

Antibody therapy in Neurodegenerative Disease

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SYNOPSIS

Advances in medical science have led to increased life expectancy and increased median age in the population. Because the symptoms of neurodegenerative diseases generally onset in mid- to late-life, a concomitant increase in the number of persons afflicted with these devastating diseases has occurred. Developing therapies for neurodegenerative diseases is of the highest priority due to the enormous cost of medical care required, as well as for the human suffering involved. Although caused by a variety of genetic and environmental insults, such diseases share commonalities. Many of these diseases are proteinopathies—diseases caused by misfolded, aggregating proteins. Antibodies that can recognize and remove misfolded proteins are ideally suited for proteinopathy therapeutics. The numerous intriguing advances in antibody-based therapies for neurodegenerative diseases are discussed in this review.

KEY WORDS

Alzheimer's disease, Parkinson's disease, Huntington's disease, immunotherapy, vaccination, immunization, intrabody

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IMMUNIZATION THERAPIES FOR ALZHEIMER'S DISEASE

Preclinical experiments with A β protein

Alzheimer's disease (AD) affects some 26 million individuals worldwide, yet the currently available drugs treat only the symptoms. Thus, the need is urgent for the development of novel therapies. Moreover, such therapies should target the upstream events involved in causing the dementia that characterizes this disorder.

The primary diagnostic signs in postmortem AD brain are plaques that contain A β protein aggregates and neurofibrillary tangles (NFTs) that contain the hyperphosphorylated tau protein. Perhaps the most novel therapeutic strategy currently under study for AD is the use of antibodies (Abs) directed at either or both A β and tau. Indeed, the results from preclinical work using this approach with various mouse AD models have been so promising that at least 13 different A β immunotherapy trials are currently in progress, enrolling an estimated 9,000 AD patients worldwide /40/.

This line of work began in 1996 with the finding by Solomon and colleagues that anti-A β monoclonal Abs can dissolve A β aggregates in vitro and prevent A β monomers from aggregating /69,70/. The finding that startled the field and brought AD immunotherapy to the fore was the dramatic result of immunizing the PDAPP AD mouse model, which as it ages develops plaques and memory deficits, with pre-aggregated A β ₁₋₄₂. This treatment prevented plaque deposition and reduced the number of existing plaques, total A β load, and gliosis /65/. Many groups have since extended these findings to a number of other mouse AD models and shown that behavioral deficits, including learning and memory, can be prevented by active immunization with various

types of A β immunogens and adjuvants. The ability of such active immunization protocols to reverse established neuropathology and behavioral deficits has been variable, however /40/.

Given that animals are heterogeneous in the ability to mount a strong immune response, a logical alternative to the active immunization method is the passive approach, wherein purified anti-A β polyclonal or monoclonal Abs are injected. In fact, studies have shown that passive Abs immunotherapy is effective in reducing established plaque and A β levels, neuritic dystrophy, behavioral deficits and, in one case, reduced aggregation of phosphorylated tau. The latter observation, among others, suggested that tau pathology is downstream of A β pathology /52/.

Clinical trials with A β

The remarkable results with the transgenic A β mouse models led immediately to several early human trials. Despite the worry that anti-A β Abs might cause autoimmune problems, in 1999 Elan and Wyeth /20/ proceeded with a Phase I safety trial of 80 patients using the AN1792 vaccine consisting of a synthetic A β_{1-42} peptide and the QS-21 saponin adjuvant. Eighty mild to moderate AD patients received 4 intramuscular injections of peptide + adjuvant, peptide alone, adjuvant alone, or placebo over 6 months. No serious adverse events were reported. In 2001, a Phase IIa 15-month double-blind, placebo-controlled trial followed involving 372 mild to moderate AD patients. This trial was stopped in 2002 after 6% of the patients developed aseptic meningoencephalitis and leukoencephalopathy. The AN1792 + adjuvant approach was designed to accentuate a cell-mediated immune response, which is appropriate for the elderly who often have a reduced immune response. Thus, it may be that the patients developing serious clinical side effects produced A β -reactive autoimmune T cells.

