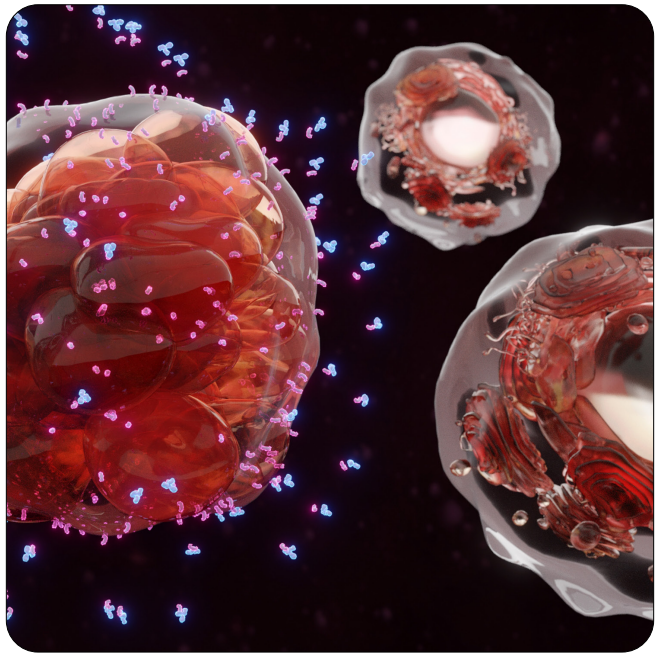
Autoimmune diseases addressed with cytokine HTRF assays

Abstract

Autoimmune diseases result from disorders in the coordination, regulation and aiming of inflammatory and immune responses. These mechanisms are complex, usually tightly regulated by numerous interactions at the cellular or protein level, as well as by significant intracellular pathways. Though this complexity makes such immune disorders difficult to address, the intricate networks of interactions, mediators and pathways involves key spots that offer attractive drug discovery targets.

Cytokines are the main mediators of inflammation. One of the most successful approach of autoimmune diseases research has been to screen for compounds inhibiting their secretion or blocking their receptors and signal transduction, thus lowering the overall inflammatory response. The most essential of these mediators and therefore the most investigated include a handful of interleukins and chemokines.



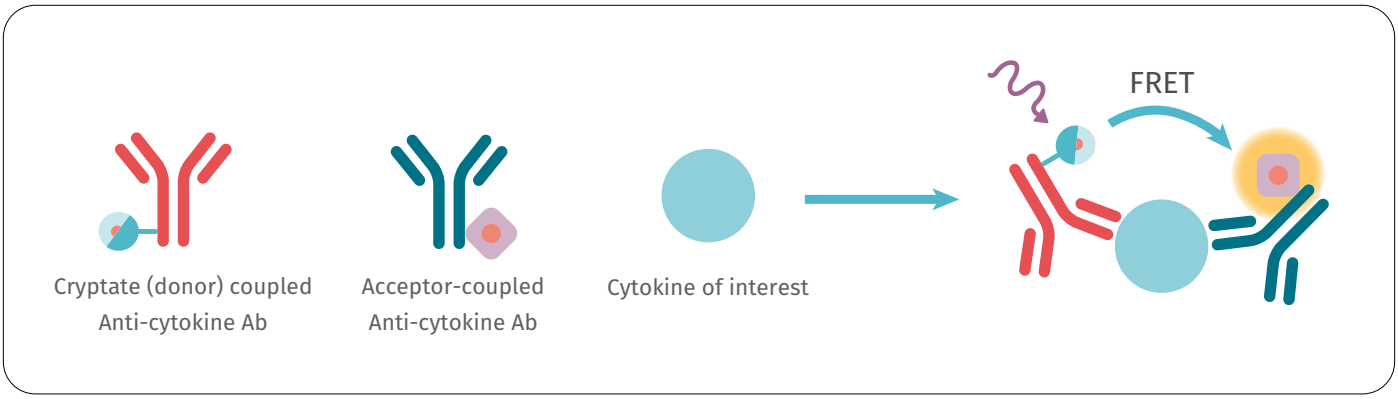


Figure 1: Principle of an HTRF® cytokine assay. Both antibodies bind to the cytokine of interest, which brings the cryptate (donor) and acceptor in proximity and triggers FRET.

revvity

Cytokine expression inhibitors discovery and characterization - Case of IL-17

IL-17 is a pro-inflammatory cytokine expressed by Th17, that has been consistently reported as an important effector in the pathogenesis of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. Its suppression has already been shown to reduce the symptoms of these diseases, making it a logical target for drug discovery that has been thoroughly investigated in the past years.

