Interaction mechanism between the HSV-1 glycoprotein B and the antimicrobial peptide Amyloid-β

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ABSTRACT

Background: Unravelling the mystery of Alzheimer's Disease (AD) requires urgent resolution given the worldwide increase of the aging population. There is a growing concern that the current leading AD hypothesis, the amyloid cascade hypothesis, does not stand up to validation with respect to emerging new data. Indeed, several paradoxes are being discussed in the literature, for instance, both the deposition of the amyloid-β peptide (Aβ) and the intracellular neurofibrillary tangles (NFTs) could occur within the brain without any cognitive pathology. Thus, these paradoxes suggest that something more fundamental is at play in the onset of the disease and other key and related pathomechanisms must be investigated.

Objectives: The present study follows our previous investigations on the infectious hypothesis, which posits that some pathogens are linked to late onset AD. Our studies also build upon the finding that Aβ is a powerful antimicrobial agent, produced by neurons in response to viral infection, capable of inhibiting pathogens as observed in *in vitro* experiments. Herein, we ask what are the molecular mechanisms in play when Aβ neutralizes infectious pathogens?

Methods: To answer this question, we probed at nanoscale lengths with FRET (Förster Resonance Energy Transfer), the interaction between Aβ peptides and glycoprotein B (responsible of virus-cell binding) within the HSV-1 virion.

Results: The experiments show an energy transfer between Aβ peptides and glycoprotein B when membrane is intact. No energy transfer occurs after membrane disruption or treatment with blocking antibody.

Conclusions: We concluded that Aβ insert into viral membrane, close to gB, and participate in virus neutralization.

Keywords: Alzheimer's Disease, amyloid-beta, HSV-1, glycoprotein B, interaction, FRET

53 **INTRODUCTION**

54

55 Alzheimer's disease (AD) is the leading cause of dementia in the world, mainly due to the
56 global increase of the aging population, as age is one of the most important risk factors for
57 developing AD. Despite intense research efforts, we still do not know what the exact causes of
58 late onset AD are. Nevertheless, there is a general agreement that AD's prime histopathological
59 signature led to the so-called *Amyloid Cascade Hypothesis* (ACH), involving plaques induced
60 by amyloid-β (Aβ) peptides and of intracellular neurofibrillary tangles (NFTs) deposition [1].
61 However, several paradoxes forced us to expand this view of ACH and reconcile it with novel
62 emerging data, e.g.: 1) Aβ peptide deposition can occur in cognitively normal individuals [2, 3,
63 4]; 2) Aβ peptides are abundant in the mild cognitive impairment stage of AD and decrease in
64 some clinically diagnosed AD [5]; 3) inflammation precedes Aβ peptide deposition [6]; 4)
65 current data support the view that aberrant processing of APP (amyloid precursor protein)
66 towards Aβ peptides, may sometimes cause human familial/early onset AD. All current data do
67 not support the conclusion that aberrant Aβ peptide expression is the cause of late onset AD but
68 likely only plays a secondary role as part of a more complex process. A compelling (yet
69 controversial) body of data is mounting, which can be reconciled with ACH and potentially
70 explain the above paradoxes. This data has its origin in a hypothesis by Dr O. Fischer in 1910,
71 which posited that infectious pathogens are involved in AD since senile plaques are reminiscent
72 of bacterial colonies [7]. Independent research (including ours) has been instrumental in
73 corroborating the infectious hypothesis by showing that, in fact, a family of microorganisms
74 (e.g. HSV-1, spirochetes, *P. gingivalis*) are potentially associated with late onset AD, also
75 hinting that sporadic AD is a syndrome [8,5]. As a case in point, DNA of HSV-1 has been found
76 in Alzheimer's Disease patients' senile plaques [9]. Moreover, several groups have
77 demonstrated that the Aβ peptide is a powerful antimicrobial peptide, secreted by neurons, in

78 response to attacks by microorganisms. This demonstration called *Amyloid Protection*
79 *Hypothesis* (APH) lends weight to the infection hypothesis (IH) [10, 11, 12]. Thus, under
80 APH/IH theory, Aβ peptide accumulation is no longer seen as Aβ being the main participant in
81 the pathophysiology of AD, but rather as, an innate immune response element (i.e. reversing its
82 role). Thus, senile plaques are possibly a by-product of Aβ fighting to contain and neutralize
83 infections [13].

