

# EUROBRAIN D

## *Prion and mad cow*

**1, 5–6 WHERE NOW  
“MAD COW” DISEASE?  
2–4 ANTIPRIONS: ANTI-  
BODIES: A STRATEGY AGAINST  
CREUTZFELDT-JAKOB DISEASE  
AND MAD COW DISEASE?**

### **WHERE NOW WITH “MAD COW” DISEASE?**

The scare over mad cow disease has had profound effects on the human food chain. When the dread disease, known formally as bovine spongiform encephalopathy (BSE), was reported in France, Germany, Italy and Spain, European consumers began to shun beef. McDonald’s stock price dropped as sales in Europe plummeted. The Parisian restaurant, L’Arpege, one of the jewels of French cuisine, announced it was going vegetarian. Meanwhile, in the U.S., health officials called for more stringent measures to prevent an outbreak there.

The cause for alarm is not BSE *per se*, but its human form, variant Creutzfeldt-Jakob disease (vCJD). Although it is one of the rarest of human diseases, with 125 cases reported to date, vCJD is apparently caused by an insidious pathogen, known as a prion, which is notoriously hard to detect, is impervious

to cooking or sterilization, and dooms its victims to a horrible death.

Scientists in Britain realized in the mid 1990s that they had a new human disease on their hands when young people—some only in their teens—began to show symptoms of Creutzfeldt-Jakob disease, which usually strikes older adults. Its victims decline over about a year, with tremors and agitation, progressing to psychosis, loss of motor control, blindness, incontinence, dementia and, finally, death. Autopsies reveal brain tissue pocked with abnormal “plaques” ringed by holes (hence “spongiform encephalopathy,” a more general term for such diseases).

The cause is now thought to be a prion, an infectious agent unlike any other. All other infectious agents are living organisms—viruses, bacteria, fungi, or protozoans—that contain DNA, but the prion has no DNA; instead, it is a protein encoded by a gene *Continued on page 5*



### **CONTACT**

**Béatrice Roth, PhD**  
Institut de Physiologie  
7, rue du Bugnon  
CH-1005 Lausanne  
Switzerland  
Tel./Fax: +41 21 692 55 25  
dana1997@iphysio.unil.ch  
www.unil.ch/edab

**Elaine Snell**  
Vicarage House  
58-60 Kensington Church Street  
London W8 4DB, UK  
Tel.: +44 207 937 7713  
Fax: +44 207 937 4314  
edab@which.net  
www.edab.net

The European Dana Alliance for the Brain is an alliance of 120 eminent scientists that promotes the importance of brain research.

# *Antiprion antibodies: a strategy against Creutzfeldt disease and mad cow disease*



Adriano Aguzzi

A humoral immune response may prevent prion disease *in vivo*. This suggests that immunotherapeutical intervention against prion diseases is not unattainable. Will vaccines and post-exposure strategies based on antibodies ever prove useful against scrapie, bovine spongiform encephalopathy (BSE), or Creutzfeldt-Jakob disease?

Vaccines are often very efficient against viral and at least some bacterial infections: from birth to adulthood, people are vaccinated against an array of diseases, ranging from measles to clostridia. Similarly, domestic animals undergo immunizations that protect them from veterinary infections. But for a long time, little evidence has come forward that prions might be attacked with immunization procedures.

Most recently, a flurry of studies have addressed the effects of antibody in prion replication and spread *in vitro* and *in vivo*. Enari and Weissmann described prevention of PrP<sup>Sc</sup> formation *in vitro* when infectious prions and a monoclonal anti-PrP<sup>C</sup> antibody were administered in concert. Only weeks later, Peretz and colleagues confirmed these results in a somewhat different study that takes advantage of antibody fragments directed against specific PrP<sup>C</sup> domains. This strategy enabled the mapping of "hot" regions of the prion

protein, which may represent putative therapeutic targets. Our laboratory has asked whether a protective humoral response against prions can be produced by the mammalian immune system. Towards this goal, we have generated transgenic mice, in which the clonal composition of the antibody repertoire was skewed toward recognition of the normal prion protein PrP<sup>C</sup>. We found that intraperitoneal inoculation of prions resulted in protection from scrapie pathogenesis. Co-expression of anti-PrP<sup>C</sup> antibodies and PrP<sup>C</sup> at physiological levels did not induce an obvious autoimmune disease—a finding of some relevance when contemplating the possibility of antiprion vaccination.

