#6501 IL10/IL2 Surrogate Cytokine Agonists

Mahalakshmi Ramadass, Sandro Vivona, Deepti Rokkam, Heiko Greb, Anu Gangopadhyay, Romina Riener, Rene de Waal Malefyt, Scott McCauley, Patrick Lupardus, Martin Oft and Robert Kastelein.

Synthekine

1505 O'Brien Dr, Menlo Park, CA, 94025

ABSTRACT

Introduction

IL10 is a pleiotropic cytokine that is secreted as a homodimer and initiates signaling on various cell types by engaging two copies of a heterodimeric receptor complex consisting of an IL-10Rα subunit and an IL-10Rβ subunit. IL10 signaling through the natural ligand has both immunosuppressive and immunostimulatory effects and this pleiotropy makes it difficult to develop wild type IL-10 for therapeutic use. In clinical trials that have studied IL10 in cancer, anemia, thrombocytopenia and hemophagocytosis associated with macrophage activation led to dose interruption and dose reduction. To drive more selective cytokine signaling only on cells of interest, we have generated surrogate cytokine agonists that bind to IL10Rα and IL2Rγ, resulting in the selective activation of T cells over monocytes. We have established a novel means of bridging the IL10Rα and IL2Rγ receptors using a IL10Rα/IL2Rγ surrogate cytokine agonist that consists of a domain that binds to IL10Rα linked to a second domain that binds to IL2Rγ. Upon contact with a cell that allows bridging of IL10Rα and IL2Rγ, the surrogate cytokine agonist causes the functional association of IL10Rα and IL2Rγ, resulting in downstream signaling in select cell types and drives unique biology that has therapeutic potential.

Experimental Procedures

Human IL-10Rα and IL-2Rγ specific single domain VHHs were generated by camel immunization and screening of VHH libraries prepared from peripheral blood cells for binding. Seven IL-10Rα VHHs and six IL-2Rγ VHHs were identified and coupled as IL10Rα/IL2Rγ dual VHHs in an all by all matrix and in both amino-carboxy and carboxy-amino orientations, yielding 84 unique surrogate cytokine agonists. These surrogate cytokine agonists were screened on a (p)STAT3 assay on primary human cells and further tested in T cell and monocyte functional assays.

Results

Several IL10Rα/IL2Rγ surrogate cytokine agonists were biologically active on primary human lymphoid cells, inducing (p)STAT3 signal in B cells, NK cells, CD4 and CD8 T cells with little to no (p)STAT3 signal in monocytes. These IL10Rα/IL2Rγ surrogate cytokine agonists were also functionally active in promoting cell survival and at inducing IFN-g and Granzyme production by CD8 T cell blasts generated upon CD3/CD28 activation. Consistent with the lack of STAT3 signaling in monocytes, the IL10Rα/IL2Rγ surrogate cytokine agonists did not inhibit LPS induced secretion of IL1β and TNFα by monocytes, suggesting selectivity and a lack of immunosuppressive activities.

Conclusions

Designing surrogate cytokine agonists that pair non-natural cytokine receptors provides the possibility of generating molecules that can decouple the pleiotropy of cytokines like IL10 by stimulating only the desired cell population. Here, we have generated IL10Rα/IL2Rγ surrogate cytokine agonists that are biologically active and signal with varying strengths in the lymphoid cells with little to no activity on monocytes, thus providing an opportunity to decouple the pleiotropy of IL10 for use in cancer therapy.

RESULTS

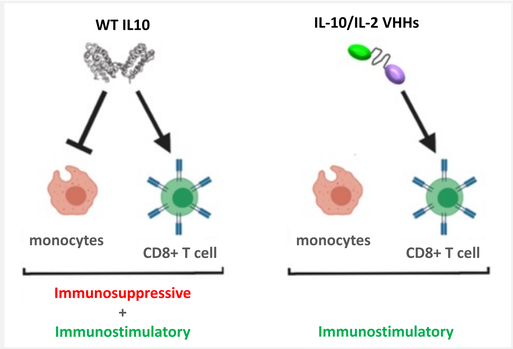

Figure 1. **IL10/IL2 Surrogate Cytokine Agonists.** In order to decouple the pleiotropy of IL10, surrogate cytokine agonists were designed to allow non-natural pairing of the IL10R1 and IL2R**γ** chains in a cytokine-independent manner. Pairing of IL10R1 and IL2R**γ** chains together generates a T-cell biased IL10 that lacks immunosuppressive functions on monocytes.