84 As HSV-1 is a recognized culprit to foment APH/IH, its interaction with Aβ is of
85 considerable interest. HSV-1 is an enveloped virus responsible mostly for cold sores, which
86 stay latent in neurons throughout life and reactivate regularly inside the brain, in case of fatigue
87 or decrease in immunity. Initially, it was demonstrated that APP, the precursor of Aβ, associates
88 intracellularly with HSV-1 and contributes to the movement of the virus towards the cell surface
89 (anterograde transport) with the help of glycoprotein D (gD) at the virus surface [14, 15]. It has
90 been shown that pathogens and particularly HSV-1, interfere with the APP metabolic pathway
91 and use C99 protein (specifically, the final 15 amino-acid sequence), rather than Aβ, to coopt
92 the intracellular transport machinery, enabling their transport along microtubules, but also
93 causing NFTs [14, 15, 16]. Moreover, studies demonstrate that APP C-terminal fragment, C99
94 protein, but not Aβ peptide is associated with neuronal death [17].

95 The interaction between viruses and amyloid peptides is under intense investigations to
96 confirm the pathogenic role of viruses in various diseases, including neurodegenerative
97 diseases. In most cases, the interaction is studied as the putative cause of the viral pathogenesis
98 by inducing amyloid aggregation. Recently, the interaction between viral proteins including
99 HSV-1 and the Aβ42 peptide, has been reassessed in different settings by taking advantage of
100 the resemblance of HSV-1 with nanoparticles, which have been shown to act as catalytic
101 surfaces, that facilitate heterogenous nucleation of amyloid fibrils *via* binding, concentrating,
102 and enabling conformational changes of amyloidogenic peptides [18, 19, 20]. It has been

103 demonstrated that HSV-1 accelerated the kinetics of Aβ42 peptide aggregation and to a lesser
104 extent that of Aβ40 peptides [21, 22]. The authors have demonstrated an interaction between
105 amyloid fibrils and the viral surface at different stages of maturation *via* early protofibrillar
106 intermediates. They also observed that HSV-1 infection led to increased accumulation of
107 amyloid plaques in a mouse model of AD. In their discussion, the authors try to reconcile the
108 antimicrobial characteristics and the virus induced nucleation process, leading to amyloid
109 plaques. Certainly, this is not mutually exclusive as we have shown that, at the beginning, the
110 Aβ42 peptide secreted by cells is antimicrobial but as the infection is progressing, it becomes
111 pathogenic, by the virtue of the process described by Ezzat *et al*. This reminds us of the capacity
112 of bacteria to form biofilm which mimic plaques and the fact that biofilms have already been
113 demonstrated in AD [23]. These studies focused only on the putative pathogenic interaction of
114 HSV-1 and Aβ42 peptides, resulting in amyloid plaque formation, rather than the antimicrobial
115 interaction of Aβ42 against HSV-1. Indeed, these studies have presented corona formation as
116 an essential step in the viral infection.

117 Enveloped viruses like HSV-1 have a core of viral nucleic acid surrounded by
118 capsomers to form a capsid, then surrounded by a membrane derived from the host. Viral
119 proteins, usually glycoproteins have been inserted into this membrane prior to encapsidation.
120 They serve to allow attachment of progeny virions to a new host and then interactions between
121 these glycoproteins promotes fusion of the viral and cellular membranes and allows the viral
122 capsid to enter the cell. We have demonstrated earlier that the Aβ peptide, as antimicrobial
123 peptide, prevents HSV-1 infection [24, 10]. Indeed, we have demonstrated that the addition of
124 Aβ peptides to the culture media before or at the same time as HSV-1, decreases virus
125 infectivity, while adding Aβ peptides when HSV-1 has already entered the neurons, shows no
126 effect [24]. Moreover, HSV-1-infected neuronal supernatants, when added to new neuronal
127 cultures, were also able to inhibit infection by HSV-1, presumably due to induced production

128 of Aβ by cells after infection and released in supernatants [10]. This accumulating evidence has
129 led us to ask about the molecular mechanisms of the action of Aβ peptides against AD
130 pathogens.