We circumvented the problem of unresponsiveness of the immune system against the ubiquitously expressed self-antigen PrP<sup>C</sup>, which most probably results from immune tolerance to the endogenous PrP<sup>C</sup>. Because B-cells appear not be intrinsically tolerant to PrP, it is probably T-helper tolerance that inhibits immunity to prions in wild-type mice. It will be interesting to investigate whether the latter might be overcome, e.g. by presenting PrP<sup>C</sup> to the immune system in a highly adjuvant context.

Finally, a study by Souan and colleagues supports the idea of inducing prionostatic immune responses: immunization of wild-

# Creutzfeldt-Jakob disease?

type mice with prion protein peptides induced anti-PrP titers, and seemed to reduce PrP<sup>Sc</sup> formation.

From a structural point of view, it appears that most of the studies discussed above identify the region encompassing codons 132-156 of the prion protein as critical to targeting prion replication. Therefore, future studies might consider this region as a promising target for developing therapeutic strategies.

## MECHANISM OF ANTIBODY-MEDIATED PRION-PREVENTION?

What is the mechanism of antibody-mediated interference with prion replication and spread? In the context of the protein-only hypothesis, at least three scenarios can be conjured to explain what happens in cultured cells, while one additional phenomenon may occur in living animals (summarized in fig. 1): Firstly, anti-PrP<sup>C</sup> antibodies might bind endogenous PrP<sup>C</sup>, which is attached to the membrane by a glycosylphosphatidylinositol anchor. As a consequence, anti-PrP<sup>C</sup> antibodies may mask PrP<sup>C</sup> and prevent prion replication, because the normal cellular prion protein PrP<sup>C</sup>—which is the necessary substrate for the conversion into PrP<sup>Sc</sup>—is no longer available. A further possibility would be that anti-PrP antibodies interact directly with PrP<sup>Sc</sup>, thus blocking the PrP<sup>C</sup>-PrP<sup>Sc</sup> interaction. Thirdly, the antibody may

effect a redistribution of the normal prion protein, at the level of subcellular compartments. In the case of anti-PrP transgenic mice, we know that the total amount of cellular PrP<sup>C</sup> is not different from that of non-transgenic siblings, but this does not exclude that PrP<sup>C</sup> is “stripped” from the cell surface, thereby becoming unavailable for pathogenesis.

Finally, a fourth mechanism may play a role in the *in vivo* experiments with anti-PrP transgenic mice: membrane-bound anti-PrP immunoglobulins of B-cells. Follicular dendritic cells (FDCs), which reside in the stroma of lymphoreticular organs, are important for peripheral prion pathogenesis. Because FDCs entertain intimate contact with B-cells, membrane-bound anti-PrP antibodies (i.e. B-cell antigen receptors) may interfere with prion replication on follicular dendritic cells.

## OUTLOOK

Only a few years ago, prion diseases were considered wholly non-treatable entities. Strictly speaking, this pessimistic view has not changed: it has still never been possible to successfully treat a patient or an animal with a prion disease. Nevertheless, exciting developments in the very recent past have evidenced several promising leads towards interventional approaches against prion diseases. On the negative side, all of what has been discussed

represents obviously early day-approaches: most of the strategies that have been tested so far only work on cultured cells, or only when prions are mixed with antibodies prior to injection into animals. Induction of protective antiprion immunity, as was attempted by our laboratory, appears to be feasible but is still very

## GLOSSARY

Prion diseases, including BSE of cows and human Creutzfeldt-Jakob disease, represent a group of degenerative diseases of the nervous system. A prominent feature of prion diseases is the accumulation of a protein called PrP<sup>Sc</sup> in the central nervous system and, in the case of new-variant Creutzfeldt-Jakob disease, in lymphoreticular organs. PrP<sup>Sc</sup> is a modified form of a normal cellular protein designated PrP<sup>C</sup>, which is produced by all mammalian organisms and is present in the brain, particularly on the surface of nerve cells. The “protein-only” hypothesis postulates that the disease-associated prion protein PrP<sup>Sc</sup> is the causing agent of prion diseases, and that it replicates by converting the normal PrP<sup>C</sup> into the pathological, disease-associated PrP<sup>Sc</sup>.