Screening for inhibitors

Fujita-Sato S, et al *(2011). J Biol Chem 286(36):31409-17 Takaishi M,* et al *(2016). J Dermatol Sci 85(1):12-19*

In 2011, Fujita-Sato et al. implemented a TR-FRET assay to screen about 700 compounds for IL-17 inhibitors that would not affect IFNg secretion.

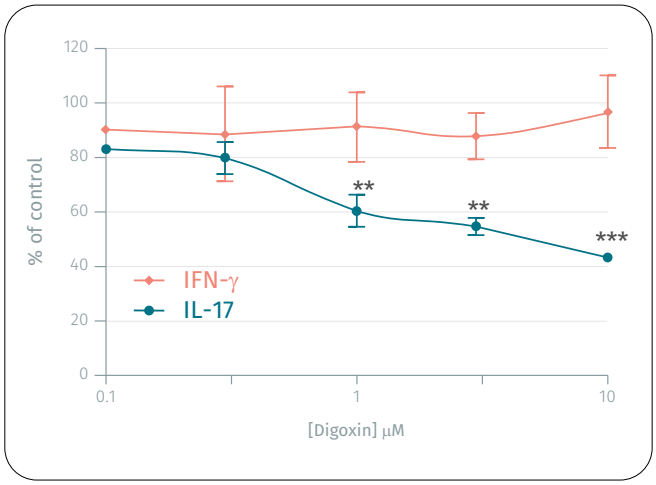


Figure 2A: IL-17 and IFN-g expression levels in CD4+ cells, in presence of digoxin. Digoxin was shown to inhibit IL-17 production without impairing IFN-g production.

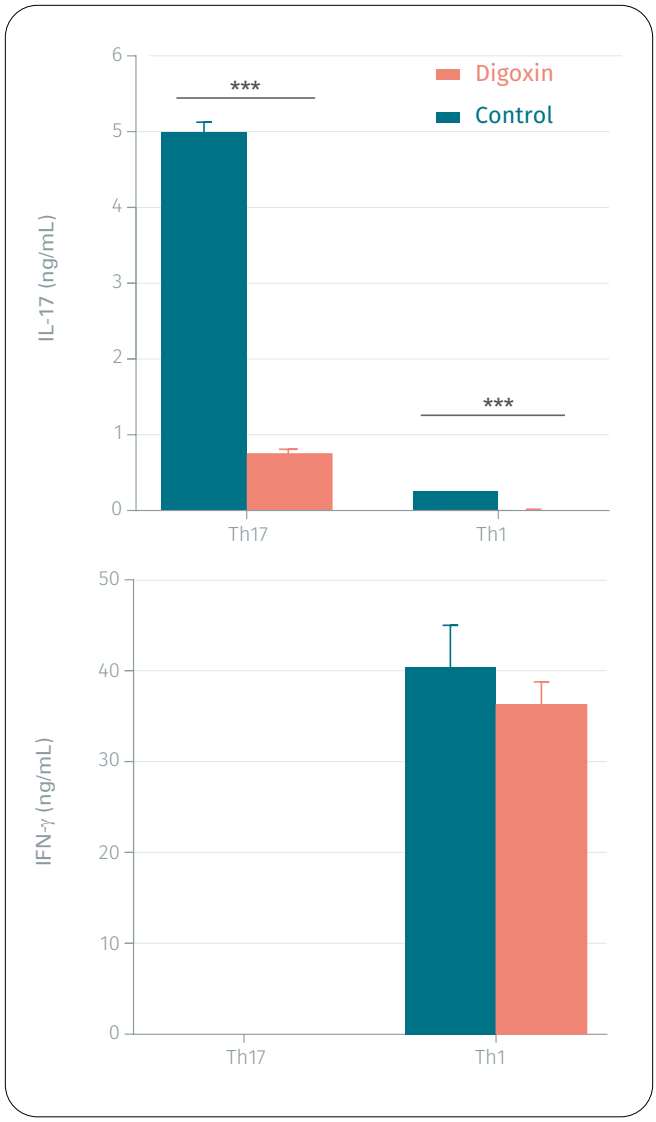


Figure 2B: IL-17 expression levels in Th17 and Th1 stimulated with ionomycin and PMA, in presence or absence of Digoxin.