131 Taking these data into consideration, it appears highly important to explore the currently
132 unknown mechanism whereby Aβ peptides render HSV-1 non-infectious. At the surface of
133 viruses, besides gD and among others, there also is the glycoprotein B (gB), a transmembrane
134 protein involved in cell attachment and fusion. In a review in 2016 [13], we have shown that
135 Aβ and gB shared sequence similarities and given the fact that Aβ could insert into microbial
136 membranes and form pores to disrupt them [25, 26], we hypothesized that Aβ could also insert
137 into the HSV-1 membrane near gB, and the pores produced would prevent viral infectivity
138 either by disrupting the mechanism of fusion of the viral membrane with the cellular membrane
139 or by allowing enzymes to enter the viral capsid and digest viral proteins or DNA. This potential
140 gB-Aβ peptide interaction could also be extended to other enveloped viruses, as well as
141 peptides, and could represent a threshold when it becomes inefficient and pathological.

142 Thus, the aim of the present study was to gather more experimental data to determine
143 the antiviral mechanism of Aβ peptides against HSV-1 virus.

144 **MATERIAL AND METHODS**

145

146 *Sequence alignment and antimicrobial prediction*

147

148 The amino acid sequence alignment between HSV-1 gB and Aβ42 was performed using
149 the PyMol software after having depicted the 3D structure of HSV-1-gB and Aβ42 peptide (The
150 PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC [27]).
151 AntiBP2 software was used, with neural networks and support vector machines (SVM)
152 to predict the amino-acid sub-sequence for a peptide with antibacterial activity. AntiBP2
153 utilizes four datasets to train their models: N-terminus based, C-terminus based, N+C terminus
154 based and amino acid composition methods. These 4 methods are SVM trained on 4 different
155 datasets, compiled using N, C, NC and full composition peptides respectively. For antiviral
156 activity predictions, we employed the AVPpred software, which computes various features (i.e.
157 motifs and alignments, followed by amino acid composition and physicochemical properties),
158 during 5-fold cross validation using SVM. In particular, we have fragmented the amino
159 sequence into subsequences of lengths 15, while taking the overlap length to be 14 and finally
160 the subsequences of length 15 were processed by AVPred [28].

161

162 *Förster Resonance Energy Transfer (FRET)*

163

164 The Förster Resonance Energy Transfer (FRET) has been performed in a
165 LightCycler480 II (Roche, Canada) with variable excitation and detection filters. 1 µg of a
166 recombinant HSV-1 containing the green fluorescent protein (GFP) integrated into gB (HSV-
167 1-gB-GFP) produced at the Cochin Institute, Paris, France and kindly provided by Pr. Flore
168 Rozenberg [29] was mixed with 1 µg of Aβ42-HiLyteFluor-555 (Anaspec, Fremont, CA), with

169 or without various pre-treatments (see below) and controls, without cells, directly in wells of a
170 96 well plate at room temperature. Plates were sealed, centrifuged, and then processed by the
171 thermocycler. Thermocycler conditions were heating 5 min to reach 37°C, then fluorescence
172 acquisition every 10 min for 30 min, before cooling. Fluorescence acquisition was measured
173 with excitation at 440 nm and emission at 580 nm (green fluorescence) and also with excitation
174 at 440 nm and emission at 660 nm (red fluorescence). Several controls were also tested with
175 the same program. Pre-treatments included mixing Aβ42-HiLyteFluor-555 with 1 μg of
176 blocking antibodies (α-Aβ42-Ab, Anaspec, Fremont, CA) for 15 min at room temperature prior
177 to adding viral particles or pretreating the HSV-1-gB-GFP for 30 min at room temperature with
178 1% NP40 diluted in PBS, pH 7, to disrupt the viral envelope, before mixing with Aβ42-
179 HiLyteFluor-555. Controls included negative controls without virus or Aβ42-HiLyteFluor-555,
180 virus alone, and Aβ42-HiLyteFluor-555 alone.
181 At the end of the program, the thermocycler software gave fluorescence values for green
182 and red fluorescence for each well. Data obtained for each measure were averaged and used to
183 create the graph.
184 Statistical analysis was performed using two-tailed unpaired T-test.