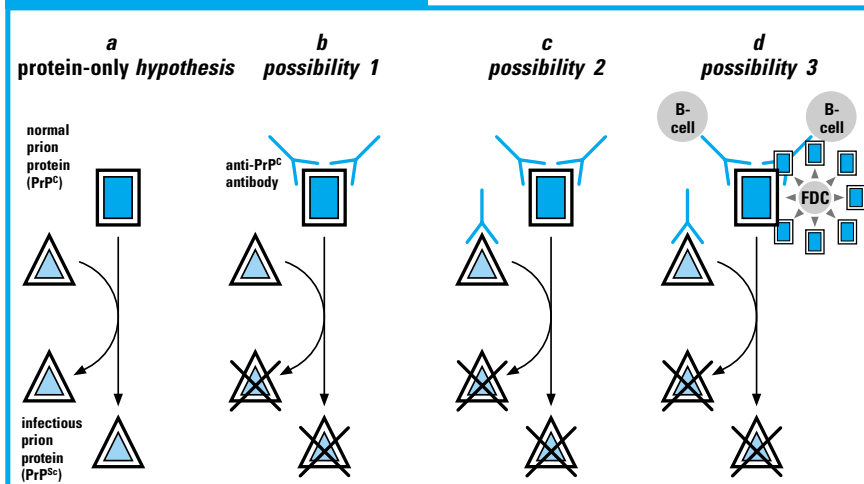
elaborate: it requires the introduction of a transgenic immunoglobulin, and will require efforts and ingenious adaptations to reduce it to the practice of everyday life.

The most promising strategies are clearly centered on pre- and postexposure prophylaxis, because once prion diseases become clinically apparent, damage to brain is typically extensive, and treatment is most likely to be ineffective. Along with the development of antiprion regimens, it will be imperative to develop sensitive diagnostic procedures that may identify individuals at risk. Several intriguing

inroads have been made recently also in this field. If research efforts in prion diagnostics and prophylaxis will continue to progress symmetrically, we might be capable of intervening against new-variant Creutzfeldt-Jakob disease—a goal that is still far away, but is now appearing less unattainable than ever before.

By **Adriano Aguzzi**,  
Institute of Neuropathology,  
Zurich, Switzerland

**Fig. 1 – The prion replication process and its inhibition by antibodies.**



*a)* The “template-directed refolding” version of the protein-only hypothesis postulates a direct interaction between exogenously introduced PrP<sup>Sc</sup> (triangles) and endogenous PrP<sup>C</sup> (squares). Heterodimerization brings about a pseudo-autocatalytic transformation of PrP<sup>C</sup> into further PrP<sup>Sc</sup>.

*b)* A possible mode of action of soluble antibodies to PrP<sup>C</sup> may be to bind and functionally mask endogenous PrP<sup>C</sup>. As a consequence, PrP<sup>C</sup> may no longer be available as a substrate for the conversion to PrP<sup>Sc</sup>, therefore hindering prion replication. Alternatively, it is possible that antibodies shift the sub-cellular distribution of PrP<sup>C</sup>, and reduce its availability and the cell surface. However, the total pool of cellular prion protein appears to be unchanged in anti-PrP transgenic mice.

*c)* Maybe the interaction of soluble anti-PrP antibodies with PrP<sup>C</sup> is not relevant to their protective effect: instead, antibodies may interfere directly with PrP<sup>Sc</sup>, thus blocking the recruitment of PrP<sup>C</sup>. This hypothesis and that outlined in panel (b) are not mutually exclusive.

*d)* Finally, it is thinkable that membrane-bound anti-PrP antibodies (B-cell antigen receptors) on the surface of B-cells interfere directly with prion replication on critical sites, such as the surface of follicular dendritic cells (FDC).

