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Janus Faces of Amyloid Proteins in Neuroinflammation

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Abstract

Amyloid forming molecules are generally considered harmful. In Alzheimer's Disease two amyloid molecules Aβ A4 and tau vie for consideration as the main pathogenic culprit. But molecules obey the laws of chemistry and defy the way we categorize them as humans with our well-known proclivities to bias in our reasoning. We have been exploring the brains of multiple sclerosis patients to identify molecules that are associated with protection from inflammation and degeneration. In 2001 we noted that aB crystallin (cryab) was the most abundant transcript found in MS lesions, but not in healthy brains. Cryab can reverse paralysis and attenuate inflammation in several models of inflammation including experimental autoimmune encephalomyelitis (EAE), and various models of ischemia. Cryab is an amyloid forming molecule. We have identified a core structure common to many amyloids including amyloid protein Aβ A4, tau, amylin, prion protein, serum amyloid protein P, and cryab. The core hexapeptide structure is highly immune suppressive and can reverse paralysis in EAE when administered systemically. Administration of this amyloid forming hexapeptide quickly lowers inflammatory cytokines in plasma like IL-6 and IL-2. The hexapeptide bind a set of proinflammatory mediators in plasma, including acute phase reactants and complement components. The beneficial properties of amyloid forming hexapeptides provide a potential new therapeutic direction. These experiments indicate that amyloid forming molecules have Janus faces, providing unexpected benefit for neuroinflammatory conditions.

Keywords

Multiple sclerosis; amyloidl; hexapeptide; neuroinflammation; experimental autoimmune encephalomyelitis

aB crystallin (cryab) was considered the most immunogenic target in MS myelin in a 1995 paper by van Noort in Nature [1]. We have found that cryab, a molecule that forms amyloid fibrils is protective, and when administered systemically can reverse experimental autoimmune encephalomyelitis (EAE) [2, 3], stroke [4], optic nerve ischemia [5], and myocardial infarction [6]. Detailed mechanistic studies have been undertaken in EAE, where we have defined a core hexapeptide structure in amyloid forming molecules that is highly immune suppressive [7]. In a series of experiments, we have established that amyloidogenic peptides including the small heat shock protein cryab, Aβ A4 [8], characteristic of Alzheimer's Disease (AD), and tau, also found in AD, as well as amylin and serum amyloid protein P, all are therapeutic EAE [2, 7]. Cryab, APP, and tau are all found in lesions that

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were laser capture micro-dissected and subjected to mass spectral analysis in our 2008 Nature paper [3, 7, 8]. The role of these amyloid forming proteins in actual lesions in MS, may be the containment of ongoing inflammation and the initiation of repair (Table I).

To better understand the molecular basis for the therapeutic effect, various amyloidogenic hexameric peptides including those from the major prion protein, tau, Aβ A4, cryab, amylin, serum amyloid P and insulin β chain, were anti-inflammatory, each capable of reducing serological levels of IL-6, and of attenuating paralysis in EAE. IL-6 is highly expressed in both acute and chronic MS lesions [7].

The small heat shock protein, cryab (also called HspB5) has been shown to be an effective anti-inflammatory that has been therapeutic in animal models of multiple sclerosis [2, 7]. More recent studies established a correlation between the molecular chaperone activity of the protein and its therapeutic function, with the therapeutic benefit of the small heat shock protein cryab (HspB5) proposed to arise from its increased capacity to bind proinflammatory proteins at the elevated temperatures within inflammatory foci. By mass spectral analysis a common set of approximately 70 ligands were precipitated by HspB5 from plasma [9]. These proteins were distinguished from other precipitated molecules because they were enriched in the precipitate compared with their plasma concentrations, and they exhibited temperature dependent binding. More than half of these ligands were acute phase proteins, or members of the complement or coagulation cascades.

Additional structure activity correlations between chaperone activity and therapeutic function were established when linear regions within HspB5 were examined. A single region, corresponding to residues 73–92 of HspB5 exhibited chaperone activity and when used as a therapeutic for EAE, was equally potent in its effects as the intact protein on a molar basis. Most importantly, only the peptide exhibiting chaperone activity was therapeutic, establishing a correlation between the two activities [10]. Tanaka and colleagues demonstrated independently that only if 73–92, or a set of analogs, formed amyloid fibrils were they also molecular chaperones [11]. This important correlation, explained how a relatively short peptide could exhibit the equivalent biologic function as a fully folded protein, and led us to analyze whether the therapeutic peptides could be simplified further and still retain their potent capacity to reduce inflammation, including attenuation of paralytic disease in EAE and the reduction of pro-inflammatory cytokines.

