Neuroinflammation research solutions with HTRF assays

Abstract

Neuropsychiatric disorders often stem from or feed on neuroinflammatory and neurotoxic environments. Unraveling the exact pathogenesis of those disorders is challenging as they involve numerous molecular and cellular players, but the understanding of their mechanisms becomes increasingly critical as aging populations make mental health concerns grow faster. This note gathers publications that exemplify various applications of HTRF® assays to advance neuroinflammation and neurotoxicity related research and show how other researchers have harnessed this technology to move their studies forward.

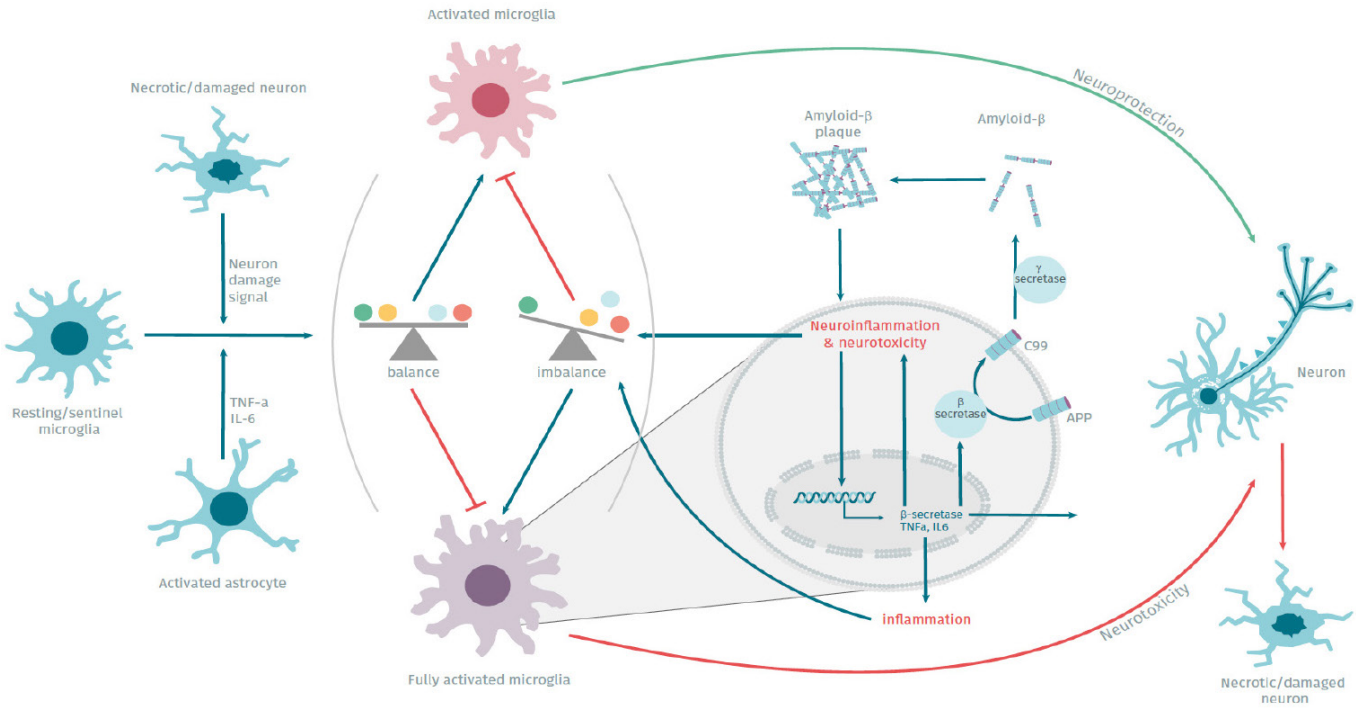

Figure 1: Amyloid-β plaque formation in microglia resting and activating states homeostasis.

Microglia are the central nervous system dedicated sentinels and protective cells. They exist in a resting surveying state from which they evolve to a spectrum of activated states when stimulated by their environment. When microglia receive stress signals from damaged neurons and astrocytes, they become activated and promote tissue clearance and repair in a neuroprotective way.