Fortunately, some data are available on the efficacy of this immunotherapy trial that was halted prematurely. In a subgroup of the 30 Phase II patients at one center, improvements in some cognitive tests were seen in the 6 patients that exhibited the highest Ab titers /26/. In a follow-up

study 4.6 years later, another subset of vaccine-treated patients displayed a much-reduced rate of functional decline on the Dependence Scale and the Disability Assessment for Dementia Scale /78/. On the other hand, a volumetric MRI study showed that, compared with the controls, the Ab responders displayed increased loss of brain volume and increased ventricular enlargement. These anatomical changes did not, however, correspond with the enhanced Neuropsychological Test Battery scores that the antibody responders achieved over the controls /20/. Although these seemingly contradictory findings remain to be explained, the cognitive testing results are encouraging, although certainly not as dramatic as those obtained in the mouse studies.

In terms of neuropathology, eight patient brains from the Phase I trial have been analyzed and showed a reduction in A β load, with 2.1% of the area of the neuropil covered by amyloid staining in the AN1792 group compared with 5.1% in the control group, a difference that reached significance /27/. Despite their lower plaque levels, however, all eight vaccinated patients displayed severe dementia at the time of death. This result is consistent with previous AD findings showing little correspondence between plaque load and dementia. In fact, individuals with normal cognition for their age group can exhibit significant plaque loads /5/. Such findings highlight the need for a better identification of the potential drivers of neurotoxicity, the A β oligomers, which can be stained by specific Abs /22/. Yet another provocative finding from 9 patients who died between 4 months and 5 years after their first immunization is the observation that 15 times as many cerebral blood vessels were coated with A β than controls. These patients also had a higher density of cortical microhemorrhages and microvascular lesions than did unimmunized controls. Unlike most of the immunized patients, however, two of the longest survivors had a virtually complete absence of plaques as well as cerebral amyloid angiopathy /7/. The authors optimistically interpreted these findings as suggesting that the injected Abs mobilize A β from the plaques, which then exits the brain via the cerebral vasculature, causing a transient increase in cerebral amyloid angiopathy.

At least seven more trials of passive immunotherapies for mild to moderate AD are underway. The results from a trial of a humanized monoclonal Ab (bapineuzimab, AAB-001) that binds the N-terminus of A β indicated that the trial did not meet its endpoints for cognitive efficacy. A post hoc analysis, however, unadjusted for multiple comparisons found significant cognitive benefits ($p < .05$) in those AD patients who did not carry the apolipoprotein E (APOE) e4 allele /64/. As this allele is a major risk factor for AD, the results suggest that the potentially more severe pathogenesis in the carriers could not be overcome by the immunotherapy. Given the lack of dramatic effects, however, this trial did not have the power to determine if this monoclonal Ab had real cognitive benefits. The possible effect of the APOE allele is an important tip for future trials. Current Phase III bapineuzimab trials have enrolled 4,000 patients.

Another humanized monoclonal antibody, solanezumab (LY2062430) that binds the middle of A β met safety standards in 52 patients, but cognitive scores were not affected. Increased levels of several forms of A β were detected at high levels in sera, however, consistent with the mobilization of these peptides. Two large Phase III studies are underway, enrolling 2,000 patients. Several new trials are also underway using a modified active immunization approach /40/.

Experiments with IVIg antibodies

An alternative immunotherapy involves intravenous immunoglobulin (IVIg). This approach has the advantage that the U.S. Food and Drug Administration has approved such injections as safe for several immune and inflammatory diseases. The Ig preparation is manufactured from the plasma of healthy subjects. This pooled mixture contains anti-A β antibodies that can block the formation of A β oligomers and fibrils, enhance the clearance of A β from the brain, and protect neurons from A β toxicity in culture /40/. Moreover, in a pilot study, IVIg treatment stabilized cognitive loss in five AD patients /14/. In another study of 8 AD patients, IVIg was administered for 6 months, stopped for 3 months, and then resumed for 9 months. The treated

patients displayed improvement in mini-mental state examination scores at 6 months, declined during the washout, and then stabilized for the final 9 months /60/. In addition, anti-A β Abs were found in the CSF, suggesting that they can cross the BBB. Several further studies of IVIg therapy are now in progress.

In addition to the clinical trials, a retrospective case-control analysis examined the incidence of AD and related disorders in people > 65 years old who had received IVIg for other indications. Five years after the start of the analysis, this group had a 42% lower risk of AD diagnosis /19/. Thus, both epidemiologic and early clinical findings with IVIg are encouraging, despite the fact that the anti-A β Abs in the preparation are only a minor component of the mixture, and they have not been optimized for efficacy.

