Chapter 6
Molecular Sequences in EAE and BSE Point to *Acinetobacter* Bacteria

6.1 Introduction

The relative increase in the 1980's of bovine spongiform encephalopathy (BSE), in cattle in the United Kingdom has evoked a general and world wide public interest in the relative safety of meat products for human consumption. Bovine spongiform encephalopathy was quickly labelled by the press as "mad cow disease".

These cases of BSE occurred after feeding animals with food preparations that had been produced using reject biological materials from slaughter houses. This practice was legally prohibited by the government and since then the number of BSE cases has steadily declined (Anderson et al. 1996).

Several theories have been proposed to explain this phenomenon, the most prominent being the prion hypothesis (Prusiner 1982).

The prion hypothesis suggests that there is an infectious particle of a virus/prion nature which is transmitted from sheep (scrapie) and cows (BSE) and maybe even to humans to produce Creutzfeldt-Jakob Disease (CJD).

Several groups of workers have suggested that there are several problems with this theory:

1. If there was an infection one should be able to demonstrate an immunological response to the agent. Yet no such immune response can be demonstrated.
2. Furthermore there are no suitable methods of culturing the agent or virus/prion (Weissmann 1996).
3. The prion sequence is actually part of the DNA of the host (Chesebro et al. 1985) and is therefore a self-protein.
4. Italian research workers have shown that the human prion sequence that accumulates in brain lesions, KTNMKHAGAAAAGAVVGGLG consists mostly of aliphatic amino acids. These protein chains readily polymerize into amyloid like fibrils (Forloni et al. 1993). Such aliphatic chains are relatively resistant to hydrolysis by enzymes found in macrophages.

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Therefore it is not surprising that such fibrils would accumulate in neurological lesions

5. The suggestion that the prion consists only of self-replicating proteins, also known as the "protein only hypothesis"(Griffith 1967) is incompatible with current concepts of molecular biology (Watson and Crick 1953).
6. Furthermore animals without an adequate or deficient immune system, such as SCID mice do not develop scrapie following immunization with affected tissues (Taylor et al. 1966). Here we encounter a most unusual paradox; absence of immune reactivity is protective since SCID mice readily succumb to viral and bacterial infections.

6.2 Experimental Allergic Encephalomyelitis (EAE) as a Model of an Autoimmune Disease Produced by a Mechanism Involving Molecular Mimicry

The development of an antirabies vaccine by Pasteur some one hundred years ago led to some patients developing severe neurological disorders. It took a few decades to identify the cause of these neurological complications but eventually they were ascribed to the presence of contaminating brain antigens evoking immune responses in the host.

In the 1930's several models of "experimental allergic encephalomyelitis" (EAE) were described in which injections of brain tissue led to immune responses producing a variety of neurological disorders in the injected test animals (Patterson 1966).

It is possible that bacteria may carry antigens cross-reacting or resembling brain tissue, which if present in sufficiently high quantities could evoke an immunological response in affected animals or humans resembling EAE.

A similar pathological sequence occurs in rheumatic fever where anti-streptococcal antibodies target cardiac tissue because of "molecular mimicry" or molecular similarity between heart tissue and *streptococcal* antigens.

A somewhat similar mechanism occurs in ankylosing spondylitis where 95 % of patients possess the HLA-B27 molecule but is only present in 8 % of the general population in the UK and the USA. However the bowel microbe *Klebsiella* has sequences resembling HLA-B27 and spinal collagens. Anti-*Klebsiella* antibodies target HLA-B27 bearing chondrocytes and spinal collagens thereby causing pathological damage and explaining the origin of the lesions found in this disease. Elevated levels of antibodies to *Klebsiella* microbes have been found in ankylosing spondylitis from 16 different countries (Ebringer 2013).

Molecular mimicry also occurs in rheumatoid arthritis where the HLA-DR1/4 antigen is found in over 90 % of rheumatoid arthritis patients but in only 35 % of the general population. However the upper urinary microbe *Proteus mirabilis* has sequences which resemble hyaline cartilage and HLA-DR1/4 antigens. Elevated levels of antibodies to *Proteus* bacteria are found in active rheumatoid arthritis patients from 14 different countries.