However, this beneficial activated state depends on a micro-environmental balance of pro- and anti-inflammatory transmitter imbalances. In the event of a neuroinflammatory context such as amyloid-b plaque accumulation, this blance is unchecked and promotes the fully activated phenotype of microglia. These exhibit increased phagocytic functions and cytotoxicity, and express proinflammatory cytokines such as IL-1β, IL-6 and TNF α, which promotes their auto-activation and sustains neuroinflammation in a vicious circle that has been linked to the pathogenesis of several disorders. In such a context of microglial overactivation and inflammation, the vitamin A metabolite retinoic acid (RA) has been identified as a potent neuroprotective and antiinflammatory compound that dampens microglia activity and decreases the expression of β-secretase (BACE1), an enzyme that promotes amyloid-β precursor and amyloid plaque formation. As a result of its anti-inflammatory properties, RA also downregulates the synthesis of inflammatory cytokines IL-6 and TNF-α, making it a hotspot in neuroinflammation research.

Retinoic acid in microglia activation

Anti-inflammatory effects of minocycline

Clemens V, et al. (2018). BMC Neuroscience, 19(1) : 58

The lipophilic tetracycline minocycline has recently been subject to investigations and clinical trials to explore its therapeutic potential in neuropsychiatric disorders as it was observed to mediate anti-inflammatory effects (notably IDO enzyme downregulation) and suggested to affect retinoic acid regulation.

In 2018, Clemens *et al* investigated the relationship between both compounds to determine how minocycline's effects are mediated and their relevance in retinoic acid signaling. To that end, the team conducted HTRF assays on PBMC-derived microglia-like cells following LPS-induced inflammation to assess the expression levels of pro-inflammatory cytokines TNF-α and IL-6 in presence of retinoids (ROL), minocycline or both (fig. 2).

The results showed that minocycline does not have inhibitory effects on TNF-α and IL-6 on its own but is RA-dependent. It is also observed that the combined use of minocycline and RA results in a stronger inhibition of cytokine expression than RA alone.

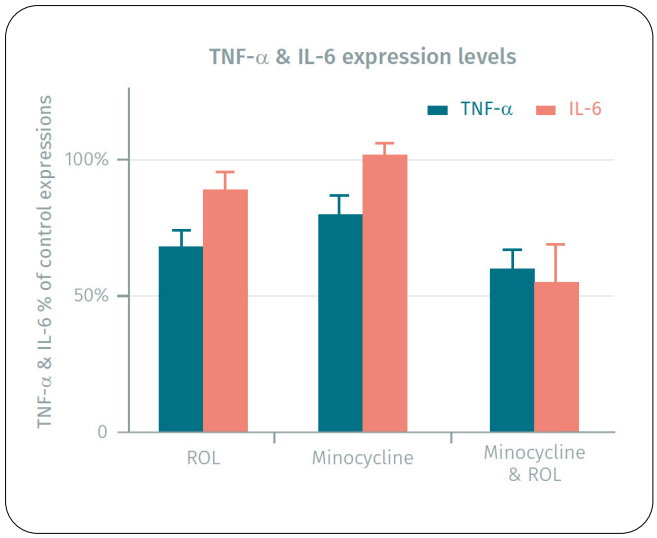

Figure 2: TNF-α and IL-6 % of control expression levels in microglia-like cells in presence of ROL, minocycline or both, following LPS-induced inflammation. Both cytokines were measured in independent experiments and compounded over their respective control expression levels.

Adapted from Clemens V, et al. (2018, September). Anti inflammatory effects of minocycline are mediated by retinoid signaling. BMC Neuroscience, 19(1), 58.

Additionally to this cytokine-based monitoring of retinoic signaling, the researchers performed an HPLC analysis showing that minocycline dampens degradation of RA in human monocytic cells and demonstrated via PCR analysis that minocycline downregulates IDO enzyme in the presence of RA alone. These findings add evidence for an RA-dependent mediation of minocycline's anti-inflammatory effects.

To further this study, the team carried out further HTRF assays to measure TNF-a expression in presence of minocycline, RA and antagonists of RAR or RXR (RA or pan-retinoid X receptor) (fig. 3).