185 **RESULTS**

186

187 We first depict the 3D structure of HSV-1-gB and Aβ42 peptides (Fig. 1A). Sequence
188 homology was observed between the viral gB protein and Aβ42 (Fig. 1B and [13]).
189 Furthermore, the antibacterial predictions show that a subsequence containing the helix and C-
190 terminus of Aβ42 peptide has antiviral activity (Fig. 1C and [22]). This result prompted us to
191 analyze the interaction between HSV-1 and Aβ peptides on *in vitro* experiments.

192 FRET experiments were performed between an HSV-1 particle with gB linked to the
193 green fluorescent protein (HSV-1-gB-GFP) and fluorescent peptides (Aβ42-HiLyteFluor-555
194 (red)). Analysis of the virus has shown that its functionality remains intact despite the presence
195 of GFP [29]. FRET experiments are a well-established way to determine whether two molecules
196 with complementary green and red fluorescent labels are in close interaction ($\leq$ 10 nm) [30].
197 When the green fluorescent label is excited, its emission can be transferred and excite a nearby
198 red fluorescent label. These experiments usually employ fluorescence microscopy, but they also
199 could be performed with thermocyclers equipped with laser induction and wavelength specific
200 detectors. They measure fluorescence amplification created by dyes and can detect FRET when
201 different fluorescents dyes are brought close together [31, 32]. If only green fluorescence is
202 observed (excitation at 440 nm and emission at 580 nm) then no energy transfer has occurred.
203 This would be expected when virus is tested alone or if the gB-GFP was more than 10 nm from
204 the red dye linked to Aβ42 peptides. If red fluorescence is measured in the channel with
205 excitation at 440 nm (which does not efficiently excite red fluorophores) and emission at 660
206 nm, then GFP has transferred its energy to HiLyteFluor-555, which can then emit, giving values
207 that are high on red axis and low on the green axis because the green fluorescence has been
208 quenched by transfer to the red fluorophore. The observed values are presented in Fig. 2 and
209 then analyzed statistically in Table 1.

210 Data showed that the green fluorescence emitted by HSV-1-gB-GFP alone (7,18 ± 0,94
211 AU) was significantly higher than that emitted by the HSV-1-gB-GFP/Aβ42-HiLyteFluor-555
212 mix (4,41 ± 0,35 AU) (Table 1), whereas the mix gave much higher fluorescence in the red
213 channel than the virus alone did. Indeed, the ratio of red fluorescence divided by green
214 fluorescence was superior to 1 for the HSV-1-gB-GFP/Aβ42-HiLyteFluor-555 mix, but inferior
215 to 1 for HSV-1-gB-GFP alone. These results are consistent with FRET. We also observed that
216 when HSV-1-gB-GFP was pre-treated with NP40 prior to mixing or when blocking antibodies
217 directed against Aβ (α-Aβ42-Ab) were pre-mixed with Aβ42-HiLyteFluor-555, then mean
218 green fluorescence values were 7,22 ± 0,47 AU and 6,58 ± 0,77 AU respectively, which are
219 significantly different from the mean values without pre-treatment (4,41 ± 0,35 AU), and
220 similar to those obtained for HSV-1gB-GFP alone. Furthermore, the ratio of red to green
221 fluorescence also decreased to values lower than 1 in the pre-treated samples. These latter
222 results corresponded to an absence of FRET.