The possibility arises that BSE could have occurred by a similar mechanism as the one described for ankylosing spondylitis and rheumatoid arthritis. The supplementary feeds given to cattle were known to contain "green offal" from the abattoirs. Such "green offal" may have contained or been inadvertently contaminated by soil and animal environmental bacteria.

Some of these bacteria could have contained antigens cross-reacting with brain tissues thereby leading to the production of anti-bacterial antibodies with activity against various brain components, similar to the situation described for ankylosing spondylitis and rheumatoid arthritis.

Cases of BSE were first described in the early 1980's reaching a peak in 1988 when the statutory prohibition of the use of supplementary feeds containing bonemeal was introduced by the Ministry of Agriculture, Fisheries and Food (MAFF). This led to a dramatic reduction in the number of BSE cases.

The question still remains as to why this ban has been successful.

Was it due to the elimination of animals infected by prions or could another explanation account for these results.

6.3 The Hypothesis That BSE Is an Autoimmune Disease

It is suggested that BSE is caused by cross-reactive autoantibodies evoked following exposure of cattle to biological material obtained from abattoirs containing bacterial antigens that cross-react or resemble bovine brain tissues. Since neurological damage is the main feature of BSE it is proposed that damage to nerve tissues occurs probably in two stages:

Firstly, the outer covering of neurones, namely the myelin sheath is damaged by bacterial antibodies having anti-myelin specificity because of molecular mimicry between myelin tissue and bacterial antigens. The question here is which bacteria show molecular mimicry to myelin tissues.

In the second stage, nerves are damaged and denatured brain and prion proteins accumulate at sites of neuronal damage. Such denatured prion proteins may consist of amyloid like fibrils previously described by the Italian group (Forloni et al. 1993).

Injection of brain tissue into experimental animals causes an autoimmune disorder, experimental allergic encephalomyelitis (EAE) which is associated with the development of neurological symptoms.

On histological examination there is an extensive vacuolar formation due to destruction of myelin and followed by subsequent degeneration of neuronal tissues. Coalescing vacuoles form larger cavities and give rise to a sponge-like or spongiform appearance.

6.4 Molecular Analysis of Myelin Sequences Point to *Acinetobacter* Bacteria

Injection of myelin preparations into experimental animals have been found to give rise to experimental allergic encephalomyelitis (EAE).

Table 6.1 Comparison of amino acids of bovine myelin and prion to microorganisms from Genbank and SwissProt, which have similar sequences in other proteins

Source	Amino acids	Positions	Locations
Bovine myelin comparisons			
Bovine myelin	LSRFSWGAE	110–118	
Acinetobacter calcoaceticus	ISRFAWGEV	41–49	4-carboxy-mucono lactone decarboxylase
Agrobacterium tumefaciens	YTRFTWGAP	693–701	Beta-glucosidase
Ruminococcus albus	YTQFEISAE	274–282	Beta-glucosidase
Prion protein comparisons			
Bovine prion	NMKHVAG	119–125	
Human prion	NMKHMAG	108–114	
Escherichia coli	QMKHMAG	340–346	*E. coli* signal recognition protein
Escherichia coli	NMKQMSG	118–124	*E. coli* colicin M

Adapted from Ebringer et al. (1997)

Abbreviations: *A* alanine, *E* glutamic acid, *F* phenylalanine, *G* glycine, *H* histidine, *I* isoleucine, *L* leucine, *M* methionine, *N* asparagine, *P* proline, *Q* glutamine, *R* arginine, *S* serine, *T* threonine, *V* valine, *W* tryptophan, *Y* tyrosine

Eylar's group from Los Angeles have identified a highly encephalitogenic peptide from bovine myelin with the following sequence FSWGAEQK (Eylar et al. 1970).