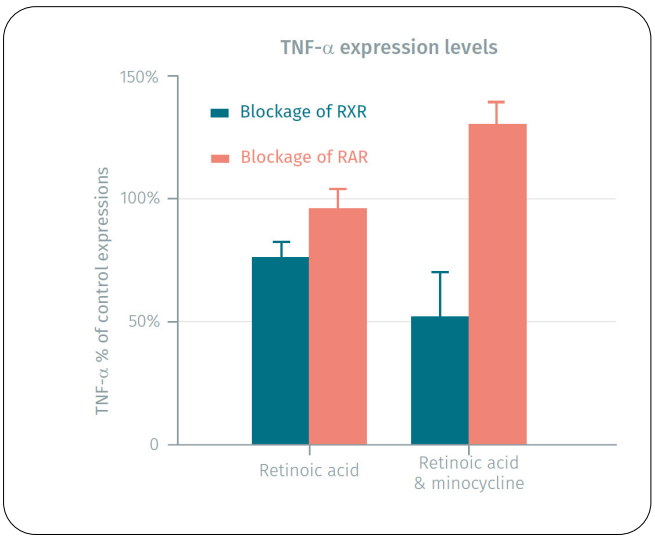

Figure 3: TNF-α % of control expression levels in microglia-like cells in presence RA or RA + minocycline. Cells were incubated with either RAR or RXR antagonists and inflammation was induced with LPS. Both RAR and RXR antagonists were tested in independent experiments and TNF-α expressions were compounded over their respective control expression levels.

Adapted from Clemens V, et al. (2018, September). Anti inflammatory effects of minocycline are mediated by retinoid signaling. BMC Neuroscience, 19(1), 58.

The results showed that blockage of RAR negates minocycline/ RA-induced inhibition of TNF-α expression and restores a controllike expression level, which is not the case for RXR blockage. These additional results led the authors to conclude the RA- and RARdependence of minocycline anti-inflammatory and neuroprotective roles through a suggested decrease in RA degradation.

Apolipoprotein and retinoic acid in amyloid beta metabolism

Clemens V, et al. (2018). Journal of Alzheimer's Diseases, 61(4) : 1295-1300

Human apolipoprotein-E (Apo-E) is primarily expressed in isoforms Apo-E2, 3 and 4, and was observed to intervene in the metabolism of amyloid-β, promoting either neuroprotection or neurotoxicity depending on said isoforms. Evidence from animal models especially suggests specific Apo-E isoforms could promote the neuroprotective properties of microglia in amyloid-b induced neuroinflammation.

Furthering their work on retinoic acid (RA) signaling and focusing on its role in amyloid-β regulation, Clemens *et al* (2018) investigated the ties between that metabolite and Apo-E in PBMC-derived human macrophages.

They relied on an HTRF assay to monitor Apo-E expression in various settings including LPS-induced inflammation, presence of RA, and a combination of both (fig. 4).

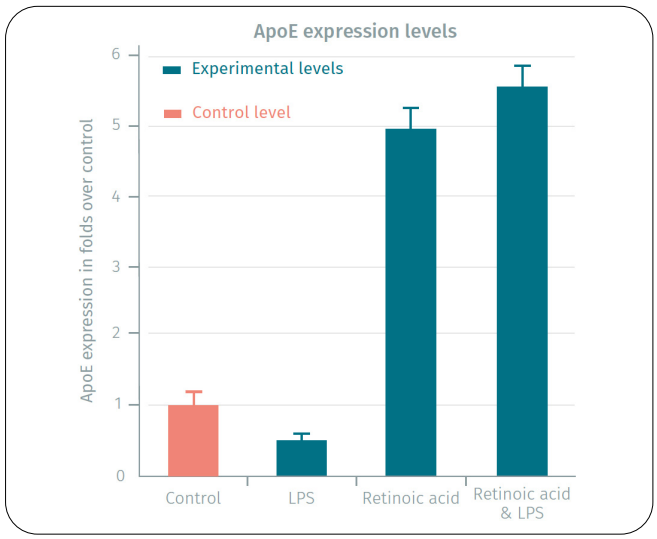

Figure 4: Apo-E expression levels in folds over control, in PBMC-derived human macrophages

Adapted from Clemens V, et al. (2018, November). Retinoic Acid Enhances Apolipoprotein E Synthesis in Human Macrophages. Journal of Alzheimer's Disease, 61(4), 1295-1300.

Results show that LPS-induced inflammation (TLR-mediated) has an inhibitory effect on Apo-E expression, whereas RA increases it by about 5 folds. Interestingly the stimulating effect of RA was observed to override inflammation-induced inhibition and resulted in a 5.5-fold increase of Apo-E.

This led the authors to conclude on a vicious circle of inflammation that promotes itself though the downregulation of Apo-E and related amyloid-b aggregation. RA was found to mediate parts of its anti-amyloid-b effects through Apo-E expression and is susceptible to override Apo-E inflammation-induced downregulation. These findings the authors say, "provide evidence for retinoids as a highly promising, possibly disease-modifying strategy".

