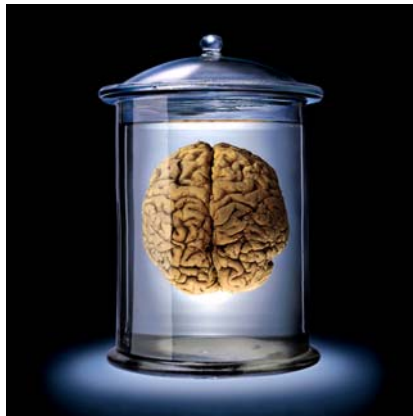


Infections and Neurodegenerative Diseases: Prions and beyond



"The story of prions is truly an odyssey that has taken us from heresy to orthodoxy." (Stan Prusiner at Nobel Banquet Speech, Stockholm 1997)
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It's a success story: All over the world, people are living longer, and effective prevention and treatment of infectious diseases has played a large part. But at the same time, cancer, autoimmune syndromes and many other chronic ailments are on the rise. This includes neurodegenerative diseases, with the number of patients afflicted with the two most prevalent - Alzheimer's and Parkinson's - estimated to double by 2030.

When the German psychiatrist Alois Alzheimer first described Alzheimer's disease in 1906, he and his colleagues suggested that microorganisms might contribute to the formation of the characteristic senile (amyloid) plaques that disrupt brain function. A century later, some researchers striving to understand and find treatments for Alzheimer's and other neurodegenerative diseases are rediscovering the connection to infection.

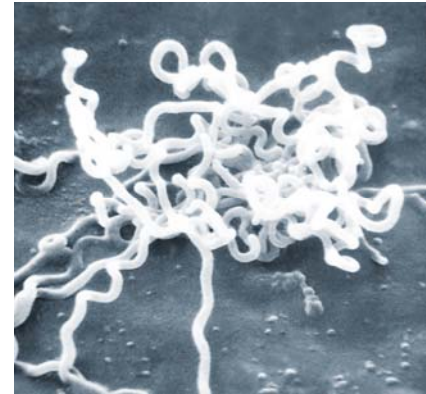
More than 600 disorders that afflict the nervous system have been identified. The clinical manifestations of a particular disease, e.g. problems with movement or deterioration of memory/dementia, depend on the specific set of neurons in the brain/spinal cord (or their myelin sheath) that is damaged. The majority of neurodegenerative disorders seem to involve abnormal neuronal proteins, which can be the result of misfolding, altered post-translational modification, aberrant proteolytic cleavage, anomalous gene splicing, improper expression or diminished clearance of degraded protein. Insoluble aggregates of misprocessed proteins that accumulate outside or inside the neuron ("plaques" and "tangles", respectively) are hallmarks of many neurodegenerative diseases. Some commonly known and studied diseases are Alzheimer's disease, Parkinson's disease, Huntington's disease, Frontal Temporal Dementia, Multiple Sclerosis and Amyotrophic Lateral Sclerosis.



Alois Alzheimer

Some neurodegenerative diseases are entirely genetic; Huntington’s disease, for example, is transmitted by a single gene and is inherited autosomal dominantly. Genetic mutations also account for a small percentage (less than 5%) of Alzheimer’s disease (familial Alzheimer’s). However, the cause(s) of most neurodegenerative disorders are not so clear-cut and are probably multifactorial, a combination of genetic susceptibility and a variety of environmental factors.

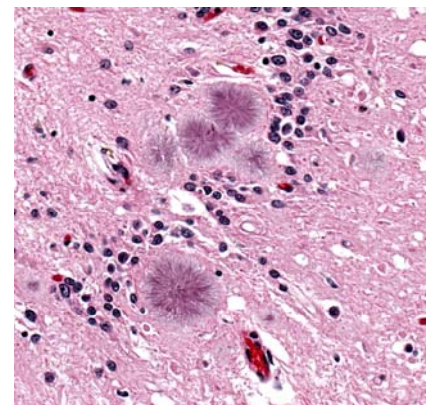
For a few neurodegenerative diseases, a clear link to a causative infectious agent exists. For example, *Treponema pallidum*, the spirochetal bacterium that causes syphilis, caused neurological symptoms (neurosyphilis) in 25–35% of syphilis patients before the introduction of antibiotics; today it is most often seen in HIV-infected patients. In its most severe form, this infection of the brain and spinal cord results in chronic dementia and subsequent death. Fellow spirochetes of the *Borrelia* species, the culprits of Lyme disease, can also infect the central nervous system and cause progressive brain dysfunction (neuroborreliosis). And prions, unique infectious agents composed entirely of misfolded protein, are generally accepted as the causative agents of transmissible spongiform encephalopathies (TSEs) such as bovine spongiform encephalopathy (BSE) and human Creutzfeldt-Jakob disease (CJD).