223 **DISCUSSION**

224

225 The purpose of the FRET experiments carried out in this study was to determine the
226 mechanism of action of Aβ peptides against HSV-1 virus. The present results indicated that the
227 fluorescent energy resulting from the excitation of the GFP associated with the gB of HSV-1
228 virions was transmitted to the fluorochrome HiLyteFluor-555 associated with the peptide Aβ42.
229 We concluded that FRET took place between the two fluorochromes, indicating that they are at
230 a distance less than or equal to 10 nm [30]. Considering the proximity of Aβ42 and gB validated
231 by FRET, the diameter of the HSV-1 virion which measures approximately 200 to 250 nm [33,
232 34], and the diameter of HSV glycoprotein tetramers are probably close to 10 nm (glycoprotein
233 D has been measured [35]), so we can therefore conclude that the Aβ42 peptide must insert into
234 the outer membrane of the virus, near the site on the viral membrane where gBs are present.
235 This conclusion is supported by the works of Kagan et al., in 2012 [25] and Lemkul et al., in
236 2013 [26]. Both works described amyloid insertion into microbial membranes.

237 Controls show us that the energy transfer is prevented by the use of NP40 detergent, a
238 non-ionic surfactant, which acts by disrupting membranes, so it cannot alter the peptides'
239 structure or direct interactions, suggesting that there is probably not a direct interaction between
240 the peptide and the glycoprotein. The most logical explanation is that Aβ42 peptide is inserted
241 into the viral envelope, near gB. When the detergent has solubilized the viral envelope, insertion
242 is prevented. Blocking antibodies also interfere with HSV-1-gB/Aβ42 interaction by preventing
243 Aβ42 peptide from inserting itself into the viral envelope.

244 Our observations support the hypothesis that Aβ peptides insert into the outer membrane
245 of enveloped viruses in the same way as the LL-37 peptide. LL-37 is an α-helical peptide which
246 inserts into lipid membranes and forms pores, deleterious to the integrity of the membrane and
247 therefore to the organism or the target cell [36, 37]. The Aβ42 peptide is also an α-helix and

248 studies on cells or bacterial membranes have shown that it is also able to insert into lipid bilayers
249 and form pores [25, 26]. It is also possible that the Aβ42-HiLyteFluor-555 has integrated the
250 corona as it has been shown to be present, but this would not explain the loss of infectivity.
251 Indeed, it has been postulated that HSV-1 must be surrounded by a corona to be infectious.

252 Very recently, Wang et al. (2022) [38] have provided *in silico* evidence that Aβ
253 associates with the HSV-1-gD. Our experimental observations by FRET of intact viral particles
254 indicates that Aβ binds to viral particles near the binding site of gB or directly to gB. We
255 proposed that this binding was *via* insertion of Aβ into the envelope of the virus akin to the
256 proposed insertion of this peptide into bacterial and fungal membranes. It is entirely possible
257 however that it binds to the viral particle *via* gD and that this binding interferes with the
258 mechanism of fusion, necessary for infection, or that binding favors agglutination of viral
259 particles, as proposed by the group of Moir and Tanzi [11]. It has been shown that gD and gB
260 are attached to the viral membrane at separate location [34], and probably not physically close
261 enough to allow FRET, so the binding of Aβ to gD observed by Wang et al., would not explain
262 our observation of FRET. Since treatment of the virus with NP40 prevents FRET, but should
263 not interfere with glycoprotein-Aβ interactions, we conclude that Aβ most probably does not
264 bind gB.

265 Previously in our lab, we have established a microglia/neurons co-culture and we have
266 measured several cytokines in supernatant after HSV-1 and/or amyloid-β adjunction [13]. We
267 concluded in this paper that innate immune system respond to the infection and the presence of
268 the peptide in the media. Our results shown here seem to confirm (at least *in vitro*) that the Aβ
269 acts as an intracerebral component of the humoral innate immune system. Thus, it would
270 recognize some molecular patterns characteristic for the neurotropic viruses (including, but not
271 limited to the HSV-1) in a non- or semi-specific way, possibly similar to the interactions
272 between the PAMPs and Pathogen Recognition Receptors.

