Review

Human and animal spongiform encephalopathies are the result of chronic autoimmune attack in the CNS: A novel medical theory supported by overwhelming experimental evidence

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Summary. Spongiform encephalopathies, also called "prion diseases", are fatal degenerative diseases of the central nervous system which can occur in animals (such as the "mad cow disease" in cattle) and also in humans. This paper presents a novel medical theory concerning the pathogenic mechanisms for various human and animal spongiform encephalopathies. It is hypothesized that various forms of prion diseases are essentially autoimmune diseases, resulting from chronic autoimmune attack of the central nervous system. A key step in the pathogenic process leading towards the development of spongiform encephalopathies involves the production of specific autoimmune antibodies against the disease-causing prion protein (PrP^{sc}) and possibly other immunogenic macromolecules present in the brain. As precisely explained in this paper, the autoimmune antibodies produced against PrP^{sc} are responsible for the conversion of the normal cellular prion protein (PrP^c) to PrP^{sc}, for the accumulation of PrP^{sc} in the brain and other peripheral tissues, and also for the initiation of an antibody-mediated chronic autoimmune attack of the central nervous system neurons, which would contribute to the development of characteristic pathological changes and clinical symptoms associated with spongiform encephalopathies. The validity and correctness of the proposed theory is supported by an overwhelming body of experimental observations that are scattered in the biomedical literature. In addition, the theory also offers practical new strategies for early diagnosis, treatment, and prevention of various human and animal prion diseases.

Key words: Prion disease, Spongiform encephalopathy, Mad cow disease, Autoimmune disease

1. Introduction

Spongiform encephalopathies (SEs), nowadays also commonly known as "prion diseases", are fatal degenerative diseases of the central nervous system (CNS) in animals and humans (Prusiner and McKinley, 1987; Kimberlin and Walker, 1988; Weissmann, 1991a,b; Prusiner, 1993, 1998, 2001; Collinge and Palmer, 1997; Collinge, 2001). Pathological studies revealed that SE diseases in animals and humans produce, usually over a long period of time, progressive neuronal loss, astrogliosis, and characteristic spongiform-like changes in the brain tissue, which is often filled with amyloid-like deposits (Prusiner and McKinley, 1987; Kimberlin and Walker, 1988; Weissmann, 1991a,b; Prusiner, 1993, 1998, 2001; Collinge and Palmer, 1997; Collinge, 2001). In animals, the most common form is scrapie found in sheep, and the other forms of SEs include transmissible mink encephalopathy, chronic wasting disease of mule deer and elk, feline spongiform encephalopathy, and bovine spongiform encephalopathy (BSE, often called "mad cow" disease) (Wilesmith et al., 1991a,b; Anderson et al., 1996; Gibbs, 1996; Nathanson et al., 1997; USDA, 2003). The commonly-known forms of the human SEs include Creuzfeldt-Jacob disease, familial fatal insomnia, Kuru, and Gerstmann-Straussler-Scheinker syndrome (Prusiner and McKinley, 1987; Prusiner, 1993, 1998, 2001; Will, 2003).

It was first suggested in the 1960s that the agent which caused scrapie in sheep might have the ability to replicate itself without the presence of nucleic acid (Alper et a1., 1966, 1967, 1968; Latarjet et al., 1970). Extensive studies by Dr. Stanley B. Prusiner and colleagues had led to a strong conclusion that the

pathogenic agent in animal and human SEs indeed lacked nucleic acid and consisted mainly, if not exclusively, of proteins (reviewed in ref. Prusiner, 1982, 1993, 1998). Their conclusion was based on their earlier finding that various procedures that were well known to destroy nucleic acid and viruses did not appear to significantly reduce infectivity, whereas procedures that denatured or degraded protein drastically reduced infectivity. In 1982, Dr. Prusiner introduced the term "prion" to distinguish this new class of proteinaceous pathogens from viruses, bacteria, fungi, and other known pathogens (Prusiner, 1982).

Studies have shown that the normal cellular prion protein (PrP^c) and the disease-causing prion protein (PrP^{sc}) are glycoproteins composed of ~250 amino acid residues, and their basic amino acid sequences appear to be identical (Prusiner and McKinley 1987; Prusiner, 1993, 1998, 2001). Although many details remain unclear, it is believed that the tertiary configurations of these two proteins are very different. Studies of the 3-D structure of the animal PrP^c proteins selectively-expressed in E. coli showed that the PrP^c contained predominantly α-helical domains and had almost no ß-sheets. It was also predicted that PrP^{sc} contains predominantly ß-sheets. Accordingly, it is generally believed that the PrP proteins have two different stable configurations (namely, PrP^c and PrP^{sc}), and the safe PrP^c configuration is normally adopted and rarely it would automatically switch to the pathogenic PrP^{sc} configuration. Studies have shown that PrP^c and PrP^{sc} have very different biochemical and biophysical properties: PrP^c was soluble in nondenaturing detergents but PrP^{sc} was not; PrP^c was readily digested by proteases but PrP^{sc} was markedly resistant to proteases; PrP^c was heat-sensitive but PrP^{sc} was partially heat-resistant (discussed in Prusiner and McKinley 1987; Prusiner, 1993, 1998, 2001). Most prion researchers believed that these different properties of PrP^c and PrP^{sc} are solely due to the different tertiary configurations adopted.

Dr. Prusiner and colleagues proposed that PrP^{sc} alone was responsible for the transmission of the SE diseases, and believed that PrP^{sc} was infectious. This theory has now become a widely-held doctrine concerning the pathogenesis of various animal and human SE diseases (Prusiner and McKinley, 1987; Kimberlin and Walker, 1988; Weissmann, 1991a,b; Prusiner, 1993, 1998, 2001; Huang et al., 1994; Bamborough et al., 1996; Telling et al., 1996a,b; Collinge and Palmer, 1997; Zhang et al., 1997; Aguzzi et al., 2001; Clarke et al., 2001; Wickner et al., 2001). Mechanistically, it is believed that the disease-causing PrP^{sc} protein can propagate itself through physically contacting the normal cellular PrP^c and causing it to unfold and flip from its usual configuration to adopt the aberrant shape(s) (Prusiner, 1993, 1998, 2001; Prusiner and McKinley, 1987; Weissmann, 1991a,b; Bamborough et al., 1996; Clarke et al., 2001; Wickner et al., 2001). It was also suggested that this change of configuration initiates a cascade in which the newly-converted PrP

would start to alter the shape of other normally shaped PrP^c molecules in an exponential manner. The conversion of normal PrP^c to PrP^{sc} has been suggested to occur inside the neurons (Ma et al., 2002; Ma and Lindquist, 2002), and PrP^{sc} accumulates within the intracellular vesicles known as lysosomes. The filled lysosomes eventually would burst and cause damage to the cells, thereby leaving small holes and PrP^{sc} deposits in the affected brain tissue. Details of the prevailing explanations concerning the mechanisms of prion-caused diseases can be found in many elegantly written review articles (Prusiner and McKinley, 1987; Kimberlin and Walker, 1988; Weissmann, 1991a,b; Prusiner, 1993, 1998, 2001; Bamborough et al., 1996; Telling et al., 1996a, 1996b; Collinge and Palmer, 1997; Aguzzi et al., 2001; Clarke et al., 2001; Wickner et al., 2001).

While I believe that PrP^{sc} (which is a long-lasting and weakly immunogenic protein) plays an important role in the development of human and animal SEs, I also think that many of the prevailing mechanistic explanations on the pathogenesis of SEs are incorrect. In this paper, a new theory is developed to provide a better mechanistic explanation of the pathogenesis of SEs. The proposed theory suggests that various human and animal SEs are essentially autoimmune-type diseases. A detailed explanation of the proposed theory, along with a discussion of the available experimental evidence in strong support of its validity, is provided below.

2. The proposed new theory on the mechanism of pathogenesis of SEs

The author of this paper hypothesizes that a key step in the pathogenic process leading towards the development of various known forms of SEs involves the production of specific autoimmune antibodies against PrP^{sc} and possibly other immunogenic macromolecules (proteins or non-proteins) present in the CNS. As precisely explained below, the autoimmune antibodies produced against PrP^{sc} are not only directly responsible for the conversion of PrP^c to PrP^{sc}, but they are also directly responsible for the accumulation of PrP^{sc} in the CNS and other peripheral tissues. The production of autoimmune antibodies against PrP^{sc} and/or other cellular macromolecules is also responsible for the antibody-mediated chronic autoimmune attack of PrP-expressing cells (such as neurons), contributing to the development of neurological lesions and clinical symptoms characteristic of various SE diseases.

2.1. Proposed mechanisms for the antibody-mediated formation and accumulation of PrP^{sc}

It is believed that most of the normal cellular PrP^c protein molecules always adopt a usual stable tertiary configuration. However, it is also conceivable that a very small fraction of the cellular PrP^c protein may transiently adopt other possible tertiary configurations, as a result of the normal intramolecular thermodynamic movements.