This short amino acid sequence was used to search the Genbank and SwissProt databases for similar sequences allowing for mismatches. The results of this genetic analysis identified three microbes which show partial molecular mimicry to bovine myelin.

The three microbes were: *Acinetobacter calcoaceticus, Ruminococcus albus* and *Agrobacterium tumefaciens* (Table 6.1).

Acinetobacter is a microbe found extensively in soil and water supplies. It is also found on human skin and in the nasal cavities.

Ruminococcus is found in the bowel flora of ruminants.

Agrobacterium is a plant pathogen causing galls which appear as excrescences on trees and shrubs. Scrapie occurs in sheep and goats in animals which are essentially nibblers of grass and shrubs. It is possible that such animals may have been exposed to large quantities of the *Agrobacteria* found in such plants.

The amino acid sequence of *Acinetobacter* contains a positively charged arginine (R) and a negatively charged glutamic acid (E). The two charged amino acids present in the sequence form a powerful immunogenic epitope which evokes antibodies with high binding affinities (Fig. 6.1).

The host protein consisting of arginine-phenyl alanine-serine and tryptophan (RFSW) would then readily bind antibodies evoked against the antigens of *Acinetobacter* and in presence of complement, produce damage to nervous tissues.

The sequences in both *Acinetobacter* and *Agrobacterium* contain tryptophan (W), an amino acid found to be important in producing experimental allergic encephalomyelitis (EAE). If the tryptophan (W) amino acid is modified this leads to loss of encephalitogenic activity (Eylar et al. 1970).