Astrocyte inflammation

Elain G, et al. (2014). Glia, 62(5) : 725-35

IL-17 receptors (IL-AR to IL-ER) are targeted by IL-17 different forms (IL-17A to IL-17F) and involved in the pro-inflammatory role of these cytokines. In 2014, Secukinumab (AIN457) was a novel anti-IL-17A monoclonal antibody undergoing clinical trials at Novartis as a potential treatment for multiple sclerosis. As of 2019, that antibody is an approved

prescription for some autoimmune disease such as psoriatic arthritis and plaque psoriasis and is still being investigated in other therapeutic contexts.

In 2014, Elain *et al* sought to study the pro-inflammatory effects of IL-17A, and the inhibitory potential of Secukinumab in human and mouse astrocytes. To that end they implemented different HTRF assays to monitor the expression of IL-6 and chemokines including CXCL-1 in various pro-inflammatory settings and in presence or absence of Secukinumab (fig. 5 & 6).

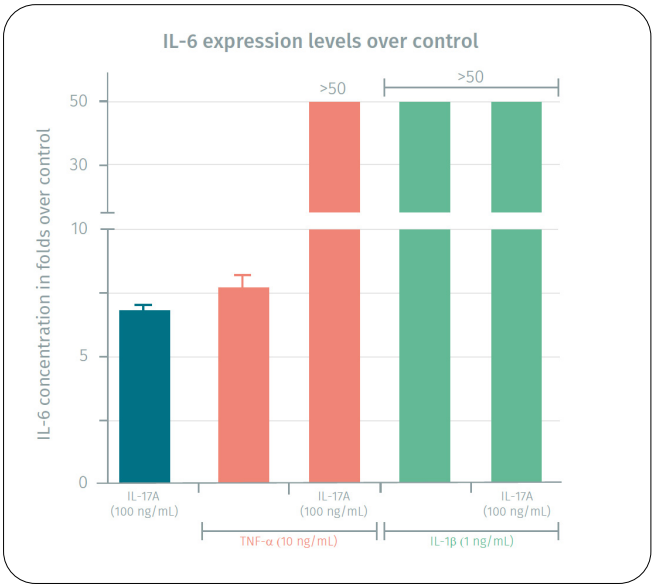

Figure 5: IL-6 expression levels in fold over control expression. Human astrocytes were incubated in presence of TNF-α, IL-1β, IL-17A and IL-17F alone of with a combination of TNF-α & IL-17A&F or IL-1 β & IL-17A&F. All conditions were tested in independent experiments and expression levels were compounded over their respective controls.

Adapted from Elain G, et al. (2014, February). The Selective Anti-IL17A Monoclonal Antibody Secukinumab (AIN457) Attenuates IL17A-Induced Levels of IL6 in Human Astrocytes. Glia, 62(5), 725-35.

These results showed IL-17A&F and TNF-α alone induce moderate increases in IL-6 (< 10 folds). IL-1 β alone, however, appeared to be a strong enhancer of IL-6 expression (about 50 folds). On the one hand, combination of TNF-α to both IL-17 showed TNF-α enhances the IL-17A&F-induced expression of IL-6 by about 10 folds compared to either one alone. On the other hand, combination of IL-1 β with either IL-17 forms failed to show improvement in IL-6 expression. This was interpreted as a result of IL-1 β being a strong promoter of IL-6 expression, that mediates maximal effects on its own. These results were later extended to mouse astrocytes.

Elain's team then conducted further HTRF IL-6 assays featuring the anti-IL-17 antibody Secukinumab to study its potential to alter the IL-17-related expression of pro-inflammatory previously observed.