The assay enabled the identification of Digoxin as an inhibitor of IL-17 expression in Th17 and Th1, without affecting IFNg secretion (fig. 2A&B). Digoxin was then successfully shown to inhibit the differentiation of Th0 cells to Th17 by binding to RORgt, a key complex partner in the signal triggering the differentiation, making it a potential target and opening exciting opportunities for the management of this cell type population.

Building upon the findings of Fujita-Sato's team, Takaishi et al. aimed to develop a RORgt antagonist to inhibit Th17 differentiation and IL-17 expression in a psoriasis context (2016).

They performed the screening of a chemical library with assays monitoring Th0 differentiation into Th17 and TR-FRET assays addressing the expression levels of IL-17 and IFNg. The results allowed for the identification of A213 as a novel RORgt agonist, stronger than the digoxin described by Fujita-Sato (fig. 3).

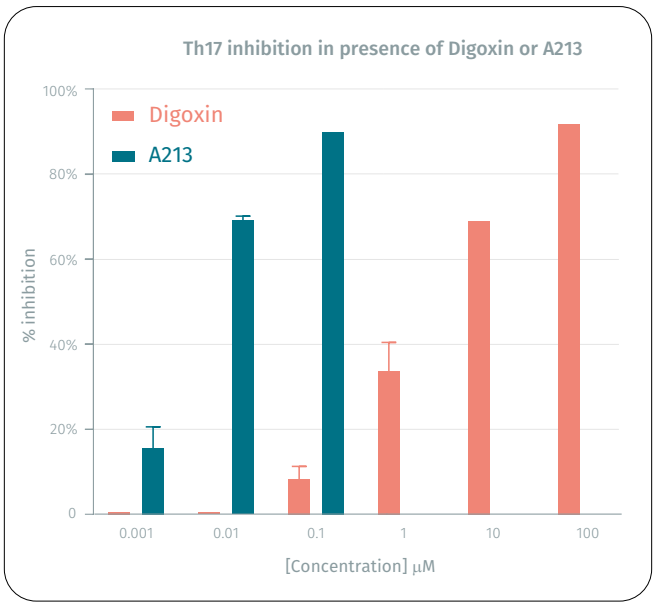


Figure 3: % of inhibition of Th17 in presence of increasing concentration of digoxin and A213. Adapted from Takaishi M, et al (2016). J Dermatol Sci 85(1):12-19

The effects of A213 were investigated in mouse models of psoriasis upon oral administration and resulted in a decrease in psoriasis-like symptoms such as skin lesions and inflammation. HTRF-monitored IL-17 expression was also reduced in the inflamed areas of the skin (fig. 4). Such findings demonstrate a strong potential application of A213 for psoriasis.