Because these transient tertiary configurations are thermodynamically unstable, PrP^c normally could not assume such unstable configurations for any significant length of time, and usually they would almost instantly revert back to the normal stable configuration. However, when specific autoimmune antibodies against immunogenic PrP^{sc} are produced and present in the body, they may also bind to the transiently-misshapen PrP^c molecules that happen to adopt the PrP^{sc}-like configuration(s), and thereby stabilize them in the aberrant configuration(s) for a much longer time (depicted in Fig. 1). It is hypothesized that when the misshapen configurations of the PrP protein are stabilized by the binding of the antibodies, some of the usually inaccessible amino acid residues may become accessible for further covalent modifications (e.g., glycosylation and/or lipid conjugates). These aberrant covalent modifications may permanently stabilize the PrP protein in the abnormal PrP^{sc} configurations.
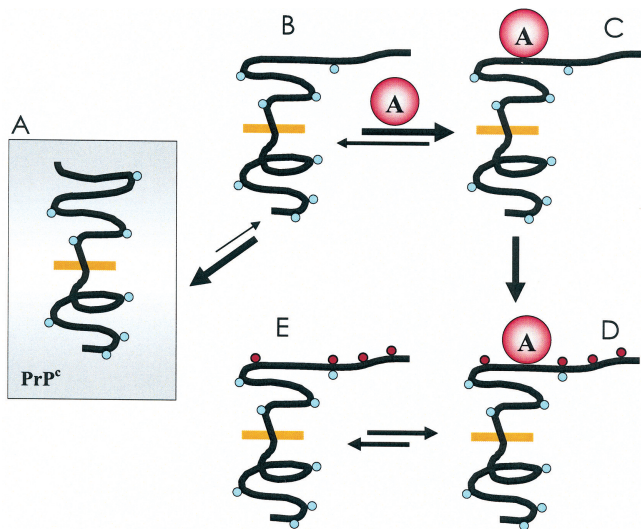
Fig. 1. Explanation of the antibody-mediated conversion of PrP^c to PrP^{sc}. The normal cellular PrP protein (PrP^c) is schematically depicted in Box A, which contains the extracellular and intracellular domains. The small circles represent the original post-translational covalent modifications (such as glycosylation) of some of the amino acid residues. B depicts the transiently-misshapen PrP protein which happens to adopt a configuration that is similar or identical to the configuration of the PrP^{sc} (depicted in E). Because configuration B is thermodynamically unstable, usually it would instantly revert back to the stable configuration **A**. However, when the anti-PrP^{sc} autoimmune antibody (Ab) is present (please note that this antibody would also have cross-reactivity for the PrP^{sc}-like misshapen configuration **B**, the configuration B could be stabilized by the binding of the antibody, resulting in the formation of the antibody-PrP complex **C**. When PrP is stabilized by the antibody in configuration C, some of the originally-inaccessible amino acid residues would become readily accessible for further covalent modifications, which eventually would lead to the formation of PrP^{sc}. Note that the additionally modified amino acid residues following antibody binding are marked with small filled circles. In the above drawing, **D** represents the antibody-PrP^{sc} complex, whereas **E** represents PrP^{sc} alone without an antibody bound to it.

Notably, although most prion researchers thought that the conversion of PrP^c to PrP^{sc} solely involves configurational changes of the PrP protein (Prusiner and McKinley, 1987; Kimberlin and Walker, 1988; Borchelt et al., 1990; Weissmann 1991a, 1991b; Pan et al., 1993; Prusiner 1993, 1998, 2001; Bamborough et al., 1996; Telling et al., 1996a,b; Collinge and Palmer, 1997; Aguzzi et al., 2001; Clarke et al., 2001; Wickner et al., 2001), I believe that the patterns of covalent modifications in PrP^c and PrP^{sc} are very different, and they are, in fact, crucial determinants of their different configurations, of their different biophysical properties, and also of their different immunogenic property. A detailed discussion of the supporting evidence for the different patterns of covalent modifications of PrP^c and PrP^{sc} is provided later in section 2.4.

Although the functions of the cellular PrP^c are still unclear at present (Bueler, 1992), it is reasonable to assume that the normal PrP^c serves certain physiological functions for those cells that express the protein (Collinge et al., 1994). When specific antibodies are tightly bound to the transiently-misshapen PrP^c proteins and stabilize them in the PrP^{sc}-like abnormal configurations (presumably functionally inactive), the cells being targeted by the antibodies would falsely sense that they only have sub-normal levels of PrP^c (as well as its associated functions), and subsequently this may lead to increased expression of the cellular PrP^c protein. Because most of the expressed PrP^c molecules, sooner or later, would be targeted by the autoimmune antibodies, more and more PrP^c would need to be expressed, and consequently a vicious cycle may ensue. Accordingly, at later stages of the pathogenic process, overexpression of the cellular PrP^c protein may occur, possibly accompanied by PrP^{sc} accumulation and plaque formation.

It should be pointed out that this novel mechanistic theory also explains that the structures of the newly-formed PrP^{sc} would be very similar and sometimes even identical to the structures of the original PrP^{sc} protein against which the antibodies were formed. To assist the readers to better understand this, a detailed schematic illustration of the explanation is provided in Fig. 2.

2.3. Proposed mechanism for the production of anti-PrP^{sc} autoimmune antibodies

As aforementioned, PrP^{sc} exists in stable yet abnormal tertiary configurations that are very different from the configuration of the normal cellular PrP^c. The abnormal configurations of PrP^{sc} would make it a new immunogenic entity that could trigger the body's immune system to generate specific antibodies. Notably, because PrP^{sc} is highly resistant to digestion by proteases, this would allow PrP^{sc} as well as some of its peptide fragments to form deposits and thus may stay in the body for a long time. As a result, the body's immune system would have ample time to interact with these immunogenic proteinaceous deposits and gradually

develop specific antibodies against the PrP^sc protein and its peptide fragments.

Here it needs to be explained as to where the immunogenic PrP^sc protein in a patient or an animal initially came from? It is hypothesized that one of the sources of the initial PrP^sc is through oral ingestion of the meat and/or offal products from SE animals. It has been documented that some of the orally ingested PrP^sc particles could be absorbed intact (undigested) across the intestinal wall at the Peyers patches (Prinz et al., 2003). These unique structures are part of the mucosal-associated lymphoid tissue (MALT) where large-size immunogenic particles (such as microorganisms) are usually presented to the body's immune system. Because PrP^sc molecules are far more resistant to various proteases than other ingested proteins (including the normal PrP^c), and also because they are usually present as clustered particles, it is possible that a fraction of the orally ingested PrP^sc molecules are taken up into the body largely undigested through the mucosal-associated
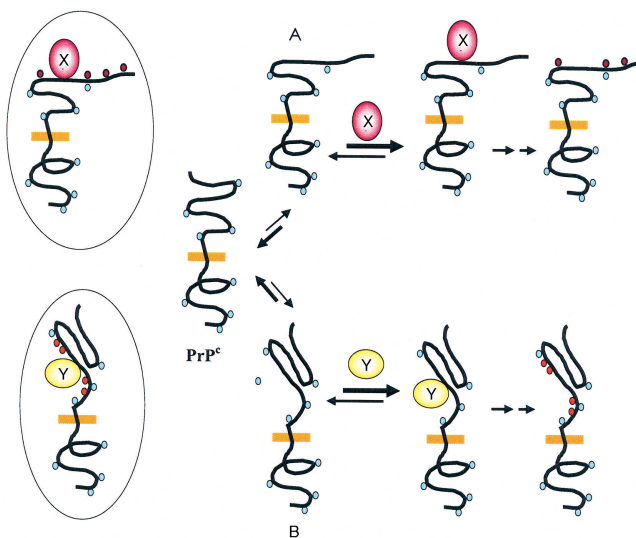
Fig. 2. Explanation of the structural similarity between the newly-formed PrP^sc and the original PrP^sc against which the antibodies are produced. The two ovular circles depict two different PrP^sc proteins which have different patterns of covalent modifications and also different 3-D configurations. Please note that each of the PrP^sc proteins in the ovular circle also has a specific antibody molecule bound to it, i.e., the antibody X is bound to the configuration-A PrP^sc, and the antibody Y is bound to the configuration-B PrP^sc. The two sets of the antibodies (X and Y) are specifically produced against these two configurationally-different PrP^sc proteins. Because each set of the antibodies would have their own specificity for recognizing the immunogenic configuration of the PrP^sc protein, the antibody X would preferentially recognize the transiently-misshapen configuration A of the PrP protein but probably would not recognize its configuration B. Likewise, the antibody Y would preferentially recognize the misshapen configuration B but probably would not recognize the configuration A. As depicted above, it is rather easily to understand that the autoimmune antibodies (X or Y) would have the tendency to copy the configuration of the original PrP^sc (against which the antibodies were specifically produced) to the newly-formed PrP^sc.

lymphoid tissue. Lymphoid cells then phagocytosize the PrP^sc particles and travel to lymphoid sites such as lymph nodes, spleen, and tonsils for inducing the production of specific antibodies. In line with this explanation, analyses of various tissues collected from animals with SE diseases have shown that their visceral lymphoreticular and secretory organs were among the organs/tissues that had the highest titers of PrP^sc at early disease stages (Farquhar et al., 1994; Kimberlin and Walker, 1989; Vandeulen et al., 1996; Hilton et al., 2002).

