIL-21 to a more bioactive form.
- 2P2 increased cytotoxicity of HIV-1 specific CD8 T cells and NK cells.
- 2P2 increased *in vivo* half-life of hIL-21.
- Only 2P2, but not control antibody, increased the number of functional virus-specific CD8 T cells and reduced viral load.
- 2P2 is a promising therapeutic to enhance IL-21-based immunotherapy for chronic infection.

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