**WHERE NOW WITH “MAD COW” DISEASE? [CONTINUED FROM PAGE 1]**

in our own DNA. Its normal function is not well understood, but mutations in the prion gene can cause the prion to assume a pathogenic shape and embark on a murderous rampage.

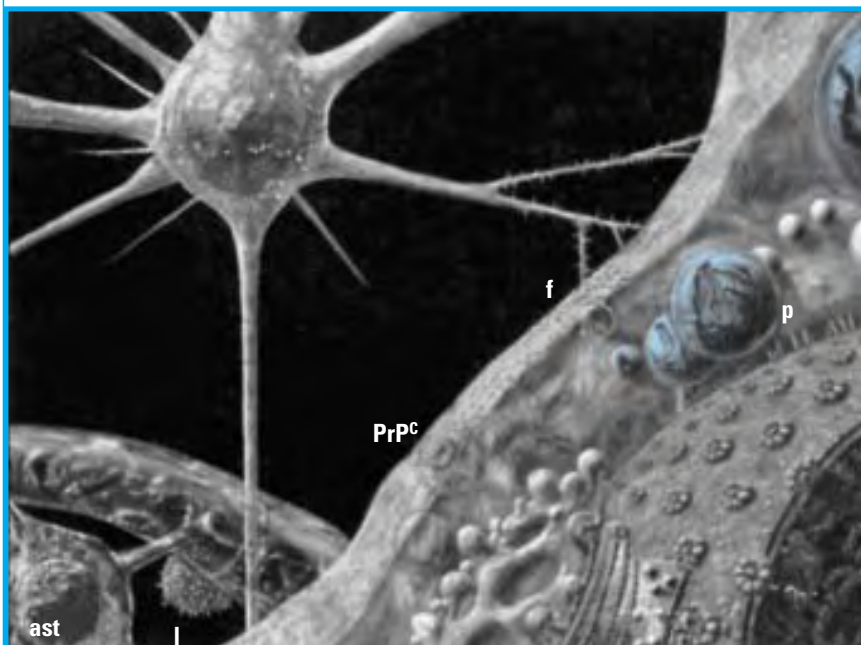
The infective prion causes normal prions to change into the malignant form and clump into the aggregates, or plaques, seen in the brains of vCJD victims. The aggregates are presumed to kill brain cells, by mechanisms as yet unknown. Fascinatingly, plaques consisting of other proteins are seen in many other neurodegenerative diseases, including Alzheimer’s and Parkinson’s.

Once changed into the disease-causing form, prions can spread to other individuals. However, what is truly alarming is that they evade easy detection and are virtually indestructible. There is not yet a cost-effective method of screening for the presence of the infective prion. To further complicate matters, normal methods of destroying germs, such as cooking at high temperatures, treating with disinfectants, and radiation, are ineffective.



What worries public health guardians is that, by the time the first cases of vCJD emerged in 1995, millions of Britons had been exposed through consumption of beef products over the preceding decade, when BSE was afflicting British cattle. Although BSE was brought under control by a 1988 ban on the processing of beef carcasses into

cattle feed (which is how the disease was presumed to have spread among cows), the time required for the disease to incubate in humans is unknown and may be decades. The number of cases reported has leaped from 3 in 1995 to 20 in 2001, but no one can predict whether this rate will taper off or suddenly explode.



What’s more, there is fear that vCJD could spread further through blood donations, organ transplants or contaminated surgical instruments. The risk is real, as the prion protein lurks in lymphoid tissue, including tonsils, lymph

**Fig. 1 – Illustration of prion replication and spread at the cellular level. The nerve comes in contact with the normal healthy prion (“PrP<sup>C</sup>” in the diagram) at site “f”. The mutated prions (“p”) are transmitted via the nerve to the healthy prions, where they force normal proteins to change their shape. The possible mechanism for prion transmission using the bloodstream is also shown in the lower left corner of the illustration. Infected lymphocytes (“l”) come in contact with astrocyte cells (“ast”) which support the production of the abnormal prions.**

*Copyright Russell Kightley Media, rkm.com.au*

nodes and spleen, and in B lymphocytes in the blood. Products derived from blood donated by three individuals who later came down with vCJD have been exported to eleven countries; these include 83,500 doses of polio vaccine given to Irish infants and children in 1998 and 1999.