After a person has orally-ingested PrP^sc-rich meat and/or offal products from the BSE cattle, the initial antibodies produced in the recipient would be highly specific for the bovine PrP^sc. However, it is conceivable that some subsets of the antibodies may retain certain degrees of cross reactivity against the human PrP molecules when they transiently adopt the bovine PrP^sc-like misshapen configurations (as a result of the thermodynamic intramolecular movements). Accordingly, the production of the initial antibodies against the bovine PrP^sc would lead to an initial antibody-mediated immune attack of the recipient's CNS and other peripheral tissues that express the PrP protein. The extent of such an antibody-mediated autoimmune attack is, in a significant part, determined by the cross-reactivity and quantity of the antibodies being produced. Following the initial attack of the human PrP^c by the anti-bovine PrP^sc antibodies, human PrP^sc would gradually be formed. The human PrP^sc, when accumulated in the body (not necessarily in the CNS) in substantial quantities, would also serve as immunogen and lead to the production of additional antibodies that would have a higher specificity for the human PrP^sc.

For most laboratory animals that were induced to develop SEs, the source of PrP^sc was usually through experimental inoculations of the brain tissues or extracts from animals or humans with SE diseases. The process for the production of initial and subsequent antibodies is essentially the same as described above. When PrP^sc from animals with SE was inoculated into the same types of healthy animals (e.g., mouse PrP^sc was inoculated into healthy mice of the same strain), the initial antibodies produced would have rather high specificity and affinity for the transiently-misshapen mouse PrP^c. As such, the disease incubation period usually would be much shorter compared to that when the PrP^sc obtained from the SE animals of one species was inoculated into the healthy animals of another species. The explanation provided here can be used to explain many of the known experimental observations related to the apparent "species barrier" of the SE diseases. For instance, laboratory studies have consistently shown that when the standard method of experimental inoculation of diseased tissue extracts was used to pass the SE disease from one animal species to another, it was generally accompanied by a significant prolongation of the incubation time compared to the passage within the same strain of animals (Pattison and

Jones, 1968). However, the subsequent passages of the disease within the new strain of animals usually became much easier, with a reduced and stabilized incubation time. Further discussion on this topic is provided later in section 3.2.

Lastly, it should also be noted that some earlier studies showed that the titers of the autoimmune antibodies produced during the development of SE diseases in animals were usually very low, which was strikingly different from the high circulating levels of the antibodies produced following tissue/organ transplantation or pathogenic organism invasion. The low levels of anti-PrP^{sc} autoimmune antibodies produced were likely due to certain degrees of host tolerance to this CNS antigen. Consistent with this explanation, an earlier study showed that PrP^{sc} became far more immunogenic in the PrP$^{o/o}$ null mice (which did not express PrP^c) than in the PrP$^{+/+}$ mice (Prusiner et al., 1993). The relatively low levels of the autoimmune antibodies for PrP^{sc} found in SE animals and patients should not be taken as evidence to exclude autoimmune attack as a crucial pathogenic factor, instead these preliminary observations only suggested that the autoimmune attack against the PrP^{sc}-expressing cells likely was very mild compared to the much stronger autoimmune response and attack usually seen following organ transplantation or pathogen invasion. The suggestion for the involvement of a chronic, mild autoimmune attack of the CNS cells during the pathogenesis of SE is fully consistent with the unusually long incubation time needed for the development of the disease in both animals and humans.

2.3. Proposed mechanism for the pathogenesis of CNS lesions in SE diseases

Loss of neurons is a salient pathological characteristic in various human and animal SE diseases. The important question here is: how does PrP^{sc} cause neuronal death? As explained below, the production of specific autoimmune antibodies is directly responsible for the development of characteristic neurological lesions and clinical symptoms of SEs through two major mechanisms:

First, when specific antibodies are tightly bound to the misshapen PrP proteins in the plasma membrane of the CNS cells, the antibody-antigen complexes would subsequently attract other components of the immune system, resulting in an antibody-mediated autoimmune attack of the PrP-expressing cells. Such chronic autoimmune attack is expected to induce inflammatory responses surrounding the targeted cells and cause chronic neurological lesions. There were several lines of experimental observations that strongly supported this hypothesis (discussed in section 3.1.).

Second, when the transiently-misshapen PrP^c molecules are bound with the antibodies and particularly when they are already converted to the functionally-inactive PrP^{sc}, it is believed that PrP^{sc} would, sooner or later, be translocated to the intracellular vesicles, and ultimately to the lysosomes where various proteases are present for the normal degradation of misfolded proteins. However, because PrP^{sc} is highly resistant to many isoforms of proteases, subsequently the lysosomes may be overloaded with undigestable PrP^{sc} or its peptide fragments. The overloading of lysosomes with undigestable PrP^{sc} would also contribute to the formation of pathogenic neuronal lesions or even neuronal death. In cases of neuronal death, the undigestable PrP^{sc} or its fragments (likely in clusters) would be released and accumulated in the region where the neurons once were, and the death of the neurons would leave small holes which could be readily seen in brain histopathological slides. In support of this explanation, there were experimental observations revealing that the structures of the lysosomes of the affected neurons were very different from normal neurons, and in some cases, the lysosomes were loaded with PrP^{sc} or its fragments.

Based on the mechanistic explanations provided here, it should be noted that although the presence of PrP plaques in the CNS serves as a useful pathological indicator for the occurrence of the SE disease, their presence is not believed to be the major cause for the accompanying neuronal lesions. It is predicted that in some cases if the antibody-mediated autoimmune attack is rather severe (due to the presence of higher-titer and/or higher-affinity autoimmune antibodies), then the CNS neurons may be damaged and ultimately destroyed within a relatively shorter period of time and there may not be enough time for PrP^{sc} to accumulate and form deposits. Another important factor that would also affect the extent of PrP^{sc} plaque formation is the degree of resistance of PrP^{sc} to various endogenous proteases. If some of the PrP^{sc} proteins, because of their different configurations and covalent modifications, are less resistant to proteases than others, then their accumulation may be significantly less than those that are more resistant to proteases. In line with this explanation, there are many experimental observations from SE patients as well as laboratory animals showing that the formation of PrP^{sc} plaques did not appear to be closely correlated with the extent of neurological impairment. For instance, it has been repeatedly observed that some prions could cause SE diseases quickly, whereas others did so slowly.

Although the precise tissue/cellular distribution of PrP protein in animals and humans is still not fully known, there are experimental data showing that, in addition to the CNS, many peripheral tissues also express this protein (Baldwin et al., 1992). It is suggested that some of the PrP-expressing peripheral tissues are actually important initial targets for the antibody-mediated autoimmune attack, resulting in the initial accumulation of human PrP^{sc} in these tissues. Since the antibodies against the immunogenic PrP^{sc} are produced in peripheral lymphatic tissues where the concentrations of these antibodies are highest, these

lymphatic tissues likely are the initial targets for the PrP^{sc} autoimmune antibodies, eventually resulting in PrP^{sc} accumulation in these tissues. In line with this explanation, studies have shown that PrP^{sc} in animals inoculated with PrP^{sc} usually first accumulate in visceral lymphoreticular and secretory organs, and later it started to accumulate in the CNS (Farquhar et al., 1994; Kimberlin and Walker, 1989; Vankeulen et al., 1996; Hilton et al., 2002). Considering the concentrations of specific antibodies present in various tissues, most CNS neurons are probably exposed to far lower levels of the antibodies (because of the blood-brain-barrier), which would be one of the factors that may determine the very slow progression of the neurological lesions. In addition to the concentrations of specific antibodies surrounding the target cells in a given tissue, another important factor that would also determine the extent of lesions and PrP^{sc} accumulation is the original levels of cellular PrP^c required for fulfilling the normal physiological functions. If a given tissue basically does not express PrP**^{c}** for its normal functions, then it is expected that this tissue may not be targeted by the autoimmune antibodies, and thus it may not accumulate PrP^{sc}. On the other hand, if a tissue normally expresses high levels of PrP^c for physiological functions than other organs, then this tissue would have a shorter incubation time for the development of characteristic pathological changes and would also have higher levels of PrP^{sc} accumulation following the anti-PrP antibody-mediated autoimmune attack. In partial support of this explanation, an earlier study has shown that transgenic mice overexpressing the mouse PrP^c became particularly susceptible to develop SE diseases with a shorter incubation time after inoculation with the mouse PrP^{sc} (Telling et al., 1996a,b).