Treponema pallidum bacteria © CDC/ Dr. David Cox

A most peculiar “bug”

It’s relatively rare, affecting only 1 out of every million people worldwide each year, but its symptoms are horrifying: impaired movement and speech, dementia, memory loss, hallucinations, personality changes. Death can follow within months after the first symptoms appear. When “mad cow disease” (variant Creutzfeldt–Jakob disease/vCJD in humans) struck Great Britain the mid 1990’s, there was public panic; anyone who had eaten meat or meat products within the previous decade, since BSE had hit the country’s cattle herds, could be infected. Since 1996, 208 individuals from 11 different countries have died from vCJD; more than 160 of the deaths occurred in the United Kingdom. In 1997, Stanley B. Prusiner was awarded the Nobel Prize in Physiology or Medicine for his research into prions, the infectious agent widely believed to be responsible for CJD.



Brain tissue with the presence of typical amyloid plaques found in a case of variant Creutzfeldt-Jakob disease (vCJD) © CDC/ Teresa Hammett

The word “prion” was crafted from proteinaceous, infectious and the ending -on (like virion). Prions are infectious, but they’re not microbes; they contain no nucleic acids. According to the prion “protein-only” theory, infection is transmitted via rogue misfolded forms of prion protein (termed PrP^{SC} for scrapie, the epidemic neurodegenerative dis-

ease of sheep and goats). In the test tube, PrP^{SC} can transform endogenous forms of the protein (PrP^C for cellular) into the abnormal form. In the brain, this change in conformation leads to protein aggregation and the formation of amyloid plaques. The disruption of normal brain structure resulting from TSEs is characterized by a holey, sponge-like appearance (hence “spongiform”).

Although prions are widely celebrated as an entirely new form of infectious agent, not every believes in the infective power of rogue proteins. Prions have had trouble fulfilling Koch’s Postulates; most problematic, purified prion preparations have not been particularly effective in transferring disease *in vivo*. However, prion researchers are working on filling the “holes” in the prion theory. In particular, Claudio Soto and colleagues, who were able to amplify infectious prions from diseased brain using a technique called protein misfolding cyclic amplification (PMCA; Castilla J. *et al.* *In vitro* generation of infectious scrapie proteins. *Cell* 2005. 121: 195–206), extended on their findings in September of last year. Using PMCA they could show that protein misfolding shapes the generation of specific prion strains and controls their species specificity (Castilla J. *et al.* Crossing the species barrier by PrP(Sc) replication *in vitro* generates unique infectious prions. *Cell* 2008. 134: 757–768). Other groups are working on defining the structural components required for the folding of infectious prions and the mechanisms by which prion infection leads to brain damage and neurodegeneration (additional information located below).

Nevertheless, a small but persistent minority supports the theory that TSEs are caused by “slow viruses”.

A case for viruses?

The group of Laura Manuelidis (Yale University) has been at the forefront in championing a role for viruses in TSEs. The features of TSEs fit with the known characteristics of diseases caused by so-called “slow” viruses, which refers to the tempo of disease (incubation periods of months or years, extended and progressive clinical course). In addition, the 25–35 nm-large virus-like particles isolated from scrapie or CJD-infected cells are large enough to hold nucleic acids 1–4 kb in length, which have been found in infectious preparations of TSE tissue.

Article: Manuelidis L, Yu ZX, Barquero N and Mullins B. Cells infected with scrapie and Creutzfeldt-Jakob disease agents produce intracellular 25-nm virus-like particles. Proc. Natl. Acad. Sci. USA 2007. 104: 1965–1970.

Review: Manuelidis L. A 25 ml virion is the likely cause of transmissible spongiform encephalopathies. J. Cell. Biochem. 2007. 100: 897–915.

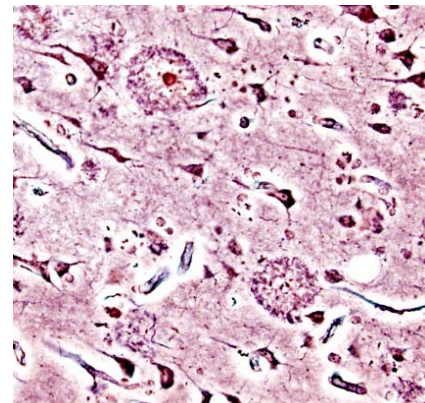
Comment: Penman S. Looking Glass Science. 2007. J. Cell. Biochem. 101: 1071–1073.

The jury is still out, but it's clear that an infectious agent - be it a prion or a virus - is responsible for TSEs. Infection hasn't traditionally played a central role in investigation of other neurodegenerative diseases, but it has been receiving increasing attention. Infections affect the course of diseases such as Alzheimer's and Parkinson's, with inflammatory responses resulting in worsening of symptoms. And a number of pathogens have been linked to such multifactorial neurodegenerative diseases.

Alzheimer's and Co.

Despite intense research into Alzheimer's disease (AD), there is little definitive information about its cause and no effective treatments. The greatest risk factor is age: after age 65, the risk of developing AD doubles approximately every 5 years. However significant links to AD have also been reported for many other factors including head trauma, early life experiences, exposure to metals (aluminum, zinc), diet and heart/circulatory health (*e.g.* blood pressure, cholesterol, diabetes). In other words, the cause is unknown - sporadic.