Anti-tau immunotherapy

The second major hallmark of AD, the NFTs containing hyper-phosphorylated tau, has only recently begun to receive significant attention as an immunotherapeutic target. According to the amyloid hypothesis of AD, tau pathology is downstream of misfolded A β , although a dual pathway hypothesis has also received support /68/. In fact, mutations in tau can cause frontotemporal dementia with parkinsonism (FTDP) in the absence of amyloid plaques, and NFTs are better correlated with dementia in late onset AD than are plaques. Moreover, in one AD mouse model, anti-A β immunotherapy cleared NFTs but not hyper-phosphorylated tau aggregates /52/, which has also been seen in human studies.

The first anti-tau immunotherapy results used a mouse model expressing a FTDP tau mutation /3/. Immunization with a tau peptide containing two phosphorylated serines associated with NFTs resulted in the reduction of insoluble tau and an increase in soluble tau. Motor performance on the balance beam and rotarod was improved in the immunized group, but cognitive tests were not informative. Tagged Abs were detected in the brains of transgenic but not wild type mice, indicating that the Abs can cross the BBB in this model. It will be important to test this therapy in a true mouse model of AD. As in the A β experiments,

the choice of the tau preparation used as antigen can be critical, as certain immunogens can cause pathology in normal mice /63/. A focus on the toxic tau oligomers may prove to be the most productive approach, as may be true for A β immunization /32/.

IMMUNIZATION THERAPIES FOR PARKINSON'S DISEASE

Among the mutations that can cause Parkinson's disease (PD), those that have received the most attention are in the protein α -synuclein. This protein is also found in the Lewy body inclusions that are characteristic of the idiopathic, non-familial form of the disease. Contemplating immunization approaches targeting α -synuclein may appear at first to be misguided, as this protein is thought to be intracellular, localized primarily in presynaptic terminals. The evidence shows that under pathological conditions, however, α -synuclein oligomers and protofibrils are detectable on the plasma membrane /13,15,38/. Moreover, a series of recent studies has demonstrated that a mutant α -synuclein protein can be transferred from cell to cell, spreading the disease in the brain in a prion-like (but non-infectious) manner /1/. Thus, circulating Abs may have access to α -synuclein.

The major immunotherapy work published thus far has employed active immunization with human α -synuclein for 8 months in a mouse model over-expressing human α -synuclein /46/. This model displays motor deficits and signs of synaptic degeneration that were attenuated by immunization. The levels of aggregated human α -synuclein were also diminished, and the effects correlated with the relative affinity of the Abs generated in the mice. The injection of tagged, purified monoclonal, anti-human α -synuclein Abs resulted in binding to cell bodies and synapses in α -synuclein-expressing but not in wild type mice. Cathepsin D was up-regulated in the labeled neurons, suggesting that the circulating Abs can recognize the aggregated α -synuclein and cause its clearance via lysosomal activation /46/.

In a different type of passive immunotherapy approach, adoptive transfer, immune cells that cross-react with myelin basic protein (MBP) were injected in the MPTP mouse model of PD /6/. The

cells were generated by stimulating donor mice with copolymer-1 (Cop-1; Copaxone, glatiramer acetate) /75/, a random amino acid polymer that protects against brain injury without the encephalitis associated with MBP immunization. The rationale for the use of these immune cells in the PD model is that Cop-1 stimulates TH2 cells, a T-cell subset that secretes anti-inflammatory cytokines, as inflammation is part of the toxic neuropathology in PD /23/. The intravenous administration of Cop-1-stimulated spleen cells to MPTP-treated mice suppressed microglial activation and protected dopaminergic neurons. This reaction could be a direct effect of the immune cells as the injected cells were found in inflamed brain regions /6/. Depletion of the donor T cells abrogated the beneficial effects, indicating that this type of cell was responsible for the therapeutic benefit. Furthermore, the injection of regulatory T (Treg) cells, which can control the level of inflammation, protects against MPTP-induced dopaminergic neurodegeneration /61/, which is contradictory to a theory of protective autoimmunity /66/.

Just as sera from healthy individuals contain anti-A β auto-Abs, such sera also contain anti- α -synuclein Abs. Whereas 31% of control sera contain anti- α -synuclein Abs, 90% and 52% of sera from familial and sporadic PD patients, respectively, contain such Abs /54/. Because endogenous anti- α -synuclein Abs can be toxic or beneficial, their physiological effects must be determined.