Here it is of note that the antibody-mediated chronic autoimmune attack of most peripheral target sites is expected to be less consequential clinically as opposed to the CNS neurons which are generally deprived of the ability to regenerate in case of neuronal death. Accordingly, it is possible that although many types of tissues/cells in the body might become the targets for the antibody-mediated autoimmune attack during the development of an SE disease, CNS symptoms may become most pronounced and the consequences likely are most devastating.

2.4. Some additional details concerning the proposed new theory

For better understanding, I have provided in this section additional clarifications of a few important issues that are related to the proposed new theory as well as its difference from the current prion hypothesis.

(1) Most proponents of the prevailing prion hypothesis hold the unwavering view that the only difference between PrP^c and PrP^{sc} is configurational, i.e., the different biophysical and pathogenic properties of PrP^c and PrP^{sc} are solely determined by their different tertiary configurations. In my view, however, the stabilization of the unusual configuration(s) of PrP^{sc} is largely determined by its different patterns of covalent modifications (such as glycosylation and/or lipid conjugation). The binding of an autoimmune antibody to the transiently-misshapen PrP^c would initially stabilize it in an abnormal PrP^{sc}-like configuration, but the subsequent covalent modifications of some of the amino acid residues that are normally inaccessible may further stabilize it in aberrant configurations.

The above suggestion is consistent with a number of experimental observations: (i) PrP^{sc} and PrP^c were both known to be post-translationally modified to an extensive degree, and PrP^{sc} was formed in vivo only after the protein had been glycosylated (Bamborough et al., 1996). Analysis of PrP^{sc} obtained from the brain of patients died of vCJD showed that the PrP glycoforms had quite different patterns from those found for sporadic or iatrogenic CJD (Caughey and Raymond, 1991). (ii) The disease-causing ability of PrP^{sc} and PrP^c was drastically different, with a ratio of ~100,000:1 (Prusiner, 1993, 1998, 2001; Prusiner and McKinley, 1987). (iii) Although pure PrP^{sc} isolated from diseased brain tissue was highly pathogenic, almost all of the studies have failed thus far to create the same degree of infectivity by only modifying the configuration of the bacterially-expressed normal PrP^c. Likewise, it has been unsuccessful thus far for researchers to abolish the structure and pathogenic activity of PrP^{sc} with specific agents or solutions, and then to restore its activity by reinstating the original structure. (iv) It should be noted that the differences in the glycosylation pattern of PrP^c and PrP^{sc} usually were not accounted for in most of the studies that were designed to determine the structural difference between PrP^{sc} and PrP^c, because the proteins were deglycosylated prior to structural and sequence analyses. Taken together, all these observations consistently suggested that the different biophysical and pathogenic properties of PrP^{sc} and PrP^c likely are not just determined by their different tertiary configurations, and in my personal view, the different patterns of covalent modifications play a crucial role in stabilizing the unique configurations of PrP^{sc} as well as in creating its immuno-genic and pathogenic properties.

(2) One of the core elements of the currently-prevailing prion hypothesis is the belief that PrP^{sc} can replicate itself through directly interacting with normal cellular PrP^c and then imparting the misshapen configuration onto PrP^c in an exponential manner. This explanation was, in part, based on some of the earlier studies which showed that in vitro incubations of PrP^c in the presence of PrP^{sc} (insoluble as deposits) appeared to increase the formation of the proteinaceous deposits compared to that when PrP^c was incubated alone. It should be noted that there are different explanations for the observations. Here I like to use the precipitation of sodium chloride (NaCl) from a supersaturated NaCl solution as an example to help make a simple point. As we know that the supersaturated NaCl solution is rather

unstable, and NaCl will tend to precipitate out over time. During the formation of NaCl precipitates from a supersaturated NaCl solution, if small pieces of the NaCl crystal are already present, then the speed for the formation of NaCl precipitates usually is much faster than that without the preexisting crystals. The reason is that before the islands of crystals are formed, it is usually more difficult for NaCl molecules in solution to precipitate out, but when small NaCl crystals are already present, the process becomes much easier. A similar explanation can be used to account for the in vitro observations that when the PrP^{sc} was already present as insoluble deposits, its presence would make it easier for the transiently-misshapen PrP^c protein to form precipitates on top of the PrP^{sc} deposits.

(3) One of the pathological hallmarks of various human and animal SE diseases is the death of CNS neurons. As discussed above, the proposed new theory provided a precise mechanistic explanation for the pathogenesis of neuronal death in SE diseases. However, the currently prevailing explanation according to the prion hypothesis is debatable, and controversies still abound. The earlier explanation suggested that the conversion of normal PrP^c to PrP^{sc} occurred inside the neurons, and PrP^{sc} accumulated within the intracellular vesicles known as lysosomes. The filled lysosomes could then burst and cause damage to the cells (Prusiner, 1993, 1998, 2001; Prusiner and McKinley, 1987). However, recently a somewhat different mechanistic explanation was fashioned (Ma et al., 2002; Ma and Lindquist, 2002), and it was suggested that a certain fraction of the misfolded PrP^c protein never reached the plasma membrane, but instead they reentered the cytosol through retrotranslocation from the endoplasmic reticulum, a process that is known for many misfolded proteins to be directed back to the proteasomes for disposal. The accumulation of the PrP protein in the cytosol would then cause rapid neuronal death. This explanation was based on earlier studies by Ma and Lindquist (Ma et al., 2002; Ma and Lindquist, 2002) showing that a form of the prion protein that was specifically targeted towards the cytosol caused rapid neurodegeneration in mice. However, there are a few problems associated with this modified mechanistic explanation. First, the particular form of the PrP protein observed in these studies did not acquire resistance to proteases, which has been one of the best known characteristics of PrP^{sc} for years. Second, the recent experimental studies (Drisaldi et al., 2003) suggested that the cytosolic PrP protein still retained its signaling peptide (which is normally removed after the protein entered the endoplasmic reticulum), and it did not appear to contain the glycosyl phosphatidylinositol anchor needed for attachment to cellular membrane (Drisaldi et al., 2003; Harros, 2003). Accordingly, these observations raised the possibility that the PrP protein described in recent studies (Ma et al., 2002; Ma and Lindquist, 2002) might actually never enter the endoplasmic reticulum, and thus it might not have undergone retro-translocation

as purported.

(4) The normal cellular PrP^c protein appears to have a rather wide tissue distribution besides the CNS (Baldwin et al., 1992). Earlier studies showed that when various tissues from SE animals were analyzed, their visceral lymphoreticular and secretory tissues were among the organs/tissues that had the highest titers of PrP^{sc} (Farquhar et al., 1994; Kimberlin and Walker, 1989; Vankeulen et al., 1996; Hilton et al., 2002). Based on these observations, most prion researchers believed that the PrP^{sc} first replicated itself within the richly-innervated peripheral lymphatic tissues, and later PrP^{sc} propagated back up along the axons to the spinal cord and eventually to the brain, causing clinical symptoms (Aguzzi, 1997; Glatzel and Aguzzi, 2000; Nicotera, 2001; Follet et al., 2002). I think this mechanistic explanation, which was fashioned according to the well-known model for the slow viral infection in the CNS, was not correct.

The proposed new theory suggests that the antibody-mediated conversion of PrP^c to PrP^{sc} may first lead to the accumulation of PrP^{sc} in peripheral tissues (particularly the lymphoreticular and secretory organs where the antibody titers are highest), and later the antibodies may slowly attack the CNS, gradually leading towards the formation and accumulation of PrP^{sc} as well as the development of typical pathological changes in the CNS. The reason for the slow progression of the CNS pathogenesis during SE diseases is, in a significant part, because the CNS neurons were usually exposed to much lower levels of the autoimmune antibodies (due to the blood brain barrier) than many other peripheral tissues.

(5) The proposed new theory suggests that other immunogenic proteinaceous and/or non-protein particles sometimes may also be present in the organs or tissues from SE animals, and these immunogens may stay in the body for a long time (due to their resistance to enzymatic digestion). Their long-term presence would also induce the production of specific autoimmune antibodies in the recipient. These autoimmune antibodies would target antigens with different cellular distribution in the CNS as compared to the PrP protein, and their involvement could be an important factor that diversifies clinical and pathological findings. The presence of other CNS immunogens likely is one of the major underlying causes for the apparent presence of different strains of prions (discussed in section 3.2.). However, when the same crude extracts (with the same combination of various disease-causing immunogens) were inoculated into the same type of animals in the same experiment, the patterns of pathological changes and clinical symptoms were, as expected, always very similar (almost identical).