We continued these studies and showed that the hexapeptides bind a group of pro-inflammatory proteins, similar to what the parent protein binds in plasma [7, 9]. Mass spectral analysis showed that these fibrils comprised of amyloid forming hexapeptide bound 24 critical serum proteins including apolipoproteins A and E, clusterin, transthyretin, components of the coagulation cascade and complement components, including C3. The anti-inflammatory properties of these fibrils represent a new class of therapeutics for inflammatory neurological disorders [7].

While we have shown in “gain of function” experiments that administration of amyloid forming proteins from molecules like cryab improve function in EAE, stroke, myocardial infarction and optic nerve ischemia [2, 4–6], ‘loss of function’ experiments with gene

deletion of amyloid forming proteins provide further support for the notion that amyloid proteins and peptides are beneficial. It is noteworthy that clinical signs and inflammation of EAE are exacerbated in knockout mice for cryab−/− [2], APP−/− [8], the major prion protein, Prp−/−[12], serum amyloid P−/− [13], and tau−/− [14] mice compared to wild type animals. As mentioned, APP, tau, and cryab are all found in MS lesions [3, 7], indicating that their presence may indeed attenuate rather than exacerbate inflammation in MS.

Experimental results from the pre-clinical development of these amyloid proteins and hexameric peptides indicates that they have potent activity in various pre-clinical models of ischemic and inflammatory disease. This approach may lead to a new class of therapeutics for acute and chronic inflammatory conditions like primary and secondary progressive MS. This strategy is actually harnessing the beneficial aspects of proteins found at the site of progressive MS [3], and then exploiting them for therapy. This work is potentially transformational, because most have thought that amyloid proteins like tau, Aβ, and even cryab are deleterious. These molecules that form amyloid structures and fibrils are actually beneficial, based on the published data we are reporting [2, 7–10]. We intend to follow the data to see where it takes us. An inescapable conclusion is that amyloid molecules can provide benefit and that they reduce inflammation in the central nervous system and elsewhere in a variety of pre-clinical animal models.

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Table I

Compilation of proteins selectively precipitated by biotinylated fibrils composed of the amyloidogenic hexamer, exemplified by Tau 623–628

Rank in ppt	Plasma protein	Plasma conc (uM)	Relative conc ppt
1,2	*Apolipoprotein A-IV*	3–6	118.2
2,10	*Clusterin* †	1–2	108.3
3,1	*Apolipoprotein B-100*	1–3	94.4
5,18	*Complement C1s* *†	1	58.6
6,20	*Beta-2-glycoprotien 1* *†	3–6	53.6
8,22	*Apolipoprotein A-1* *	30–70	46.7
9,21	*SerpinG1 Plasma Protease C1 inhibitor* *†‡	3–5	43.7
12,17	*Coagulation factor V* ‡		35.8
14,12	*Complement C1r* *†	1	28.8
19,33	*Alpha-2-HS-glycoprotien* *	9–30	17.9
20,13	*Vitronectin*	1–3	14.9
23,24	*Transthyretin* *	15–30	12.9
24,29	*Apolipoprotein E* ‡	0.6–2	10.9
28,28	von Willebrand factor ‡	0.04–0.08	8.9
29,36	*Vitamin K-dependent protein S* ‡		7.9
33,27	*SerpinC1 Antithrombin-III* ‡	3–5	7.0
34,42	*Histidine-rich glycoprotein* *	1–3	7.0
35,31	*SerpinA3 alpha-1-antichymotrypsin* *	4–9	6.0
42,43	*Coagulation factor X* ‡	0.2	4.0
43,53	*Serum paraoxonase/arylesterase 1*	2	3.0
48,62	*Angiotensinogen* *	1	2.0
52,58	*SerpinA4 Kallistatin*	0.2	2.0
56,55	*Coagulation factor IX* ‡	0.1	2.0
57,11	*Coagulation factor II Prothrombin* *‡	1.5	1.0

The proteins are listed in the rank hierarchy based on the relative concentration in the precipitate from three separate plasma samples from MS patients. Acute phase (*), complement (†), and coagulation proteins (‡) are demarcated, and those proteins also selectively precipitated by HspB5 are italicized. The concentration of the proteins in plasma from healthy individuals is listed along with the relative amounts found in the streptavidin precipitate (from ref. 7)