

# Virobiome derived peptide t: anti-inflammatory peptides for treating neuro-aids and neurodegenerative diseases

## Abstract

The identification of biologically significant, receptor-targeting epitopes from the “virobiome”, the diverse population of viruses which engage the host immune system, especially those determinants which may control host immunity or be viral entry receptor binding sites, is an important goal for the development of anti-viral drugs, vaccines, and immunomodifying therapies. We<sup>1</sup> and others<sup>2</sup> have observed that numerous viruses, including members of the herpesvirus, poxvirus, and lentivirus families encode peptides that block innate immunity, presumably to help them overcome immune surveillance. A virus, especially a lentivirus, which is well adapted to exist in a nuanced balance within the complete, natural, and physiological host immune system, would be able, over millions of iterations (viral replication cycles), to perfect an escape from immune surveillance by modulating the entire innate immune network. Identifying those innate immune modifying peptide epitopes provides a rational basis for drug development. Contrast this to a typical “pharma” screening approach that a priori seeks to identify a “pure” or specific receptor target, to then be tested in artificial systems, yielding few hits, which typically are of low potency. These types of screens are most suited for detecting either agonist or antagonist activity. However, a more desirable pharmacologic feature, which the virobiome might provide, is partial or mixed agonist/antagonist activity, as this is most suited to provide a balanced modulation of immunity, and avoids substantial suppression of what are certainly useful host immune function(s). Small peptides derived from the HIV envelope protein have been identified that block HIV entry at chemokine receptors, protect neurons, and which antagonize cytokine, chemokine, and TLR/MyD88 inflammatory pathways. This has allowed creation of orally active, potent, peptides to provide treatments in diverse conditions with an underlying inflammatory pathogenesis such as psoriasis, Neuro-AIDS, neuropathies, and Alzheimer’s disease (AD), to name a few examples.

**Keywords:** virobiome, peptide t, neuro-aids, neurodegeneration, neuroinflammation, chemokine antagonist, oral peptide

Volume 5 Issue 2 - 2017

Michael R Ruff

Creative Biopeptides, USA

**Correspondence:** Michael Ruff, Creative Biopeptides, Rockville, MD, USA, Tel 240-893-7400, Email [mruff@creativebiopeptides.com](mailto:mruff@creativebiopeptides.com)

**Received:** April 26, 2017 | **Published:** June 21, 2017

**Abbreviations:** DAPTA, d-alal-peptide t-amide; VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; GHRH, growth hormone releasing hormone; HIV, human immunodeficiency virus; MMSE, mini-mental state examination; FDG, fluorodeoxyglucose; DBPC, double-blind placebo controlled; HAART, highly active antiretroviral therapy

## Introduction

We have exploited the HIV virobiome, specifically the envelope protein gp120, to identify highly potent receptor-active peptides that act as functional antagonists of multiple chemokine receptors. This was accomplished by searching the gp120 sequence for small local homologies to known signaling peptides, such as neuropeptides of the VIP/PACAP/GHRH family.<sup>1,3-5</sup>

We identified a discreet octapeptide domain (peptide T site) near the V2 stem of gp120<sup>1</sup> and related peptides derived from these sequences that preferentially block infection of R5-tropic HIV isolates.<sup>6-8</sup> Our initial studies used an early passage patient isolate, later shown to have an R5/X4 (dual-tropic) receptor phenotype, although none of the chemokine receptors, nor the significance of chemokine receptor utilization, were known at the time of this work (circa 1985). The predominant antiviral effect for R5 vs X4 HIV isolates explains the early controversy related to lack of antiviral effects with X4 lab adapted isolates.<sup>9</sup>

While peptide T was the first receptor targeted anti-viral for HIV (this class came to be called “entry-inhibitors”), as the AIDS epidemic expanded in the mid-1980’s it became evident that the virus, while establishing infection in the CNS, did not replicate in neurons. Nevertheless, profound cognitive and motor deficits were being reported, adding to the great stigma and suffering this disease carried at the time. In our initial reports<sup>1</sup> we noted a cortical distribution of gp120 binding to receptors in primate brain and hypothesized that gp120 killing of susceptible neurons would explain the observed patient cognitive deficits. We proposed that peptide T, by blocking gp120 binding,<sup>1</sup> later shown to be at CCR5,<sup>8</sup> would be efficacious in preventing neuronal loss in HIV infection, now known to occur very early after infection.