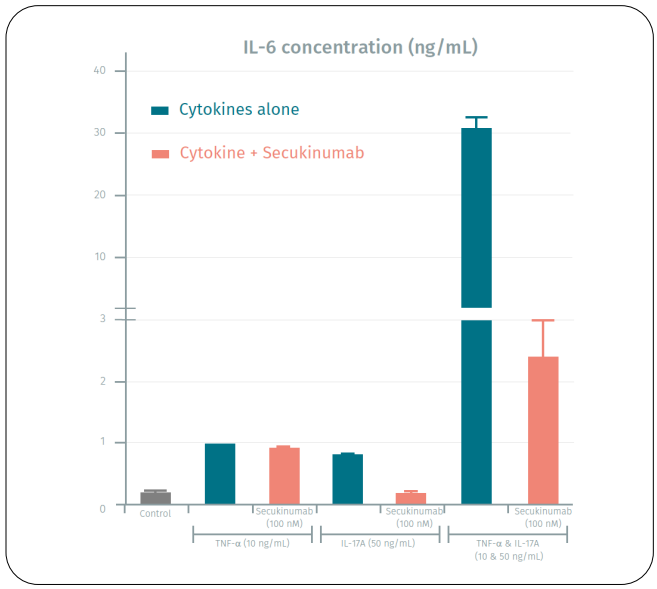

Figure 6: IL-6 expression levels in primary human astrocytes. Data showed are from cells treated with the anti-IL17A antibody Secukinumab (100 nM), with or without IL17A (50 ng/mL), TNFa (10 ng/mL) and IL17A/TNFa.

Adapted from Elain G, et al. (2014, February). The Selective Anti-IL17A Monoclonal Antibody Secukinumab (AIN457) Attenuates IL17A-Induced Levels of IL6 in Human Astrocytes. Glia, 62(5), 725-35.

Researchers found Secukinumab had no effect on TNF-a-signaling but negates IL-17A effects on IL-6 expression. Additionally, it decreased the combined TNF-a & IL-17A-induced expression of IL-6 back to a level similar to that of TNF-a alone.

Besides monitoring pro-inflammatory phenotypes via IL-6 expression, the author assessed the translocation of transcription factor NF-κB into the nucleus in presence of TNF-α & IL-17A, with or without a selective IL-17A inhibitor. Results showed an increased translocation in presence of TNF-α & IL-17A, which the inhibitor dampened. NF-kB translocation was also found to increase IL-6 expression. These findings are complementary to all previously described experiments as they suggest TNF-α/IL-17A-induced IL-6 increased is mediated via the NF-κB axis, which can be altered and reduced by selective IL-17A inhibitors.

Finally, building upon this previous identification of NF-κB as a key pathway in the pro-inflammatory effects of TNF-α and IL-17A, the authors sought to establish the effectiveness

of NF-κB and MAP kinase pathway inhibitors (inhibitors of p38 kinase, IkBβ, IKKβ) on TNF-a and IL-17A -induced increases in IL-6 and CXCL-1.

These experiment used HTRF assays to monitor IL-6 and CXCL-1 levels and the results were consistent with previous findings as they showed once again higher IL-6 levels after TNF-α and IL-17A combined treatment. They also allowed to observe a partial restoration of control levels by the MAP kinase inhibitor and complete restoration by NF-κB inhibitors. The authors therefore concluded that the combined use of TNF-α and IL-17A mostly increase IL-6 via the NF-κB pathway. Similar results and conclusion were reached with the measures of CXCL-1 levels.

Overall, this study opened perspectives on the roles of IL-17A in neuroinflammation and "support the hypothesis that selective inhibition of IL17A signaling, by selective drugs such as secukinumab, may be effective in controlling astrocyte-mediated neuroinflammatory signaling." the authors say.

Conclusion

The growing understanding of neuroinflammatory disorders has led the latest research and therapies to investigate and address the delicate balance of pro- and anti-inflammatory balances of transmitters that rules microglia cells and astrocytes behavior toward neurons. HTRF assays have been successfully used to that end for years and 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 investigating neuroprotective and neuroinflammatory mechanisms, as well as running experiments that improve the understanding of potential drug candidates.

Works cited

- Clemens V, et al. (2018, September). Anti inflammatory effects of minocycline are mediated by retinoid signaling. BMC Neuroscience, 19(1), 58.
- Clemens V, et al. (2018, November). Retinoic Acid Enhances Apolipoprotein E Synthesis in Human Macrophages. Journal of Alzheimer's Disease, 61(4), 1295-1300.
- Elain G, et al. (2014, February). The Selective Anti-IL17A Monoclonal Antibody Secukinumab (AIN457) Attenuates IL17A-Induced Levels of IL6 in Human Astrocytes. Glia, 62(5), 725-35.

HTRF assays cited

Product	Tests	Cat. No.#
Human TNF-a kit	500	62HTNFAPEG
Human IL-6 kit	500	62HIL06PEG
Human CXCL-1 kit	500	62HCXC1PEG
Human Apolipoprotein E kit	500	63ADK004PEG

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