A good example for the suspected involvement of other immunogenic proteinaceous particles is the pathogenic ß-amyloid protein found in Alzheimer's disease. It is well known that ß-amyloid protein shares similar biophysical and pathogenic properties as PrP^{sc},

but it is a totally different protein. The proposed theory on the pathogenesis of SEs also naturally leads to the suggestion that Alzheimer's disease likely is another autoimmune-type disease that preferentially affects the CNS of the elderly. In fact, there is considerable amount of experimental evidence in scientific literature that provides support for this novel hypothesis (a detailed discussion provided elsewhere (Zhu, 2005). In this context, I also like to note that there may be potentially unavoidable untoward effects associated with the recent clinical trials that were designed to stimulate the body's production of antibodies for ß-amyloid plaques as a prevention and/or treatment strategy for Alzheimer's disease. Given the new mechanistic understanding developed here, it is almost certain that such a strategy would not work as intended, rather it may do the opposite, accelerating the development of Alzheimer's disease, as some of the clinical trial data have already indicated.

3. Experimental evidence that supports the proposed theory

There is a large body of laboratory and clinical evidence (discussed below) which all points to a coherent, unified pathogenic mechanism that various human and animal SE diseases are autoimmune-type diseases largely affecting the CNS. Notably, although the experimental data and findings discussed in this paper are scattered in the biomedical literature and often they were incorrectly construed in the original papers, these data, when interpreted in the clear context of the proposed new theory, form a collective body of coherent evidence that provides strong support for the validity of the theory.

(1) Studies have shown that mice with severe combined immunodeficiency (SCID) generally were highly resistant to the development of SE following inoculations with the PrP^{sc}-rich extracts (Fraser et al., 1996; Brown et al., 1997; Taylor et al., 1996; O'Rourke et al., 1994; Klein et al., 1997; Mabbott and Bruce, 2001).

(2) Consistent with the above observations, other studies have further shown that a functionally-proficient B lymphocyte system (which produces specific antibodies) was required for the development of SE in an animal model following inoculations of extracts from diseased brain tissue (Klein et al., 1997). Notably, earlier studies have also suggested that mice deficient in B lymphocytes might still develop SE diseases (with lower incidences) when PrP^{sc} was either directly injected into the brain or very high doses of PrP^{sc} were used (Brandner et al., 1996; Fraser et al., 1996; Taylor et al., 1996; Klein et al., 1997; Brown et al., 1999; Frigg et al., 1999; Mabbott and Bruce, 2001). It was possible that small amounts of the autoimmune antibodies against the mouse PrP^{sc} might still be formed in these animals when they were either intracerebrally challenged with PrP^{sc} or received very high doses of PrP^{sc}.

(3) Studies have shown that the PrP null mice (PrP$^{o/o}$), i.e., those animals with both alleles of the PrP^c gene disrupted, would not develop SEs following inoculations of the brain extracts from diseased mice (Bueler et al., 1993; Sailer et al., 1994). The explanation for the observation is rather simple on the basis of the proposed new theory. Although specific antibodies against the mouse PrP^{sc} were believed to be produced in PrP$^{o/o}$ null mice following inoculations with the mouse PrP^{sc}, these antibodies were unable to cause usual pathogenic lesions in the recipient's brain tissue because the brain cells did not express PrP^c (the precursor for the misshapen PrP^{sc}), and accordingly, the antibodies also could not cause an autoimmune attack of the brain tissue.

Additional studies showed that following inoculations with diseased brain tissues which contained PrP^{sc}, the PrP$^{o/o}$ mice with a reconstituted PrP$^{+/+}$ lymphoreticular system and carrying a PrP$^{+/+}$ mouse graft developed the typical pathological changes only in the graft, but not in the recipient's brain (Brandner et al., 1996; Fraser et al., 1996). To explain the above observation, it should be noted that the normal cellular PrP^c protein present in the brain graft is not immunogenic to the PrP$^{+/+}$ mouse lymphoreticular system, and accordingly, antibodies against mouse PrP^c would not be produced. However, when the animals were inoculated with diseased brain tissue containing mouse PrP^{sc}, specific antibodies against PrP^{sc} would be produced because the mouse PrP^{sc} is weakly immunogenic to the mouse lymphoreticular system. The produced antibodies would then target the PrP-expressing brain graft but not the recipient's brain which did not express PrP^c, and thus the pathogenic changes would only be seen in the brain graft but not in the recipient's brain.

(4) Studies have shown that the mouse PrP gene differs from the hamster PrP gene at 16 codons (out of a total of 254 codons). Normally, mice inoculated with hamster PrP^{sc} rarely developed an SE disease. However, when the transgenic mice were made to carry the Syrian hamster PrP gene in addition to its own PrP gene, the animals started to make both mouse and hamster PrP proteins (Prusiner et al., 1990; DeArmond and Prusiner, 1995; Scott et al., 1989). These transgenic mice after selectively inoculated with the hamster PrP^{sc} developed the SE disease. Their brains were tested positive only for the hamster PrP^{sc}, but were essentially negative for the mouse PrP^{sc}. Similarly, when these animals were selectively inoculated with the mouse PrP^{sc}, they also developed SEs, but their brains were tested positive only for the mouse PrP^{sc}. These observations had been incorrectly construed by prion researchers to suggest that the PrP^{sc} preferentially converted the cellular PrP^c with a homologous composition to the corresponding PrP^{sc}. They believed that the preferential ability of PrP^{sc} to physically attract PrP^c with the same amino acid sequence for direct intermolecular interactions between them was the main reason for the described experimental

observations.

The proposed new theory offers a totally different mechanistic explanation. It is rather clear that in transgenic mice that expressed both mouse and hamster PrP^c proteins, inoculations of the mouse PrP^{sc} would only lead to the production of antibodies specific for the misshapen mouse PrP^c, eventually leading to the formation of the mouse PrP^{sc}. The hamster PrP^{sc} would not be formed assuming that the antibodies had little cross-reactivity for the misshapen hamster PrP protein. Likewise, when these mice were inoculated with the hamster PrP^{sc}, only the hamster PrP^{sc} would be formed.

Here it is tempting to also point out that in the above-described experiments, if the hamster PrP transgene in mice can produce hamster PrP^c that serves similar physiological functions as does the mouse PrP^c, then we can predict that the accumulation of the mouse PrP^{sc} in the CNS would be rather minimal. The reason is that when the mouse PrP^c is functionally inactivated by the binding of the autoimmune antibodies, the targeted cell may not need to drastically increase the expression of the mouse PrP^c because the hamster PrP^c protein can still fulfill much of the needed physiological functions that are normally provided by the mouse PrP^c.

(5) Another line of important evidence for the proposed new theory came from studies using the PrP$^{o/o}$ null mice that received a hamster PrP transgene, which was precisely placed under the transcriptional control of either a glial cell-specific promoter or a neuron-specific promoter so that the hamster PrP^c could be selectively expressed in either type of the cells (Race et al., 1995; Raeber et al., 1997). Following an intracerebral challenge with the hamster PrP^{sc}, the glial cells and the neurons each could develop characteristic disease-state histopathology independently, but the same PrP^{sc} inoculation did not cause pathological changes in PrP$^{o/o}$ mice. The observations from these elegant experiments agreed perfectly with the proposed new theory that the production of specific antibodies is needed for the development of SE. When the hamster PrP was selectively expressed in neurons, the specific antibodies would only target neurons; but when PrP was selectively expressed in glial cells, the antibodies would only target glial cells.

(6) There were some experimental findings that are in line with the hypothesis that the antibody-mediated chronic autoimmune attack on the CNS, along with chronic inflammatory responses, is closely associated with the development of SE diseases. First, elevated presence of lymphocytes and other white blood cells were present in the affected regions of the CNS (Betmouni et al., 1996; Betmouni and Perry, 1999; Mabbot and Bruce, 2001). Second, the cytokine levels were elevated following prion inoculation (Mabbott and Bruce, 2001), along with other serological indicators of a CNS inflammation (Coe et al., 2001). Third, earlier studies showed that the complement system of the recipient body could facilitate prion pathogenesis (Klein et al., 2001). This finding was particularly interesting because it is consistent with the suggested role for the antibody-mediated immune attack of CNS in SE pathogenesis. Lastly, an early study showed that administration of a corticosteroid markedly inhibited the development of an SE disease in mice (Outram et al., 1974). Taken together all the evidence discussed here, I think the antibody-mediated chronic autoimmune attack, along with the associated inflammatory responses, plays a crucial role in the development of neuronal lesions seen in SE diseases.

Lastly, it is also worth noting that the CNS inflammatory responses described above usually only occurred at a very mild level during the pathogenesis of SE diseases, which was in contrast to the massive white blood cell infiltration commonly seen during CNS viral infections. The mild inflammatory responses in the CNS likely reflected the very low levels of the autoimmune antibodies produced for PrP^{sc}. However, in most of the biomedical literature, these experimental observations were only construed by prion researchers as the supporting evidence for excluding viral infections as the etiological origin for SEs.