IMMUNIZATION THERAPIES FOR HUNTINGTON'S DISEASE

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disease resulting from the expansion of a normally occurring glutamine repeat in exon 1 of the huntingtin protein (Htt). When this expansion is greater than 36, the protein misfolds and accumulates as aggregates /11,28/. The mutant Htt protein (mHtt) is deficient in performing vital functions of the wildtype Htt (wtHtt), including mediating axonal transport, transcriptional regulation, and mitochondrial metabolism. In addition, mHtt also engages in aberrant interactions, such as

sequestering important cellular proteins, including transcription factors and vesicle-associated proteins /29/. These interactions lead to striatal and cortical atrophy as well as to motor and cognitive decline /50/. Despite the simple genetic nature of HD, available therapies are aimed at symptom management rather than disease modification. Recent work has shown, however, that Ab-based therapeutic approaches can be developed against the most upstream target in HD, the mHtt protein itself. Such approaches have the potential to prevent the onset or delay the progression of HD.

The intracellular localization of the mHtt protein makes it a less than ideal target for immunization therapies. Nonetheless, systemic vaccination with DNA for an N-terminal fragment of mHtt ameliorates the diabetic phenotype of the R6/2 transgenic mouse model of HD and normalizes pancreatic insulin levels /47/. This effect is reminiscent of the positive effects of peripheral injection of the cytoplasmic protein α -synuclein discussed above. The immunized mice, however, displayed highly variable serum responsiveness; no clear effects on brain pathology or behavior were observed, limiting the therapeutic potential of this approach.

Intrabody therapy

Intrabodies (iAbs), intracellularly expressed antibody fragments consisting only of antigen recognition domain(s), are a powerful potential therapeutic for proteinopathies, including AD, PD, and HD. These highly specific reagents can be developed to recognize an infinite number of targets, including amino acid sequence, protein conformation, post-translational modifications, and specific protein interactions. In addition, trafficking sequences can be used to target iAbs to specific sub-cellular compartments /44/. A number of therapeutic iAbs recognizing either distinct conformations or distinct epitopes in proteins that cause neurodegenerative diseases have been developed.

Conformation-specific intrabody therapies

Each of the mutated proteins discussed here can adopt a number of conformations, including

monomers, oligomers, protofibrils, and the fibrillar structures found in A β plaques, Lewy bodies, and large mHtt aggregates (Figure 1). Although some conformations confer toxicity, others perform the beneficial functions of these proteins. Amyloid precursor protein, which gives rise to A β , is involved in normal neuronal physiology, and perturbations could lead to unknown consequences. The α -synuclein protein is natively unfolded and although the oligomeric form is cytotoxic, the monomeric form performs vital beneficial functions. The mHtt protein is also a dynamic protein forming multiple structures. Targeting specific conformations of these disease-causing proteins with iAbs should allow therapeutic intervention with minimal disruption of normal function. Of note, a number of Abs have been isolated that bind well to oligomeric or fibrillar species of several different toxic proteins that do not share amino acid sequence homology /8,31/. Thus, these Abs apparently bind to a conformation shared by the amino acid backbone of several proteins, without regard to the diversity in amino acid side chains.

D5 is an iAb raised from a phage display library against oligomeric α -synuclein. This iAb reduces *in vitro* aggregation and extracellular toxicity of α -synuclein in neuroblastoma cells /16/. When fused to a secretion signal (D5-SEC), this iAb causes the removal of intracellular α -synuclein, which in turn reduces the toxicity of overexpressed α -synuclein in co-transfected 293 cells /81/. D5 also recognizes an oligomeric form of mHtt. Although it reduces aggregation of mHtt *in vitro*, D5 binding increases toxicity in a cell culture model of HD /51/. Another iAb, syn-10H, recognizes a stage of α -synuclein oligomer that is larger and later than D5. This iAb recognizes aggregates in PD but not in normal human brain and reduces α -synuclein-induced toxicity in neuroblastoma cells /17/.

6E is an iAb selected for binding to fibrillar α -synuclein but also recognizes only the fibrillar form of mHtt. This iAb increases fibril formation, aggregation, and toxicity of mHtt in a cell culture model of HD /36/. The extreme selectivity of conformation-specific iAbs that allow differentiation, not only between mutant and wt proteins but also between various species of mutant proteins,

warrants further investigation in both structural and therapeutic contexts.