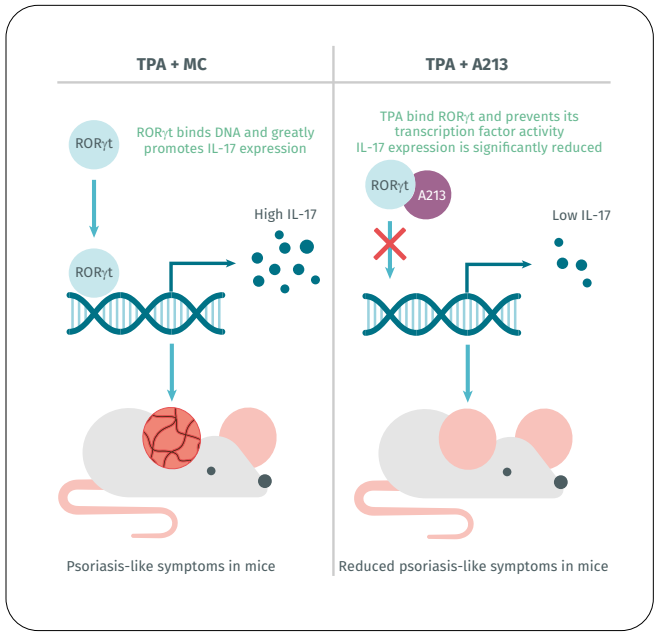


Figure 4: Effects of A213 on TPA-induced psoriasis-like symptoms in mice ears. Methylcellulose (MC) for control..

Pharmacological characterization of inhibitors

Dobritsa S.V, et al *(2013). J Biomol Screen 18(1):75-84*

In 2013 Dobritsa et al. developed, optimized and validated an HTS compatible TR-FRET assay to screen for IL-17 and/or Th17 inhibitors.

Using the HTRF IL-17 kit, they assessed the reliability of the assay in HTS compatible 96- and 384-plate formats, concluding on the robustness, ease of use and discriminatory potency of the system.

They further validated it with dose-response analysis involving rapamycin and cyclosporin A, two compounds known as IL-17 inhibitors, whose *in vitro* potency was measured for the first time in this study (IC50s of 80 ± 23 pM and 223 ± 52 nM, respectively) (fig. 5).

Finally, the assay allowed for the identification of IKK16 as an inhibitor of IL-17 production, with an IC50 of 315 ± 79 nM.

Blockade of a cytokine receptor - Case of IL-21R

Vugmeyster Y, et al *(2010). MAbs 2(3): 335-346*

Though most cytokine-related assays in the literature focus on identifying inhibitors of cytokine secretion, other drug discovery approaches are possible, such as reducing a cytokine activity via blocking its receptor or transduction signal pathway.

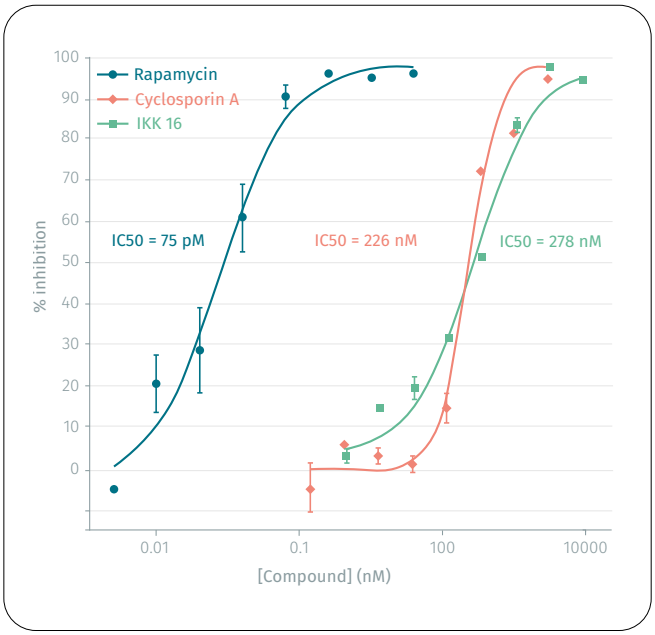


Figure 5: Dose-response curves of rapamycin (IC50 = 74 pM), cyclosporine A (IC50 = 226 nM) and IKK16 (IC50 = 278 nM).

IL-21 is a cytokine that is mainly secreted by CD4+ T-cells and NKT cells. It has been increasingly thought to be part of the pathogenesis of autoimmune diseases characterized by chronic inflammation such as lupus, rheumatoid arthritis and inflammatory bowel diseases. Neutralizing IL-21 bioactivity has already been shown to decrease these diseases' symptoms in murine models.

In 2010, Vugmeyster et al. seeked to develop optimized antibodies suited for the blockade of the IL-21 receptor. Using a phage display approach, they produced numerous antibody variants (Single-chain variant fragments or ScFv) from the 18A5 anti-IL-21R antibody.