4. Explanation of the existence of multiple "prion strains" and "species barriers"

Many studies have shown that animals inoculated with brain extracts from SE animals or humans usually have distinguishable pathological characteristics on the basis of the disease incubation time, location of the brain lesions, patterns of the spongiform changes, and the profiles of PrP^{sc} glycoforms (Fraser and Dickinson, 1968; Dickinson and Meikle, 1969; Outram et al., 1974; Kimberlin and Walker, 1978; Bruce and Dickinson, 1987; Bruce et al., 1989, 1991, 1994; Robinson et al., 1995; Somerville et al., 1997; Hill and Collinge, 2001; Klein et al., 2001; Lloyd et al., 2001; Race et al., 2002). The time course for the development of various CNS pathological changes appeared to be largely determined by the structural characteristics of the inoculated PrP^{sc} and the recipient's PrP^c protein (Prusiner and McKinley, 1987; Kimberlin and Walker, 1988; Bruce et al., 1989; Bruce et al., 1991; Weissmann 1991a,b; Race et al., 2002). To explain these phenomena, it was suggested that PrP^{sc} has different "strains", which carry information directly encoding their own biological properties (Prusiner and McKinley, 1987; Bock and Marsh, 1988; Kimberlin and Walker, 1988; Bruce et al., 1991; Weissmann 1991a,b; Ridley and Baker 1996). Enormous efforts have already been made in the past to characterize the disease incubation time and the profiles of spongiform changes in various strains of mice (Fraser and Dickinson, 1968; Dickinson and Meikle, 1969; Kimberlin and Walker, 1978; Bruce and Dickinson, 1987; Bruce et al., 1989, 1991, 1994; Hill and Collinge, 2001). For instance, it has been suggested that there were as many as 15 or more different strains of scrapie (on the basis of the different latency and lesion patterns) which could be propagated in the same inbred mouse

strain.

In addition to the apparent existence of multiple strains for the disease-causing PrP^{sc}, it appeared that there are clear species barriers, i.e., PrP^{sc} from one animal species often is very difficult to cause similar diseases in animals of another species. It was first reported in the 1960s that it was very difficult to make rodents to develop SE diseases by inoculating them with the brain extracts from scrapie sheep. Studies using laboratory animals have also shown that when the hamster PrP^{sc} was inoculated into mice, many (but not all) of the animals lived a long, SE-free life and did not accumulate PrP^{sc} in their brains. Similarly, many of the hamsters inoculated with mouse PrP^{sc} did not develop SEs.

The prevailing explanation for the multiple prion strains has been that the PrP^{sc} could adopt multiple configurations and it could serve as a precise template and directly copy its structural information to the recipient PrP^c through direct physical interactions between the PrP^{sc} and PrP^c, and ultimately transforming PrP^c into PrP^{sc}. When PrP^{sc} was folded in one way, it might convert PrP^c to the pathogenic PrP^{sc} with a higher efficiency, giving rise to a shorter incubation time. Folded in another way, it might work less efficiently. It was further suggested that one PrP^{sc} might be attracted to neuronal populations in one part of the brain, whereas another form of PrP^{sc} may be attracted to neurons elsewhere, thereby producing different profiles of pathogenic brain lesions and clinical symptoms. Similarly, the explanation for the species barriers according to the prion hypothesis was that PrP^{sc} preferentially interacts with the PrP^c protein of a homologous composition and converts it into PrP^{sc}, whereas PrP^{sc} cannot convert the PrP^c protein from a different species with a slightly different structure.

The proposed new theory offers sound but very different explanations for the apparent existence of multiple PrP^{sc} strains. It is suggested that the apparent existence of different strains of prions was mostly caused by a combination of the following two factors:

First, the presence of PrP^{sc} with different immunogenic determinants, which are largely determined by the steric structures/configurations of the PrP^{sc} proteins. Consequently, the produced autoimmune antibodies may preferentially recognize the PrP molecules with a certain misshapen configuration. Besides, these antibodies may target different regions (i.e., intracellular vs extracellular) of the PrP protein. Depending on the regions of the PrP that the antibody targets, the antibody-stabilized misshapen PrP may have different configurations and different patterns of covalent modifications. Also, these differently-modified PrP proteins might have different susceptibility to digestion by various proteases contained in lysosomes. These variables, when present in different combinations, are expected to diversify the pathological changes seen in SE diseases.

Second, the possible involvement of other long-lasting immunogenic macromolecules. This possibility would become more likely when crude extracts from diseased brain tissues were used to inoculate animals to induce SE diseases. Many earlier studies have noted that the pathogenic features of the SE disease in the same strain of animals inoculoated with isolated PrP^{sc} were noticeably different from those in animals inoculated with the diseased whole brain tissue. When the whole tissues or the crude extracts from SE animals were used for inoculation, it is expected that varying titers of the autoimmune antibodies for various immunogenic cellular macromolecules would be produced and they may have varying degrees of cross-reactivity against the recipient's brain tissue. In addition, because these immunogens may have quite different distribution in the CNS, eventually the localization of the pathogenic lesions may vary significantly. Notably, the presence of ß-amyloid plaques in the brain of Alzheimer's patients serves as a good example in support of the possible involvement of other protease-resistant immunogenic proteinaceous particles.

According to the proposed new theory, it is suggested that the apparent "species barriers" of SE diseases are largely attributable to the dissimilar immunogenic determinants of the PrP^{sc} proteins (as well as the presence of other immunogenic macromolecules) from different animal species. If the immunogenicity of the PrP^{sc} proteins from two animal species is highly similar, then the anti-PrP^{sc} antibodies produced for one species may also have considerable cross-reactivity with the PrP from another species, and thus there would be no apparent species barriers between these two species. However, if the antibodies could not recognize the PrP protein of the recipient animal, then they may not cause significant pathogenic lesions in the recipient's CNS. Studies have also revealed that the passage of the SE disease from one species to another using experimental inoculations of the diseased tissue extracts, if ever occurred, was generally accompanied by a significant prolongation of the incubation time relative to the passage of the SE disease within the same species. According the proposed new theory, the markedly longer incubation time is due to the fact that two different sets of antibodies would need to be produced. As explained earlier, the initial set of antibodies were produced in response to the inoculated PrP^{sc}, and the second set of antibodies were produced at a later time after the recipient's PrP^c molecules had been converted to the immunogenic PrP^{sc}. However, when some of the recipient animals from another species had already developed SE, the subsequent passage of the disease within the new species usually would have very high incidence and also have a consistent, shorter incubation time.

Here it is also worth noting that the similarity in the amino acid sequences of different PrP proteins from different animal species is not entirely proportional to their similarity in immunogenicity. The immunogenicity is determined not only by the amino acid sequence of a

protein, but also by its tertiary structure (configuration). There are many known examples that certain amino acid residues in a given protein play a more important role than other amino acid residues in determining the steric configurations of a protein and thus its immunogenicity. According to the proposed new theory, it is reasonable to suggest that the so-called "species barriers" are probably not as strict as usually thought. There are many experimental observations in line with this suggestion. For instance, the brain extracts from BSE cattle could cause similar SE diseases in cattle, sheep, mice, pigs, and mink after intracerebral inoculations. Similarly, the brain extracts (containing PrP[sc] protein) from cattle, nyala, kudu, and domestic cats have all been found to cause SE diseases in C57BL, VM, and F1(C57BLxVM) mice following intracerebral inoculations, and all of these extracts gave very similar disease incubation time.

Along the same line of thinking, it is also reasonable to suggest that the PrP[sc] proteins from different poultry animals may share varying degrees of similarity to the human PrP[sc] in certain antigenicity-determining regions. If this is the case, then humans would be susceptible to the development of SE diseases if they are exposed to sufficiently-high doses of PrP[sc] (along with other pathogenic proteinaceous particles) from diseased cattle or other poultry animals, although the pathogenic activity of the immunogens from various animals may be substantially different from each other.

5. Explanations of the autoimmune origin of various animal and human SE diseases

Clinical observations have shown that the development of various animal and human SE diseases, whether sporadic, inherited, or acquired, are consistent with the hypothesis that they are autoimmune-type diseases primarily affecting the CNS. A brief overview of the animal and human SE diseases is provided below.

5.1. SE diseases in animals

In animals, the most common form of SEs is scrapie found in sheep, and the other forms of SEs include transmissible mink encephalopathy, chronic wasting disease of mule deer and elk, feline spongiform encephalopathy, and bovine spongiform encephalopathy (BSE). BSE, also called "mad cow disease", was first identified by G.A. Wells and J.W. Wilesmith after it began striking cows in Great Britain, causing them to become uncoordinated and unusually apprehensive (reviewed in Wilesmith and Wells, 1991). It was estimated that as many as ~1 million cattle have been sick with BSE in the past several decades, and near two hundred thousand cattle (primarily dairy cows) have died of BSE within the past two decades (USDA, 2003). The mean incubation time for BSE was estimated to be ~5 years, and most cattle usually did not manifest the disease since they were slaughtered between 2 and 3 years of age.