EPITOPE-SPECIFIC INTRABODY THERAPIES FOR ALZHEIMER'S DISEASE

As discussed above, the major component of plaques in AD brains is A β , one of the products generated from the proteolytic processing of the amyloid precursor protein (APP). APP is processed by one of two mutually exclusive cleavage pathways. In the first, more common pathway, APP is cleaved by α -secretase generating the non-toxic P3 fragment. In the second much less common pathway, APP is cleaved by β -secretase generating A β (Figure 2A) /25/. The two common forms of A β , are the 40 AA form, A β ₄₀, and the 42 AA form, A β ₄₂. The longer form, A β ₄₂, is less soluble and has a greater propensity to form oligomers and fibrils, leading to enhanced toxicity /37/.

As the differential proteolytic processing of APP can lead to either toxic or non-toxic products, emulating or enhancing the α -secretase cleavage of APP has therapeutic potential. A screen of iAbs for α -secretase-like activity identified two iAbs with possible therapeutic applications for AD. A serine-protease-like iAb, c23.5, mimics α -secretase cleavage, producing the 1-16 and 17-40 amino acid fragments of A β . A carboxypeptidase-like iAb, hk14, sequentially cleaves the C-terminal residues of A β ₄₂, generating the less toxic A β ₄₀ /59/. Another iAb, sFv β 1, recognizes an epitope adjacent to the α -secretase site of APP at AA 3-6 of A β . This iAb shifts APP processing toward the more favorable α -secretase product, reducing A β production and toxicity in co-transfected 293 cells. When fused to an endoplasmic reticulum (ER) retention signal, sFv β 1 prevents the newly generated APP from leaving the ER, leading to its degradation and virtually abolishing A β production /53/.

Other iAbs have been selected from phage display libraries for A β -binding efficacy. H1v2 is an iAb that recognizes AA 17-28 of A β and reduces its *in vitro* aggregation. The co-incubation of this iAb with A β prevents extracellular toxicity to neuroblastoma cells /43/. The adeno-associated virus (AAV)-mediated delivery of anti-A β iAb scFv59 to the cortico-hippocampal region of the

brains of Tg2576 AD model mice results in strong iAb expression and reduced A β plaque load as long as 1 year post-injection /21/.

Immunization studies have identified monoclonal Abs that when passively delivered, prevent A β plaque deposition in the brains of AD transgenic mice. Three iAbs have been generated from such Abs; scFv9, which recognizes AA 1-16 and binds both A β ₄₀ and A β ₄₂, and scFv40.1 and scFv42.2, which respectively recognize A β ₄₀ and A β ₄₂, /42/. An AAV-mediated delivery of any of these iAbs to the ventricles of P0 CRND8 AD model mice resulted in a 25% to 50% reduction in A β plaque deposition /41/. The beneficial effects of scFv42.2 were enhanced by the addition of an ER retention signal. The AAV-mediated delivery of the modified iAb to the hippocampus of 3xTg-AD model mice resulted in not only reduced A β ₄₂ plaque load but also reduced levels of hyperphosphorylated tau, once again indicating that tau pathology is downstream of the A β pathology in AD /74/.

EPITOPE-SPECIFIC INTRABODY THERAPIES FOR PARKINSON'S DISEASE

The primary component of the Lewy body inclusions that characterize PD is α -synuclein, and a portion of this protein, the non-amyloid component (NAC), is found in AD plaques (Figure 2B). Although far less common than sporadic PD, familial PD is most often caused by mutations in the α -synuclein gene. Moreover, the overexpression of wt α -synuclein is sufficient to cause a PD-like toxicity in cells, flies, and mice /12/. For these reasons, α -synuclein is the primary therapeutic iAb target for PD.

NAC32 is an iAb selected from a phage-display library against the NAC of α -synuclein. NAC32 expression reduces the aggregation and toxicity of a mutant form of α -synuclein in co-transfected ST14A cells /45/. Another iAb selected from a phage display library is D10, which recognizes monomeric α -synuclein. D10 expression reduces the aggregation and toxicity of overexpressed wt α -synuclein in co-transfected 293 cells. When fused to a nuclear localization signal (NLS), this iAb re-targets α -synuclein to the

nucleus, but has no effect on the localization of the highly homologous α -synuclein, demonstrating the extreme specificity of iAbs /82/. The beneficial effects on toxicity are augmented when D10 is fused to a secretion signal (D10-SEC), leading to the removal of α -synuclein from cells /81/. Interestingly D10-SEC leads to the removal of larger quantities of α -synuclein than the oligomer-specific D5-SEC, but is not as beneficial, indicating lower cellular levels of the more toxic oligomeric species.