Generation and Construction of IL-10R1/IL-2Rγ Surrogate Cytokine Agonists

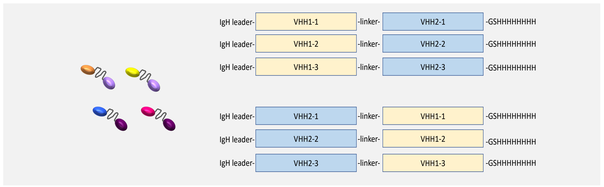

Figure 2. Seven IL-10R1 VHHs and six IL-2R**γ** VHHs were paired together in an all by all matrix and in both (IL-10Rα amino/ IL-2Rγ carboxy and IL-10Rα-carboxy/IL-2Rγ-amino) orientations, yielding 84 unique dual VHH dimers.

Binding Kinetics of IL-10R1 and IL-2Rγ specific VHH segments

Ligand	k_{ON} (1/Ms)	k_{OFF} (1/s)	Affinity (nM)
IL-10R1	9.84E+04	1.36E-04	**1.4**
	1.06E+05	1.90E-04	**1.8**
	3.27E+05	1.97E-03	**6**
	1.68E+05	1.07E-03	**6.4**
	1.54E+05	1.19E-03	**7.7**
	9.97E+04	1.15E-03	**11.6**
	1.04E+05	1.39E-03	**13.4**
IL-2Rγ	3.66E+05	8.12E-04	**2.2**
	2.85E+06	6.93E-03	**2.4**
	5.54E+05	3.54E-03	**6.4**
	1.92E+05	2.70E-03	**14.1**
	6.56E+04	1.13E-03	**17.2**
	9.68E+04	2.51E-03	**26**

Figure 3. Binding kinetics of IL10-R1 and IL-2Rγ VHHs. Kinetics table summarizing the association and dissociation rate constants and affinity constants of each of the seven IL-10R1 VHHs and six IL-2R**γ** VHHs. The experiments were conducted on a Biacore T200 instrument equipped with a Protein A chip. VHH-Fc fusions were captured on the chip and IL-10R1 and IL-2R**γ** extracellular domains were injected ranging from 3.7 to 300 nM. Measurements were performed in single cycle kinetics mode.

IL-10R1/IL-2Rγ Surrogate Cytokine Agonists signal in CD8 T cells but not Monocytes

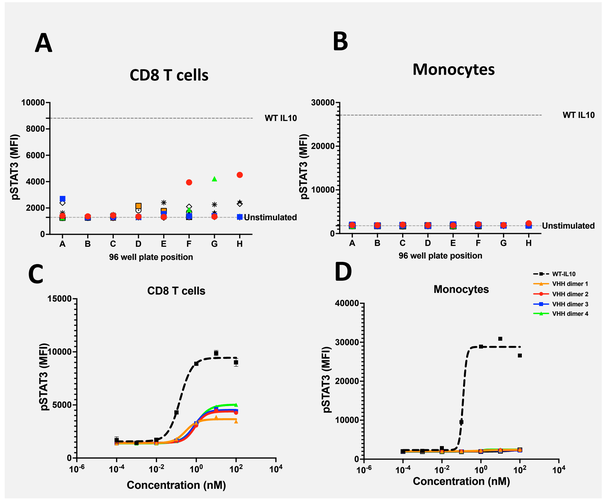

Figure 4. IL10-R1/IL-2Ry Surrogate Cytokine Agonists signal in CD8 T cells but not Monocytes. 84 unique VHH dimers were screened for pSTAT3 signaling on human CD8 T cells **(A)** and human monocytes **(B)** at a 100nM concentration. Several Surrogate Cytokine Agonists activated pSTAT3 signaling in CD8 T cells but not on monocytes. The top 4 hits from the screen were tested in a dose response for pSTAT3 signaling in CD8 T cells **(C)** and monocytes **(D).** The Surrogate Cytokine Agonists signaled with varying potencies and Emax in the CD8 T cells while not activating monocytes.