Although sporadic or inherited SEs may occur in animals, the source of the apparent epidemic in the past few decades has been traced to a food supplement that included meat and bone meal (MBM) from dead sheep, among which a few of them might have developed SEs. The MBM was prepared from the offal of sheep, cattle, pigs, and chickens and used as a protein-rich nutritional supplement, and it was primarily fed to dairy cows (Wilesmith et al., 1991; Wilesmith and Wells, 1991; Anderson et al., 1996; Gibbs, 1996; Nathanson et al., 1997; USDA 2003). The animals could be exposed to PrP[sc] from sick animals with SE diseases as a result of oral feeding of the MBM.

Notably, there are a number of possible reasons for the higher occurrence of SE diseases in cattle than in other animals in recent decades: (i) The MBM was primarily fed to dairy cows (Wilesmith et al., 1991; Wilesmith and Wells, 1991; Anderson et al., 1996; Nathanson et al., 1997; USDA, 2003). (ii) The development of SEs usually took a long incubation period (Dickison et al., 1975). Comparing to other poultry animals (such as pigs, sheep, and chickens), dairy cows usually were kept alive for a much longer time than other animals and thus they would have a much higher chance to develop full-blown SE diseases.

It is suggested that the sporadic SE diseases in animals (and also humans) are due to aberrant formation of autoimmune antibodies against the PrP and possibly other CNS proteins. It occurs rarely, and usually it has a long and slow progression period. It is conceivable that the risk for an animal to develop sporadic SEs may be markedly elevated due to genetic predispositions (such as mutations or polymorphism) related to the target proteins (e.g., PrP[c]) and/or the production of antibodies. There are many cases of human PrP gene mutations that are presently known to be linked to an elevated risk for SE diseases (discussed later). However, it should also be noted that mutations of the PrP gene, in some rare cases, can reduce the risk for SE diseases. For instance, it is known that sheep with the Arg/Arg polymorphism at position 171 are essentially resistant to scrapie (Parry, 1962; Oppenheimer, 1983; Hunter et al., 1993, 1997a,b; Westaway et al., 1994; Belt et al., 1995; O'Rourke et al., 1997). The possible explanation for this phenomenon is that the Arg/Arg polymorphism of the sheep PrP[c] may alter its structure or its ability to transiently adopt certain misshapen configurations, thus allowing it to evade the recognition and attack by the antibodies produced against PrP[sc] from cattle or other sheep.

5.2. SE diseases in humans

Kuru

Kuru was found among the Fore highlanders of Papua New Guinea (Wilesmith et al., 1991; Wilesmith and Wells, 1991; Prusiner, 1993; Collinge and Palmer, 1997; Gajdusek, 1997). Many highlanders became afflicted with a strange, fatal disease marked by the loss

of coordination (ataxia) and often later by dementia. It is believed that the affected individuals acquired Kuru through ritual cannibalism, i.e., the Fore tribe reportedly honored the dead by eating their brains. Since the practice was stopped several decades ago, Kuru has virtually disappeared. The pathogenic explanation according to the proposed theory is that oral ingestion of PrP^{sc} and/or other long-lasting immunogenic macromolecules from diseased human brains would lead to the production of specific autoimmune antibodies against the human PrP and/or other macromolecules present in the human brain. These autoimmune antibodies would lead to accumulation of PrP^{sc} and/or other protease-resistant proteins in the CNS. Moreover, the antibodies would also bring on a chronic autoimmune attack of the CNS cells, ultimately resulting in the development of the disease.

Creutzfeldt-Jakob disease (CJD)

Cases of CDJ have been found worldwide, with dementia as a usual clinical manifestation. Often it occurred sporadically, roughly striking one person in a million, typically at age ~60 (Prusiner and McKinley, 1987; Collinge and Palmer, 1997). For the apparently sporadic CJD, it is suggested that the likely cause is due to the abnormal production of autoimmune antibodies against the human PrP protein or other immunogenic components in the CNS. Similar to the production of autoimmune antibodies involved in many other types of autoimmune diseases, the mechanism for the aberrant production of autoimmune antibodies against CNS neurons is not clear at present. When the antibodies against the CNS components are produced, they would cause a chronic autoimmune attack of the CNS cells that express these proteins, gradually leading towards the development of CJD.

It is also known that a small number of the CJD cases are iatrogenic. The known causes for iatrogenic CJD mainly included the use of human growth hormone and gonadotropins derived from cadaveric pituitaries (before recombinant hormones became available), dura mater grafts, transplanted corneas, and improperly sterilized depth electrodes (Duffy et al., 1974; Thadani et al., 1988; Centers for Disease Control, 1989; Miyashita et al., 1991; Brown et al., 1992; Baker et al., 1993; Esmonde et al., 1993; Lane et al., 1994; Bateman et al., 1995; Britton et al., 1995; Billette de Villemeur et al., 1996; Will et al., 1996; Bernoulli et al., 1997; Cousens et al., 1997; Heckmann et al., 1997; PHS Interagency Coordinating Committee, 1997; Lugaresi et al., 1986; Will, 2003). More than 90 young adults have developed iatrogenic CJD after treatment with cadaveric human growth hormone, with incubation time ranging from 3 years to over 20 years (Brown et al., 1992; PHS Interagency Coordinating Committee, 1997; Billette de Villemeur et al., 1996). Dura mater grafts implanted during neurosurgical procedures seemed to have caused more than 60 cases of CJD, with an incubation time

ranging from 1 year to over 14 years (Parry, 1962; Duffy et al., 1974; Gajdusek, 1977; Oppenheimer, 1983; Hunter et al., 1997; O'Rourke et al., 1997). It is suggested that the development of iatrogenic CJD was caused by autoimmune antibodies produced against the brain tissue of the recipient. Since many of the iatrogenic CJD have apparent sources of the pathogenic human immunogens, the case for the role of autoimmune antibodies in its pathogenesis is somewhat clearer. However, a few intriguing questions related to this explanation are worth noting here: If the main immunogen that eventually led to the development of iatrogenic CJD was the human PrP^{sc}, then this would mean that a small but significant fraction of people might actually have considerable amounts of PrP^{sc} in their brain and other tissues yet without clear clinical symptoms of an SE disease at the time of death. If this was the case, then the further question is: what were the natural causes that had led to the formation of PrP^{sc} in these elderly donors? It is possible that age-related oxidative damage to various proteins (including PrP^{c}) may be among the causes for increased conversion of PrP^{c} to PrP^{sc} over time. On the other hand, it may also be possible that other immunogenic proteins or even non-protein macromolecules may be involved in the development of autoimmune antibodies that target the recipient's brain, causing similar neurological lesions seen in iatrogenic CJD. Somewhat in line with this suggestion, the clinical manifestations of the iatrogenic human CJD were noticeably different from those of sporadic CJD. Also, some recent studies indicated that PrP^{sc} from the brain of patients who died of iatrogenic CJD had a different pattern of glycoforms than PrP^{sc} from sporadic CJD or vCJD patients.

Cases of new variant CJD (called vCJD) occurring in recent years in Great Britain and France have prompted suspicion that these human cases were causally-linked to the occurrence of BSE in those countries (Cousens et al., 1997). While most of the sporadic CJD cases occurred around age 60 or older, the majority of the vCJD patients were about 40 years of age or even younger (quite similar to iatrogenic CJD). The neuropathology of vCJD patients was found to be unusual, with numerous PrP amyloid plaques surrounded by intense spongiform generation. It is suggested that vCJD patients might have previously been exposed to bovine PrP^{sc} due to consumption of the meat and/or offal products from diseased cattle. It is expected that the titers of the autoimmune antibodies for human PrP protein in vCJD patients might be comparatively higher than the antibodies present in sporadic CJD patients. Consequently, vCJD would progress more rapidly, due to stronger antibody-mediated autoimmune attacks of neurons and other peripheral tissues that express the PrP protein. This hypothesis can be experimentally tested in different ways, such as through determining the titers/affinities of the autoimmune antibodies for both bovine and human PrP^{sc} proteins and also determining the chronic CNS inflammatory responses mediated by

the autoimmune antibodies.