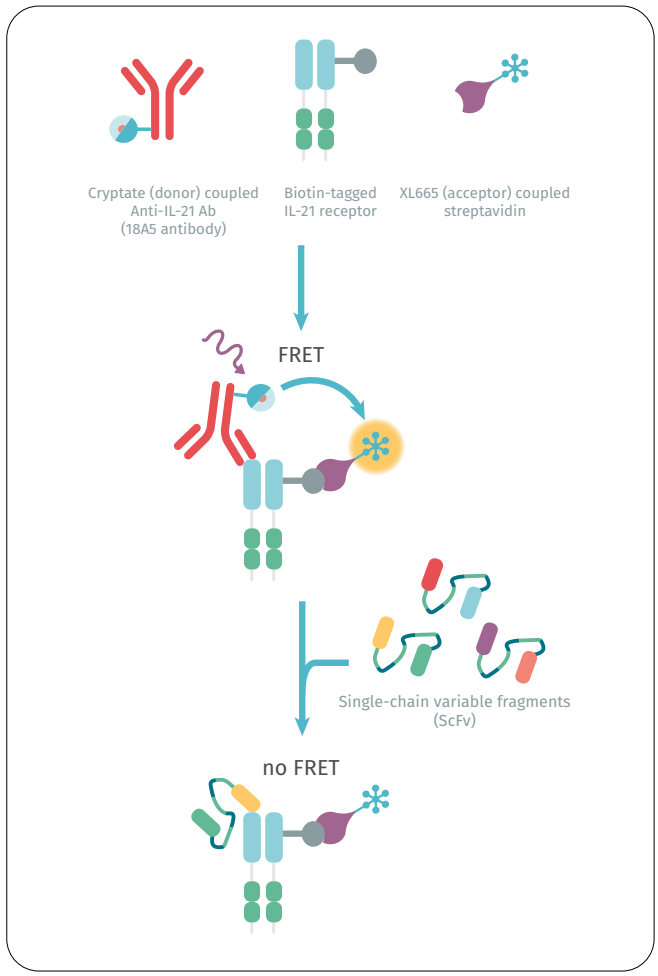


Figure 5: Dose-response curves of rapamycin (IC50 = 74 pM), cyclosporine A (IC50 = 226 nM) and IKK16 (IC50 = 278 nM).

Copyright: Dobritsa S.V, et al *(2013). J Biomol Screen 18(1) pp 75-84, copyright © 2019 by Revvity Bioassays, Reprinted by permission of SAGE Publications, Inc.*

These ScFv were tested against the original 18A5 antibody in a TR-FRET competitive assay to identify the ones exhibiting an interesting binding profile (fig. 6).

Thanks to this assay, the authors were able to identify a set of potential antibody variants, two out of which stood out while differing by only four amino acids. Further investigations and tests of these two in mouse lupus model led them to conclude that very minor variations in such antibodies could results in significant *in vitro* and *in vivo* effects in terms of pharmacological activity and pharmacodynamic profiles.

Conclusion

Cytokines offer multiple screening targets for drug discovery in autoimmune diseases. HTRF assays have been successfully used to address these over the last decade and have demonstrated an acute relevance for the various requirements and formats of drug discovery.

The publications featured in this note report the effective implementation of such assays for pharmacological characterization and screening, as well as the significant robustness and consistency they provided to HTS steps.

Works cited

- Takaishi M, et al. (2016). Oral administration of a novel RORgt antagonist attenuates psoriasis-like skin lesion of two independent mouse models through neutralization of IL-17. Journal of Dermatological Science 85(1):12-19
- Fujita-Sato S, et al. (2011). Structural Basis of Digoxin That Antagonizes RORgt Receptor Activity and Suppresses Th17 Cell Differentiation and Interleukin (IL)-17 Production. The Journal of Biological Chemistry 286(36):31409-17
- Dobritsa S.V, et al. (2013). Development of a High-Throughput Cell-Based Assay for Identification of IL-17 Inhibitors. Journal of Biomolecular Screening 18(1):75-84
- Vugmeyster Y, et al. (2010). *In vitro* potency, pharmacokinetic profiles and pharmacological activity of optimized anti-IL-21R antibodies in a mouse model of lupus. Monoclonal Antibodies 2(3): 335–346

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com