## Benefits in neuroinflammation and neurodegeneration

Initial studies that showed peptide T, and its degradation resistant analog D-alal-peptide T-amide (DAPTA), was protective to the neurotoxic effects of gp120 in neuronal cultures,<sup>10-13</sup> in part by releasing protective chemokines.<sup>14</sup> The neuro-protective effects of DAPTA were also shown in animals treated with gp120, where the loss of synapses and dendritic arbor, along with behavioral delays, were reversed.<sup>15</sup> The neurotoxic effects of gp120 were therefore shown to be a cause of cortical neuronal loss and dendritic pathologies<sup>16,17</sup> in neuro-AIDS. The mechanisms of gp120 neurotoxicity were gradually revealed by us and others to be indirect, and related to activation of microglia by gp120,<sup>18,19</sup> effects which are blocked by DAPTA.<sup>20</sup> Activated micro-

glia and astrocytes are now well appreciated to be mediators in diverse neuropathologies, which peptide T analogs can treat.

Although the anti-viral actions of these peptides was the initial subject of investigation, it gradually became clear that the peptide T family of peptides had useful immunomodulating<sup>14,21-24</sup> and neuronal sparing<sup>14,25</sup> effects beyond HIV that could be exploited therapeutically, especially in neurodegenerative conditions with an inflammatory pathogenesis. DAPTA lowered M1 cytokines IL-1, IL-6, IL-8, IL-23, TNF $\alpha$ , and enhanced M2 cytokines such as IL-4 and IL-10.<sup>26</sup> Some examples of possible DAPTA treatment benefits include Alzheimer's disease,<sup>20,25</sup> neuropathies of diverse origin,<sup>27-29</sup> cancer pain,<sup>30</sup> excitotoxicity<sup>31</sup> and stroke/cerebral ischemia.<sup>32</sup> Clinical benefits have been shown for neuro-AIDS (below), suppression of growth hormone,<sup>5</sup> a cause of developmental delays in pediatric HIV, which DAPTA has restored,<sup>33</sup> as well as in non-HIV related skin conditions like psoriasis.<sup>34,35</sup>

### Clinical trial results in neuro-aids

Peptide T, or more correctly DAPTA, entered human clinical trials for neuro-AIDS endpoints in 1986. Improvements in MRI brain scans and cognitive testing were subsequently reported.<sup>36,37</sup> The cognitive benefits in neuro-AIDS were confirmed in further controlled testing, which showed significant group (active vs. placebo,  $p=.003$ ) and time ( $p=.001$ ) effects,<sup>38</sup> absent any toxicities. A three-site DBPC trial in 215 randomized subjects of intra-nasal spray DAPTA (2 mgs, TID) was conducted in the early 1990's. The main endpoint was change in global cognitive score at 6 months on a battery of 23 tests. While no significant difference was found between the DAPTA and the placebo group on the global cognitive score, 2 of 7 domains, working memory ( $p.04$ ) and speed of information processing ( $p=.008$ ), did show improvement in the DAPTA group. A treatment effect was also reported for patients whose CD4 cell numbers were above 200 cells/ $\mu$ L at baseline (non-AIDS).

Overall, this cohort was minimally cognitively impaired (MMSE=28). However, among those with greater and clinically significant cognitive deficit  $>0.5$  on the global Z-score, a pre-planned sub-group analysis showed that DAPTA was associated with improved performance while deterioration was more common in the placebo group ( $P=.02$ ).<sup>39</sup> Although anti-viral measurements were not a primary endpoint in this trial, those data were reported and showed that DAPTA reduced the viral load ( $-0.54$  log,  $p<.03$ ).<sup>40</sup> The finding of anti-viral effect in this study is remarkable as the patients were not enrolled based on viral load, there was no dose optimization for anti-viral effect, their R5 or X4 viral phenotypes which would determine sensitivity to an anti-viral effect was unknown, and since the study was done in the pre-HAART era, DAPTA was essentially tested as monotherapy.