EPITOPE-SPECIFIC INTRABODY THERAPIES FOR HUNTINGTON'S DISEASE

Exon 1 of Htt (HDx-1), the site of the mutation that causes HD, consists of 17 N-terminal amino acids, a polyglutamine (polyQ) tract, the proline rich region (PRR) that includes two polyproline (polyp) tracts separated by a proline rich (P-rich) sequence, and 13 C-terminal amino acids (Fig. 2C). Potential therapeutic iAbs recognizing each of the four regions of HDx-1 have been developed.

Intrabodies recognizing N1-17

The N-terminus of Htt is the site of many important interactions including membrane association and post-translational modifications /4, 62/. This domain regulates the localization, aggregation and degradation of Htt /76,77/. Two iAbs recognizing this region, C4 and VL12.3, have been developed as potential therapeutics.

C4 is an iAb isolated from a phage-display library that preferentially binds to diffuse but not to aggregated N-terminal Htt fragments /48/. The expression of this iAb in 293 and ST14A cells reduces the level of both wt and aggregated mHtt, but increases the level of soluble mHtt /48/. The C4 iAb was originally shown to reduce the aggregation and toxicity of mHtt in cell culture models of HD, but it required a 5:1 ratio to mHDx-1 for optimal effects /39/. Re-engineering C4, which involved mutagenizing and selecting for greater Htt binding affinity, resulted in an iAb that protects against malonate-induced toxicity in mHDx-1-transfected organotypic slice cultures /49/. In a *Drosophila* model of HD, the C4 iAb

also rescues the eclosion deficit, increases adult survival, and decreases photoreceptor degeneration /80/.

VL12.3 is a single domain, light chain iAb selected from a yeast surface display library that reduces mHDx-1 aggregation in a cell-free system as well as in mHtt co-transfected 293 cells /9/. Although originally requiring a 5:1 ratio to mHDx-1 for optimum effects, the VL12.3 iAb was re-engineered by removal of the disulfide bond, random mutagenesis and selection for greater Htt binding affinity, yielding a very stable, soluble and potent iAb /10/. The mature VL12.3 iAb reduces mHDx-1- induced toxicity and aggregation at low iAb:HDx-1 ratios in both cell culture and organotypic slice culture models of HD. Consistent with the cytoplasmic retention function of the Htt N-terminus, VL12.3 binding also causes an increase in nuclear HDx-1 /71/. The VL12.3 iAb was also tested in vivo in a variety of mouse models of HD. In one model involving the lentiviral delivery of mHDx-1 to the striatum of 4-week-old wt mice, the AAV-mediated striatal delivery of VL12.3 dramatically reduces mHtt aggregation and striatal neuron death and rescues the abnormal amphetamine-induced rotation phenotype of these mice. Despite such promising results in acute cellular and in vivo models of HD, the AAV-VL12.3 treatment of R6/2 and transgenic models of HD results in no change or even a worsening of phenotypic severity, including decreased survival of R6/2 mice despite a dramatic reduction in striatal HDx-1 aggregates /72/.

The target of C4 and VL12.3, the N-terminus of Htt, is required for cytoplasmic retention. Compromising the cytoplasmic retention this function of the Htt N-terminus results in increased nuclear Htt that is associated with increased toxicity /56,62/. The N-terminus domain is also required for aggregate seeding, which may be a protective mechanism /2,67,75,76/. The N-terminus is also the site of several important post-translational modifications, including phosphorylation, which increases the degradation of mHtt and is neuro-protective /24,76,77/. For these reasons, the iAb blockade of the N-terminus of Htt may not be an ideal long-term therapeutic strategy for HD, as suggested by in vivo results with VL12.3.

Intrabodies recognizing polyQ

The expanded polyQ tract is the only AA sequence difference between wt and mHtt, and as the polyQ tract alone can confer toxicity, this epitope should be an ideal target for iAb therapy directed selectively at mHtt.