IL-10R1/IL-2Rγ Surrogate Cytokine Agonists are functional in CD8 T cells but not Monocytes

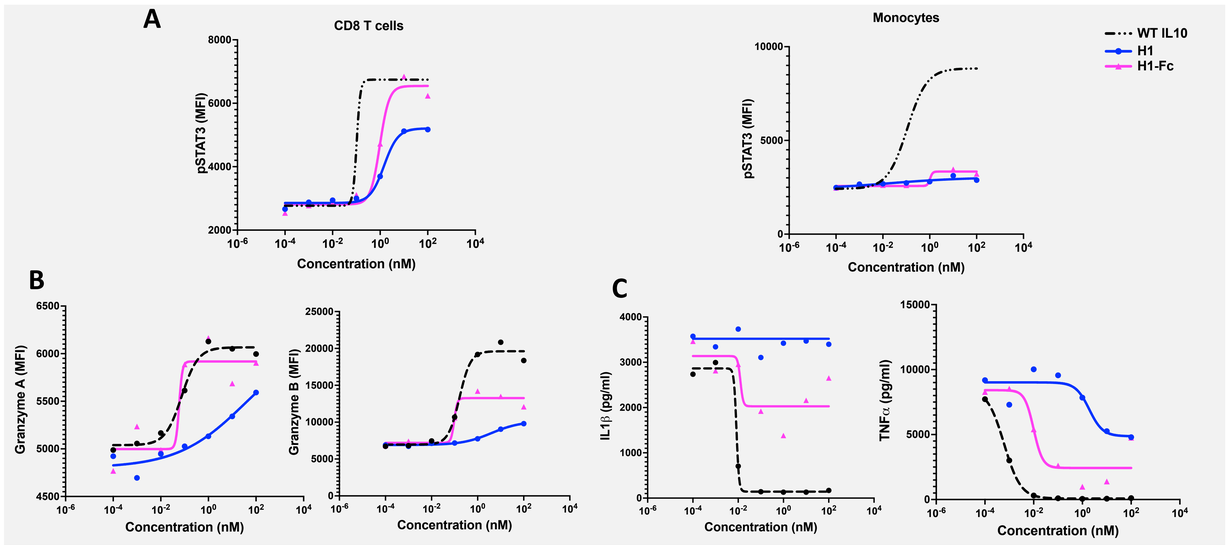

Figure 5. IL-10R1/IL-2Rγ Surrogate Cytokine Agonists activate CD8 T cells but not Monocytes. IL-10R1/IL-2R**γ** Surrogate Cytokine Agonist (H1) and the Surrogate Cytokine Agonist on an Fc backbone (H1-Fc) activate pSTAT3 signaling in human CD8 T cells but not in human monocytes. The H1-Fc molecule has an enhanced Emax equal to that of the WT IL10 on CD8 T cells **(A).** CD8 T cell blasts activated for 3 days with **α**-CD2/**α**-CD3/**α**-CD28 were treated with WT IL10 or Surrogate Cytokine Agonists for 72 hours. The Surrogate Cytokine Agonist (H1) induces Granzyme A and Granzyme B at low levels while H1-Fc is more potent at inducing Granzyme A and Granzyme B in human CD8 blasts **(B).** The Surrogate Cytokine Agonist (H1) did not inhibit LPS-induced secretion of IL1**β** and TNF**α** in human Monocytes treated with LPS for 48 hours. H1-Fc, though a weak suppressor, did not completely inhibit LPS-induced monocyte secretion, including at high concentrations **(C).**