An in-hospital phase 2 study of DAPTA at 15 or 1.5mg/day for 4 weeks in nine IV drug users with early AIDS dementia also showed improved neurocognitive performance, at the higher dose compared with the lower dose or the placebo ( $P<.05$ ).<sup>41</sup> In a further study, benefits on functional brain imaging were reported in a 39-year old man with AIDS Dementia Complex who received 12 weeks of intranasal DAPTA (0.4mg TID, 1.2mgs per day). This study demonstrated that 34 of 35 brain regions having low FDG activity showed remission after therapy,<sup>42</sup> consistent with the MRI and cognitive benefits identified in the phase 1 and 2 studies. (op.cit., above).

### Orally active peptides

While DAPTA is substantially protected from degradation in plasma it is rapidly digested in the stomach.<sup>43</sup> Furthermore, its clinical use

has primarily been by nasal spray, which requires long term storage of a liquid drug product. DAPTA has been shown to lose activity by aggregation upon storage. Aggregation of the drug product was a concern in the DBPC trial<sup>39</sup> as stored product was used for the several years it took to complete the study and gelling of study medication was reported by the patients. This has limited DAPTA further clinical development. To overcome this significant obstacle, we created an analog of DAPTA (RAP-103) that is fully protected to degradation, and retains picomolar potency.

A proof-of-concept study in rats showed that oral administration of RAP-103 (0.05-1mg/kg) for 7 days fully prevents mechanical allodynia and inhibits the development of thermal hyperalgesia after partial ligation of the sciatic nerve in rats.<sup>27</sup> In this study, we further showed that DAPTA and RAP-103 blocked both CCR2 as well as the closely related chemokine receptor CCR5. Moreover, RAP-103 could reduce spinal microglial activation and monocyte infiltration, and inhibit the inflammatory cytokine responses evoked by peripheral nerve injury, the cause of neuropathic pain.

Our findings suggest that targeting CCR2/CCR5 should provide greater efficacy than targeting CCR2 or CCR5 alone, and that the dual CCR2/CCR5 functional antagonist RAP-103 has the potential for clinical use in neuropathic and other pain conditions. Because this analog shares multiple DAPTA mechanisms to reduce microglial activation, shift the cytokine balance, protect neurons and spare dendritic arbor, it is a prime candidate for further clinical development in the multiple neuroinflammatory conditions already discussed, for which benefits in pre-clinical animal testing models have been shown, as noted in the many citations of this review.

### Conclusion

Virobiome derived peptides have led to creation of an entirely new and novel class of innate-immune modulating peptides with proven human benefits in neuro-AIDS that may be useful in many other neuroinflammatory diseases with few, if any, effective treatments. Of particular interest are findings that DAPTA prevented neuronal losses and protected synapses in neuro-AIDS and aging rodent models, and blocked microglial and astrocyte activation in brain, results highly suggestive of a beneficial effect in Alzheimer's disease and other neurodegenerative conditions. Benefits in animal models of AD, as well as ischemic stroke and neuropathic pain result in part from DAPTA's action to shift the cytokine profile from an M1 (inflammatory) to an M2 (repair) response, actions which have particular utility in brain injuries and neurodegeneration from diverse causes.

Virobiome-derived peptides like DAPTA, have been shown in multiple human studies to be safe and efficacious by blunting innate immune cytokines and chemokines. Next-generation orally active peptides have shown proof-of-concept benefits in neuropathic pain models, via effects at clinically validated receptor targets. Virobiome-derived oral peptides can therefore provide significant treatment and patient benefits over current antibody-based pharmaceuticals which seek to reduce inflammatory cytokines including IL-1, IL-6, IL-8, IL-23, and TNF $\alpha$ . Their small size allows rapid tissue distribution and entry into the brain, and peptides in general have an excellent safety profile, compared to many small molecule therapeutics.

### Acknowledgements

The author wishes to acknowledge the contributions of the late Dr. Candace Pert, co-creator of Peptide T, DAPTA, and the oral peptides here described, champion of safe and effective treatments for HIV, and co-author of numerous research articles on the topics here discussed.

## Conflicts of interest

The author is an inventor of the subject peptides.

## Funding

None.

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