MW1 and MW2 are iAbs derived from monoclonal antibodies that recognize polyQ in HDx-1. The iAbs preferentially bind mHDx-1 and mHtt and recognize both the native and denatured proteins. Despite the ability of the expanded polyQ domain to confer toxicity, the treatment of cell culture and organotypic brain slice models of HD with MW1 or MW2 increases mHDx-1-induced toxicity and aggregation /33/. This increase in toxicity could potentially be the result of an iAb-mediated stabilization of a toxic conformation of mHDx-1 or an iAb-mediated cross-linking of mHDx-1 molecules, which could accelerate oligomer formation. An exploration of such possible mechanisms may shed light on the molecular basis of mHtt toxicity. Despite the potential selectivity of targeting this domain, the results indicate that iAb binding to the polyQ tract is more likely to potentiate than to ameliorate toxicity.

Intrabodies recognizing the proline rich region

The PRR of mHtt mediates a number of aberrant protein-protein interactions, including the sequestration of SH3 and WW domain-containing transcription factors and vesicle proteins /18,58/. These domains are the site of interactions with IKK γ , a regulatory subunit of the I κ B kinase complex. Activating this complex promotes aggregation and nuclear localization of mHtt /34/. The PRR of Htt is also the site of its interactions with P53 and the CREB-binding protein and the PRR is required for mHtt-induced transcriptional repression of P53-regulated genes /73/. All these toxic interactions are strengthened in response to increased polyQ length. Three iAbs recognizing the PRR domain, MW7, Happ1 and Happ3, have been developed as possible HD therapeutics.

MW7 is an iAb derived from a monoclonal antibody that recognizes polyP. This iAb reduces mHDx-1-induced toxicity and aggregation in cell culture, *Drosophila*, and organotypic brain slice

models of HD /30,33,71/. MW7 binding results in an increased clearance of soluble mutant but not wtHDx-1, a mechanism with great therapeutic potential /71/. The MW7 iAb is, however, not very potent as it requires a 4:1 ratio to mHDx-1 for optimal effects /33/. Although no evidence of MW7 binding to proteins other than Htt has been observed, the specificity of this iAb for pure polyP could potentially allow binding to other polyP domain-containing proteins.

Happ1 and Happ3 are single domain V_L iAbs selected from a phage-display library that recognize the P-rich domain of Htt lying between the two polyP tracts (Fig. 2C). These iAbs have the beneficial properties of MW7, including the reduction of mHtt-induced aggregation and toxicity in cell culture and organotypic brain slice models of HD, as well as an increased clearance of mHtt. Moreover, the recognition of a single, unique sequence found only in Htt increases the likelihood of extreme binding specificity. In addition, Happ1 and 3 display increased potency over MW7, requiring a 2:1 ratio to mHtt for optimum effects /71/. Although these iAbs bind to both mutant and wtHDx-1 in denaturing conditions, the selectivity for the increased clearance of only the mutant protein in cells in native conditions indicates a strong preference for this form. This preference may be due to the increased availability of the PRR epitope in expanded polyQ Htt conformations. This idea is supported by the findings of increased binding of other Htt PRR-interacting partners with increased polyQ length /55,58,73/.

The results obtained with five different HD mouse models further support the therapeutic utility of Happ1 /72/. AAV-mediated intrastriatal delivery of this iAb in the lentiviral mouse model dramatically reduces mHDx-1 aggregation and striatal neuron death and completely rescues the amphetamine-induced rotation behavioral phenotype. In addition, delivery of AAV-Happ1 to the striatum of the R6/2, N171-82Q, YAC128, and BACHD transgenic HD models ameliorates many aspects of the HD-like phenotype. Normal motor performance is restored in N171-82Q, YAC128, and BACHD mice, and motor performance is improved in R6/2 mice. The cognitive effects of mHtt are another important aspect of HD, and