The IL-10/IL-2 Surrogate Cytokine Agonist selectively activates T cells

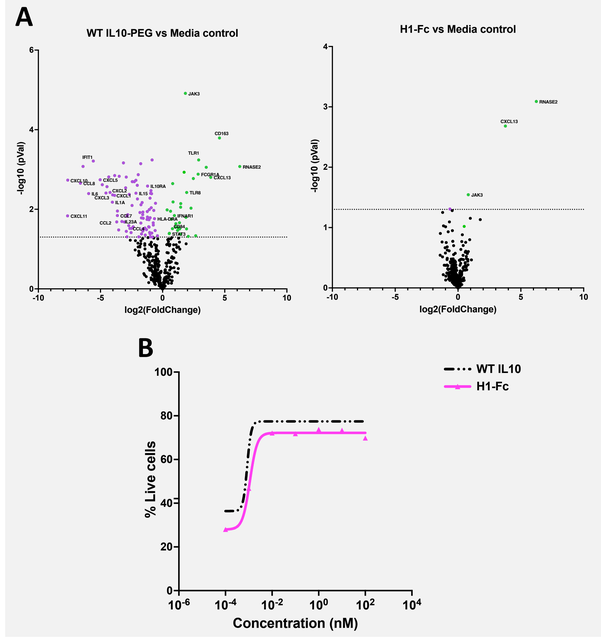

Figure 6. The IL-10/IL-2 Surrogate Agonist selectively activates T cells. Nanostring analysis of human monocytes treated with 10 nM WT IL10 or 10 nM H1-Fc (for 6 hours) using the myeloid innate immune panel that includes 770 genes shows that WT IL10 alters several genes in monocytes, while the surrogate cytokine agonist (H1-Fc) is generally silent on monocytes **(A).** CD8 human blasts activated for 3 days with **α**-CD2/**α**-CD3/**α**-CD28 were treated with either WT IL10 or H1-Fc for 72 hours. The surrogate cytokine agonist (H1-Fc) was as potent as the WT IL10 at inducing survival of activated CD8 T cell blasts **(B).**

The IL-10/IL-2 Surrogate Cytokine Agonist expands human CD8 T Cells in-vivo

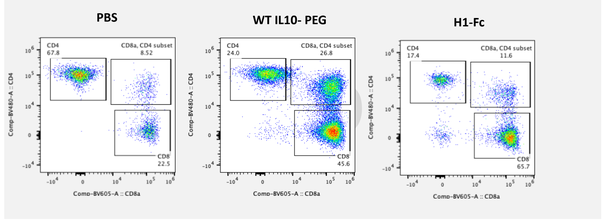

Figure 7. The IL-10/IL-2 Surrogate Agonist expands CD8 T Cells in-vivo. 10^7 human PBMCs were implanted into the tail vein of NSG mice and the mice were dosed either with PBS, WT IL10 (3.3 **μ**g) or H1-Fc (100 **μ**g), twice a week for 8 weeks in a Graft-vs-host disease (GVHD) model. The proportion of CD8 T cells in the blood was analyzed by staining and flow cytometry during final takedown. At the concentration tested, IL10/IL2 surrogate cytokine agonist (H1-Fc) was more potent than WT-IL10 at expanding CD8 T cells.

CONCLUSIONS

We have generated a series of IL10/IL2 surrogate cytokine agonists that are capable of signaling through the non-natural receptor pair IL10R1 and IL2Ry. Functional characterization of the IL10/IL2 surrogate cytokine agonist on the Fc backbone shows that the molecule

- Induces pSTAT3 signaling in human CD8 T cells with similar Emax values as the WT IL10 without signaling on monocytes.
- Activates human CD8 T cells blasts to secrete Granzymes and induces their survival.
- Causes expansion of human CD8 T cells in vivo in a Graft-Vs-Host Disease (GVHD) model.
- Does not cause gene expression pattern changes in monocytes and fails to inhibit LPS induced cytokine secretion in monocytes.

REFERENCES

IL-10 exacerbates xenogeneic GVHD by inducing massive human T cell expansion Abraham et. Al., Clin Immunol. 2015 Jan; 156(1): 58–64.