Although no case of vCJD has been traced to a blood transfusion, many countries, including the U.S., now bar blood donations from people who have lived for extended periods in regions with BSE, including Britain, Ireland, France and Portugal. Yet such measures carry their own risks to life, including a shortage of blood products and organs for transplantation.

The answers lie in further research. A simple reliable method is needed to screen animals and humans for the presence of infectious prion and an early diagnostic test is essential for developing treatments. Swiss researchers recently developed a rapid

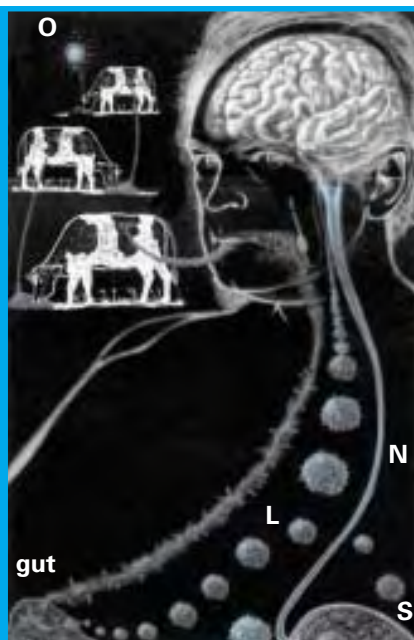
screening method that uses an enzyme to cleave the infective prion, leaving a telltale fragment that can be easily detected (*Arch. Virol. Suppl.* 2000; (16): 189-95).

Prospects for treatment seem distant, but not impossible. The laboratory of prion discoverer, Stanley Prusiner, recently reported that compounds called branched polyamines can eliminate pathologic prions, at least in the test tube. Such compounds might point the way to an effective therapy.

By **June Kinoshita**,  
Belmont MA, USA,  
adapted from *Brain Work*, Vol. 11, No. 3, 2001.

**Fig. 2 –** This diagram charts the possible route of transmission of BSE prions from cows to the human brain. The mutated prion ("O" in the diagram) passes from animal to animal (often through feed containing infected parts from other cows). The human consumes the contaminated beef, where the prions may attach themselves to lymphoid tissue in the gut. Lymphocytes ("L") travel to the spleen ("S") where the prions are replicated. Nerves ("N") that connect from the spleen to the brain then carry prions into the brain. Prions may also concurrently travel to the brain through the bloodstream while attached in the lymphocytes.

Copyright Russell Kightley Media, rkm.com.au



Editorial Board:  
**Pierre J. Magistretti, Chairman,**  
**Colin Blakemore, Leslie Iversen,**  
**Wolf Singer, Piergiorgio Strata,**  
**Jacques Glowinski,**  
**Norbert Herschkowitz**  
Production Manager:  
**Beatrice Roth**  
Contributing Editor:  
**Elaine Snell**

A Dana Alliance for the Brain Inc  
Newsletter prepared by EDAB,  
the European subsidiary of DABI



**The  
European  
Dana Alliance  
for the Brain**

Chairman  
**William Safire**

Vice Chairmen  
**Colin Blakemore,**  
*PhD, ScD, FRS*  
**W. Maxwell Cowan,**  
*BM, BCh, DPhil, FRS*

Chief Executive  
**Colin Blakemore,**  
*PhD, ScD, FRS*

President  
**Edward F. Rover**

Executive Committee  
**Alain Berthoz, Dr ès Sci, Dr Ing**  
**Albert Gjedde, Dr Med**  
**Malgorzata Kossut, MSc, PhD**  
**Pierre J. Magistretti, MD, PhD**  
**Richard Morris, DPhil, FRSE, FRS**  
**Wolf Singer, MD, PhD**  
**Piergiorgio Strata, MD**

**Dana Alliance  
for Brain Initiatives (DABI) – US**

Executive Director  
**Barbara E. Gill**

European Dana Alliance for the Brain Limited  
Registered Office: 165 Queen Victoria Street,  
London EC4V 4DD  
Registered in England: 3532108