273 These experiments complement our previous and present results. When cells secret
274 appropriate quantity of the Aβ42 peptides in the presence of enveloped viruses, this will initiate
275 an interaction resulting in the neutralization of the virus infectivity. With age, the most
276 important risk factor of AD, microglial clearance capacity decreases, and cellular and viral
277 waste accumulate in brain. When the quantity of virus or the resulting synthesis of Aβ42
278 peptides increases, not only will the Aβ peptides inactivate the virus, but pathological
279 interactions between the viruses e.g. HSV-1, RSV or SARS-CoV2 and Aβ peptides will result
280 in the formation of traditional amyloid plaques. Upon further consideration, it can also be
281 surmised that a biofilm or plaque may be advantageous both for the virus (protection) and for
282 the cells (protection). The elucidation of these processes is of the most importance prior to using
283 these conditions in a therapeutic perspective.

284

285 *Conclusions*

286

287 We concluded that there is indeed a close interaction, likely nonspecific or semi-
288 specific, between the two types of molecules, which result in virus neutralization. Aβ peptides
289 are antimicrobial peptides (AMP) that belongs to innate immune response and insert into viral
290 envelop to disrupt them and prevent infection.

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295

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306

307 **CONFLICTS OF INTEREST**

308

309 The authors declare no conflicts of interest.

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	Mean ± SD	Difference *vs.* HSV-1-gB-GFP	Difference *vs.* HSV-1-gB-GFP/Aβ42-F555
HSV-1-gB-GFP	7,183 ± 0,94	-	< 0,0001 ****
HSV-1-gB-GFP/Aβ42-F555	4,415 ± 0,35	< 0,0001 ****	-
HSV-1-gB-GFP/Aβ42-F555 + α-Aβ42-Ab	7,222 ± 0,47	No	< 0,0001 ****
HSV-1-gB-GFP/Aβ42-F555 + NP40	6,584 ± 0,77	No	< 0,0001 ****

418 **Table 1. Documentation of FRET between HSV-1-gB-GFP and Aβ42-HiLyteFluor-555.**

419 Significant differences were observed in the mean fluorescence between HSV-1-gB-
420 GFP/Aβ42-HiLyteFluor-555 mix and HSV-1-gB-GFP, but also when Aβ42-HiLyteFluor-555
421 was pre-treated with blocking antibodies or HSV-1-gB-GFP was pre-treated with NP40 prior
422 to mixing. Unpaired T-test, two-tailed. ****: p<0,0001. n=2-5 independents experiments in
423 triplicate.

424 **LEGENDS**

425

426 **Fig. 1. Molecular structure, sequence alignment and anti-pathogenic property of Aβ.** Panel
427 (A): 3D crystal structure of HSV-1-gB and Aβ-42; data from PDB (RCSB Protein Data Bank,
428 http://www.rcsb.org) are shown using PyMol software. Center panel shows zoom on HSV-1
429 gB intermembrane sequence with formed coils. Panel (B): sequence alignment between HSV-
430 1-gB and Aβ-42 (using PyMol software). Panel (C): prediction of amino-acid sub sequences of
431 Aβ42 possessing antibacterial activity *via* AntiBP2 software
432 (http://crdd.osdd.net/raghava/antibp2) and antiviral activity with AVPpred software
433 (http://crdd.osdd.net/servers/avppred).

434

435 **Fig. 2. Energy transfer between HSV-1-gB-GFP and Aβ42-HiLyteFluor-555**.
436 Panel (A): Förster Resonance Energy Transfert process. Panel (B): ratio of emitted green and
437 red fluorescence in each well. Green fluorescence was emitted by HSV-1-gB-GFP particles and
438 red fluorescence was emitted by Aβ42-HiLyteFluor-555 when FRET occurs. Circles represent
439 background measure, triangles represent HSV-1-gB-GFP/Aβ42-HiLyteFluor-555 mix, stars
440 represent HSV-1-gB-GFP particles alone, squares show results after NP40 pre-treatment of
441 virus and diamonds represent results after pre-treatment of Aβ42 with blocking antibodies. The
442 dotted line identifies where green and red fluorescence emission are equal. n=2-5 independent
443 experiments in triplicate.