It is of note that while the bovine PrP^{sc} has been suspected of causing human vCJD cases (Cousens et al., 1997), the general perception has been that the contaminated meat and/or offal products from other SE animals (such as sheep, pigs, and chickens) may not be as pathogenic or not pathogenic at all to humans. This perception was, in part, based on some epidemiological studies suggesting the lack of a link between sheep scrapie and the occurrence of human CJD in sheep-farming countries, while a few apparent cases were widely reported that farmers who had "mad cows" in their herds had died of vCJD. According to the proposed new theory, although it is not impossible that the human antibodies produced against the PrP^{sc} proteins from non-cattle poultry animals may have little or no cross-reactivity against the human PrP protein, the more likely scenario is that the antibodies also possess certain degrees of cross-reactivity against the human PrP protein. If so, humans most likely would also develop SE diseases through consuming contaminated meat and/or offal products from non-cattle poultry animals, although the pathogenic activity of PrP^{sc} from different poultry animals may vary. Notably, there are a number of good reasons that might well explain the apparent lack of an association between sheep scrapie and human vCJD. In the past several decades, the numbers of cattle with BSE were far greater than the numbers of other poultry animals with SE diseases. This was due to the fact that the MBM was primarily fed to dairy cows, and the dairy cows usually were kept alive for a much longer time than other animals and thus they would have a much higher chance to develop full-blown SE diseases. Also, since PrP^{sc} usually would accumulate at high levels in the CNS and other tissues at late stages of the SE disease, this would mean that tissues from BSE cattle likely were more pathogenic than tissues from other poultry animals that may only have early-stage SE diseases. In addition, the human consumption of beef worldwide was far greater in quantity than sheep meat. All these underlying factors might have contributed to the fact that there has been no reported case of human vCJD that could be directly linked to the consumption of meat and/or offal products from sheep or other poultry animals.

It is of note that the banning of the widespread practice of feeding the meat and bone meal (MBM) to cattle and sheep in 1988 has contributed, in a very important way, to the sharp decrease of the SE cases in animals (Wilesmith et al., 1991; Anderson et al., 1996, Gibbs, 1996; Nathanson et al., 1997; USDA, 2003), which was somewhat reminiscent of the disappearance of Kuru in the Fore people of New Guinea several decades ago. This ban is believed to have also helped to reduce the risk of human SE diseases.

GGS and FFI

Gerstmann-Straussler-Scheinker syndrome (GGS)

and fatal familial insomnia (FFI), first described by Lugaresi, Medori and colleagues about two decades ago (Lugaresi et al., 1986), usually are inherited and typically appear in midlife. Most of the GGS and FFI cases seemed to occur in humans without any indications of having been spread from one host to another, and in some families they appeared to be inherited (Lugaresi et al., 1986; Monari et al., 1994). Cloning of the PrP gene obtained from a man who had GGS in his family and was dying of it himself revealed a point mutation in codon 102, with leucine substituted for proline in the PrP protein. Following this initial finding, later it was further found that the same genetic mutation was present in other GSS patients, and the incidence in the affected families was significantly high, thus suggesting a genetic linkage between PrP mutations and human SE disease.

Now ~20 mutations of the PrP gene in families with inherited SE diseases have been uncovered, and several of these mutations have been found to be genetically-linked to the SE disease. It is hypothesized here that these mutations may make the PrP^c protein more readily adopt other aberrant configurations, which may have a higher chance for stabilization through covalent modifications, gradually leading towards the formation and accumulation of protease-resistant, immunogenic PrP^{sc} and its fragments. When PrP^{sc} and its fragments are formed and gradually accumulated in the body, it would lead to the production of autoimmune antibodies. The anti-PrP^{sc} antibodies would lead to autoimmune attacks of the CNS and peripheral tissues that express the PrP protein, and cause accumulation of the PrP^{sc} in the targeted cells.

6. Early diagnosis, treatment, and prevention of various SE diseases

6.1. Early diagnosis of SEs

Based on the proposed theory on the mechanism of pathogenesis of various SEs, there are far easier ways to determine whether a person or an animal has developed certain forms of SE diseases by measuring the titers of antibodies for human and animal prions as well as for other proteinaceous and nonprotein antigens present in the brain tissue from animals and humans died of various forms of SE diseases. Here it should be noted that when a person develops SE because of prior consumption of the meat and/or offal products from diseased cattle, that person may produce antibodies against both bovine PrP^{sc} and human PrP^{sc}. The initial antibodies produced are for the bovine PrP^{sc} because of the exposure to bovine PrP^{sc} in contaminated beef, and these antibodies likely also have some cross-reactivity for the misshapen human PrP (which transiently adopts the bovine PrP^{sc}-like structure). However, when the initial antibodies against the bovine PrP^{sc} are produced, they would gradually lead to the formation of human PrP^{sc}. The human PrP^{sc}, when formed in substantial amounts in the body, may further serve as an

immunogen and induce the formation of specific antibodies against it. These antibodies are expected to have a higher affinity for human PrP^{sc} than for bovine PrP^{sc} (different from the initial antibodies produced against bovine PrP^{sc}). Accordingly, it would be necessary to compare the titers and affinities of the antibodies for both animal and human PrP^{sc}. A comparison of their titers and affinities may provide useful clues about the origin and stages of the disease. It is suggested that at early stages of the human SE disease that resulted from ingesting contaminated beef, the antibodies were expected to have a higher titer and affinity for the bovine PrP^{sc} than for the human PrP^{sc}, but at late stages, the antibodies might have increased affinity for the human PrP^{sc}. The proposed antibody-based serological assays would enable us to provide very rapid early diagnosis. Notably, the presently-used bioassays for detecting bovine PrP^{sc} in mice are very insensitive and usually take a long time (up to years) to yield results.

6.2. Treatment strategy for SE diseases

According to the proposed new theory on the pathogenesis of SEs, it is suggested that some of the immune suppressants may be highly effective for the treatment of SEs. In support of this novel notion, an early study has shown that administration of a corticosteroid markedly inhibited the development of an SE disease in mice (Outram et al., 1974). It is possible that immune suppressants with preferential activity towards antibody formation and antibody-mediated autoimmune attack and inflammation may be particularly useful. Also, certain anti-inflammatory agents with a strong CNS activity may also be highly useful as part of the drug therapy.

6.3. Prevention of SE diseases

The earlier implementation of the ban on the use of MBM as part of the animal feed was a very effective measure to reduce the incidence of SE diseases in poultry animals (Anderson et al., 1996; Gibbs, 1996; Nathanson et al., 1997), and such a ban should be strictly reinforced for the purpose of preventing SE diseases. In November 1989, the bovine offal ban which prohibited human consumption of CNS and lymphoid tissues from cattle older than 6 months of age would also help further reduce the risk for humans to acquire SE diseases. This specific legislation was partly based on an earlier study showing that the highest titers of PrP^{sc} were found in these sheep tissues (Hadlow et al., 1982). Although all these measures would assist in reducing the risk of human SE diseases through ingesting contaminated animal meat and offal products, it should be noted that it is vitally important to test all cattle and other poultry animals for SE diseases before they are slaughtered for human consumption. Rapid serological assays as proposed in this paper may provide a means for very

rapid, reliable testing for SE diseases in live animals.

Lastly, it is of note that since PrP^{sc} is partially heat-resistant, thoroughly cooking the meat (particularly beef and ram) may help destroy the antigenic structures of the PrP^{sc} and possibly other similar antigenic macromolecules, and thus may help reduce the risk. Conversely, eating raw or undercooked beef may increase the risk for acquiring the immunogenicity-proficient PrP^{sc} protein.

7.1. Conclusions

Extensive earlier investigations by Prusiner and colleagues over the past three decades have led to the conclusion that the pathogenic agents in animal and human SEs consisted mainly of proteins. Their conclusion was, in part, based on their earlier findings that various procedures that were well known to destroy nucleic acid and viruses did not appear to significantly reduce infectivity, whereas procedures that denatured or degraded protein drastically reduced infectivity. Dr. Prusiner and colleagues proposed that PrP^{sc} alone was responsible for transmitting an infectious disease. This theory has now become a widely accepted doctrine concerning the pathogenesis of various animal and human SE diseases.

While I believe that PrP^{sc}, possibly along with other immunogenic proteinaceous and/or non-protein particles, plays an important role in the development of human and animal SEs, I think that the explanation of pathogenesis according to the prion hypothesis is totally incorrect. Based on the available experimental evidence, a novel, sound theory has been developed in this paper which suggests that various known forms of human and animal SE diseases are the result of chronic autoimmune attack in the CNS. A key step in the pathogenic process leading towards the development of SE involves the production of specific autoimmune antibodies against PrP^{sc} and possibly other long-lasting antigenic macromolecules present in the brain. As precisely explained in this paper, the autoimmune antibodies produced against PrP^{sc} are not only responsible for the conversion of the normal cellular PrP^{c} protein to the disease-causing PrP^{sc}, but they are also responsible for the accumulation of PrP^{sc} in the brain and other peripheral tissues. This mechanistic theory also explained that the antibodies have the tendency to copy the configurational features of the parent PrP^{sc} to the newly-formed daughter PrP^{sc}. Most importantly, the antibodies are responsible for the initiation of an antibody-mediated chronic autoimmune attack of targeted cells (such as neurons), which would produce pathological changes and clinical symptoms characteristic of the SE diseases. As discussed in this paper, the proposed novel theory is strongly supported by an overwhelming body of existing experimental data scattered in the biomedical literature. The theory also provided novel practical strategies for early diagnosis, prevention, and treatment of various SE diseases.

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