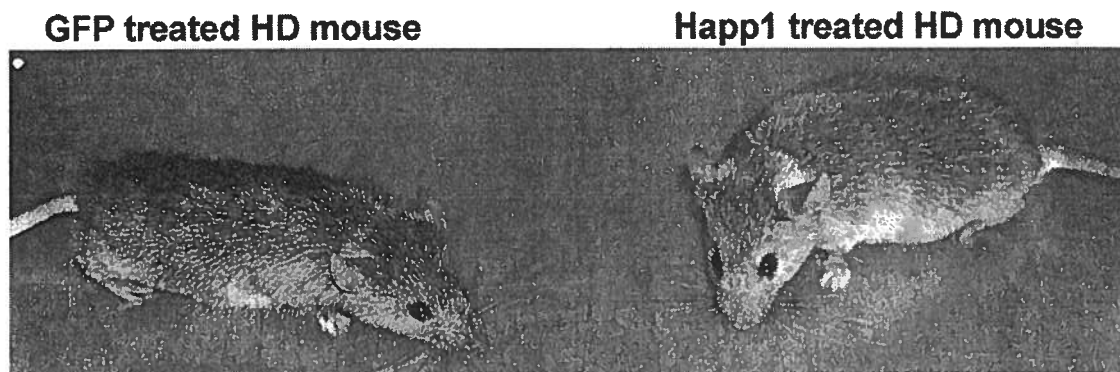


Fig. 3: Happ1 treatment improves body weight of N171-82Q HD model mice. The GFP-treated N171-82Q mouse (left) displays reduced body weight, crouched posture and ruffled coat, whereas the Happ1-treated N171-82Q mouse (right) displays normal body weight and appearance. The mice shown are male littermates. /72/

Happ1 treatment restores normal cognitive performance in YAC128 mice and improves cognitive performance in BACHD mice. Happ1 treatment also ameliorates neuropathology in these models. HDx-1 aggregation is strongly reduced in the R6/2 model, and ventricle size is normalized in R6/2, YAC128, and BACHD mice. Although Happ1 has no effect on R6/2, YAC128, or BACHD body weight or R6/2 survival, this iAb does significantly increase both body weight and life span of N171-82Q mice (Figure. 3) /72/. Engineering to increase stability and binding affinity could potentially further improve this very promising iAb.

One potential mechanism that could mediate the positive effects of the anti-PRR iAbs could involve the blockade of a number of aberrant interactions of mHtt with other proteins that lead to a gain of toxic function. Another mechanism involves the regulation of mHtt stability because iAb binding to the PRR leads to increased clearance of the mutant but not the wildtype protein.

Intrabodies recognizing the C terminus of HDx-1

Although the functions of the C-terminus of HDx-1 are unclear, results with two iAbs, EM48 and MW8, which bind this region, indicate that this domain contributes to mHtt toxicity.

EM48 is an iAb derived from a monoclonal antibody that recognizes an epitope in the C-terminus of HDx-1 that is adjacent to the PRR. This iAb preferentially binds mHDx-1 and increases its ubiquitination and turnover in a cell-culture model of HD. Adenoviral delivery of EM48 to the striatum of R6/2 and N171-82Q HD model mice reduces striatal neuropil aggregates, increases Htt cleavage products, and improves the motor performance of N171-82Q mice. Yet, EM48 gene therapy has no effect on intranuclear inclusions, body weight, or survival /79/.

MW8 is an iAb derived from a monoclonal antibody that recognizes a unique epitope in the C-terminus of HDx-1. This iAb preferentially binds aggregated rather than diffuse Htt, allowing selectivity for the mutant form. MW8 treatment reduces mHtt-induced toxicity and aggregation in cell culture models of HD, although its beneficial effects are modest in comparison to those of several other iAbs, making MW8 a poor choice for further development /35/.

Taken together, these studies demonstrate that the iAb gene therapy strategy for the treatment of HD can be very effective. Those iAbs directed at the regions flanking the HD mutation rather than at the mutation itself, and particularly those that can distinguish between mutant and wtHtt, display the greatest promise.

PERSPECTIVES

Clearly, a wide variety of antibody-based therapies can have striking efficacy in animal models of AD, PD, and HD. Clinical testing of passive and active A β immunization for AD is quite advanced. Regrettably, the early safety results have been somewhat mixed, and the early efficacy results have certainly not been as impressive as in the mouse models. Nonetheless, this therapy is still in its very early days, and one should recall the many missteps and the fine-tuning that was required to achieve the nearly uniform success we now enjoy with bone marrow, kidney, and heart transplant procedures. Many patients gave their lives in those clinical experiments, which has not yet been the case in the context of immunotherapy for AD, PD, and HD. Indeed, more than fine-tuning must be done to test immunotherapy for neurodegenerative diseases. Clinical experiments are needed to sort out the relative efficacy of non-specific versus highly specific antibody approaches, systemic versus intracranial delivery, gene therapy versus active or passive immunization, and antibody versus intrabody approaches. Hopefully, the large clinical trials of immunization for AD now underway will yield enough positive results to sustain the momentum of the immunotherapy approach to these devastating diseases.

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