After age, the greatest risk factor for AD identified to date is the e4 allele of the APOE gene. There are three common alleles of ApoE (e2, e3, e4), which encodes apolipoprotein E, a protein that - among other functions - helps carry blood cholesterol throughout the body. Between 25 and 30% of the general population have the e4 allele, while it's found in approximately 40% of patients with sporadic (late-onset) AD. The risk appears greatest (up to 20-fold greater) for individuals with two e4 alleles (e4/e4), who also tend to develop disease at a younger age than those with one (e2/e4 or e3/e4) or no e4 alleles. Of course, many individuals with the e4 allele do not develop AD, and the disease can also strike those without the allele. Nevertheless, it's a striking finding that also seems to have an infection connection, namely with herpes simplex virus type 1 (HSV1).



Histopathologic image of senile plaques seen in the cerebral cortex of a person with Alzheimer's disease of presenile onset. © KGH

HSV1, the neurotropic virus that causes cold sores, is an almost ubiquitous companion of human beings. In a large percentage of elderly individuals, latent HSV1 DNA can be detected in brain tissue (it's rare in young people), but - if assessed separately - there seems to be no association with ApoE genotype or AD. However, a high risk for AD has been shown when HSV1 infects the brain of individuals with the ApoE4 genotype. Recent research has strengthened the connection.

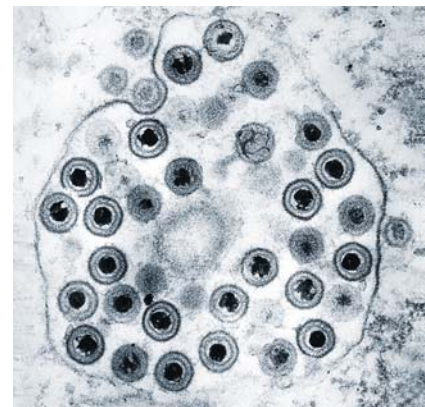
The ApoE4 allele supports the lytic cycle of HSV1, possible enabling the virus to be more active in the brain. (Miller RM and Federoff HJ. Isoform-specific effects of ApoE on HSV immediate early gene expression and establishment of latency. Neurobiol. Aging 2008. 29: 71–77)

In AD brain specimens 90% of plaques contain HSV1 DNA, and most of the viral DNA is located within amyloid plaques; the association of viral DNA with plaques in non-AD “aged” brains is much weaker. (Wozniak MA, Mee AP and Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. J. Pathol. 2009 217:131–138)

ApoE is involved in proteolytic clearance of the peptide beta-amyloid, the main component of AD plaques, in the brain; compared to the ApoE2 and ApoE3 isoforms, ApoE4 is impaired in beta-amyloid proteolysis and removal. (Jiang Q et al. ApoE promotes the proteolytic degradation of Aβeta. Neuron. 2008. 58: 681–693)

The case for HSV1 involvement in AD is getting stronger and is by far the most solid connection of an infectious agent to a multifactorial neurodegenerative disease.

But it is not alone. Species of *Chlamydia* (particularly *C. pneumoniae*), *Mycoplasma*, *Borrelia* and *Brucella* – among others – have been linked to disorders including Alzheimer's disease, Parkinson's disease, Multiple Sclerosis and Amyotrophic Lateral Sclerosis (see the Nicolson review below). In diseases influenced by so many environmental factors, it cannot be expected that a “magic bullet” will be found soon, but the identification of microbes involved in neurodegenerative disorders can open new avenues for therapy with the potential to help millions.



A transmission electron micrograph (TEM) of numerous herpes simplex virions © CDC/ Dr. Fred Murphy; Sylvia Whitfield

For further information:

- A recent general review of pathogens and neurodegenerative diseases:
Nicolson, GL. Chronic bacterial and viral infections in neurodegenerative and neurobehavioral diseases. *LabMedicine*. 2008 39(5): 291-299. (<http://labmed.ascpjournals.org/>)
- Different models for the mechanism by which infectious prion protein (PrP^{Sc}) causes brain damage and neurodegeneration are discussed in a review by C. Soto (Endoplasmic reticulum stress, PrP trafficking, and neurodegeneration. *Dev. Cell* 2008 15 :339–341.)
- C. Soto has also written an informative commentary to a recent article about the structural requirements of infectious prions (Original article: Sigurdsson CJ, *et al.* *De novo* generation of a transmissible spongiform encephalopathy by mouse transgenesis. *Proc. Natl. Acad. Sci. USA* 2009. 106: 304–309; Commentary: Constraining the loop, releasing prion infectivity. *Proc. Natl. Acad. Sci. USA* 2009 106: 10–11).
- For discussion of the Pathogen Hypothesis of late-onset Alzheimer's disease, with access to some interesting articles, see:
<http://www.alzforum.org/res/for/journal/detail.asp?liveID=65#{5976B70D-206A-40A7-AC4D-A62F88C6F2F5}>
- Several reviews covering the potential role of infection were published in the May 2008 issue of the *Journal of Alzheimer's Disease*.