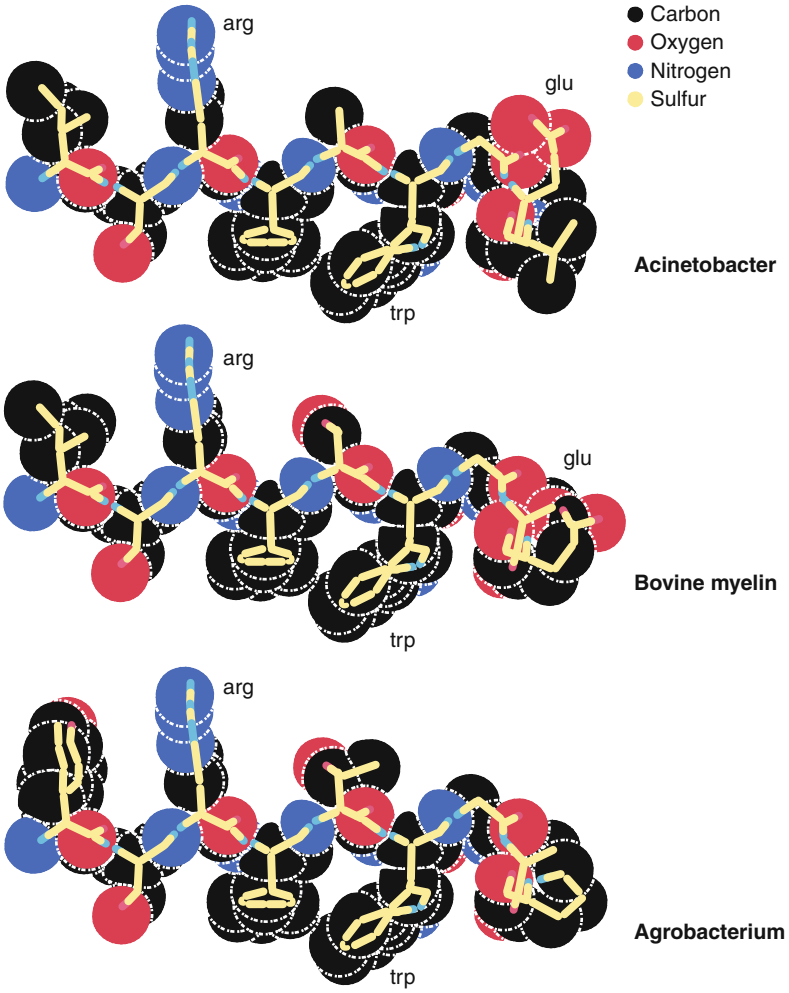

Fig. 6.1 Comparison of space filling models, using Alchemy III (Tripos ASSOC Inc, St. Louis, MO) of *Acinetobacter calcoaceticus*, bovine myelin and *Agrobacterium tumefaciens* which shows molecular mimicry between myelin and bacterial antigens. The immune response to these bacteria, over time, may cause spongiform changes characteristic of chronic experimental allergic encephalomyelitis and neurological symptoms of bovine spongiform encephalopathy. **Abbreviations**: *arg* arginine, *glu* glutamic acid, *trp* tryptophan (Adapted from Ebringer et al. (1997))

The immunological and biological activity of the encephalitogenic peptide remains after it is heated to 100 °C for one hour or treated with 8 M urea. Resistance to heat at 100 °C and to 8 M urea are also, properties found in other proteins such as prions (Weissmann 1996).

The bovine prion sequence NMKHVAG was used to search for similar sequences in microbes. Three sequences were found, all in the same microbe: NMKQMSG in *Escherichia coli* colicin M, QMKNGG in *Escherichia coli* signal recognition

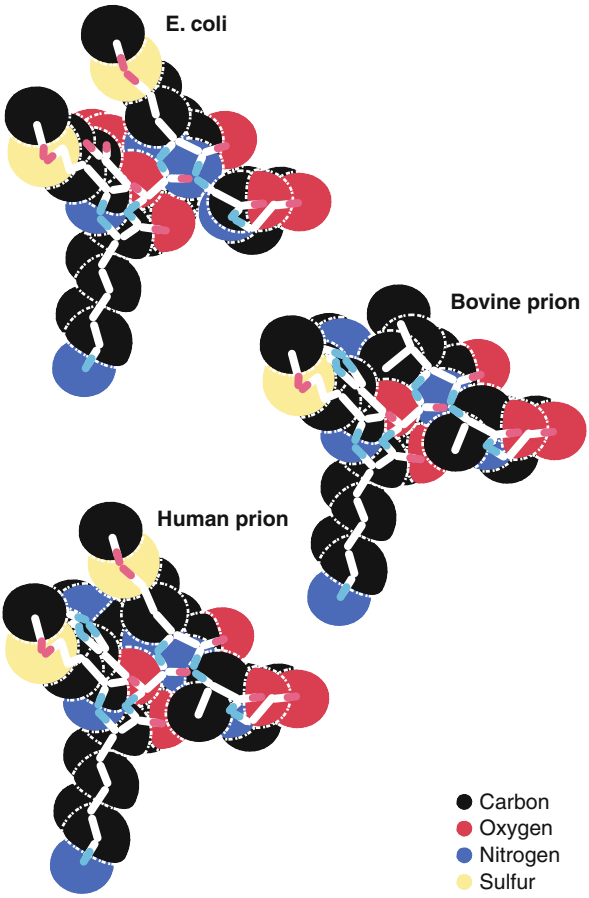

Fig. 6.2 Comparison of space filling models of *Escherichia coli* signal recognition protein, bovine prion and human prion. The nonaliphatic sequence of the prion molecule shows molecular mimicry with the *E. coli* recognition protein. The immune response to *E. coli* may contribute to the neuronal lesions found in spongiform diseases (Adapted from Ebringer et al. (1997))

protein (Fig. 6.2) and NMQHVAG in *Escherichia coli* maltodextrin glucosidase (Ebringer et al. 1997). The question arises whether this microbe is involved in BSE. It can only be answered if elevated levels of antibodies are found against this microbe in BSE animals.

6.5 Discussion and Conclusions

If BSE is an autoimmune disease, caused by bacteria showing molecular mimicry to brain antigens, then elevated levels of anti-bacterial antibodies should be present during active phases of the disease. Evidence of inflammatory disease activity could be demonstrated by elevated levels of C-reactive protein, as occurs in humans (Cowling et al. 1980).

The pathological mechanism could be similar to that found in other autoimmune diseases such as rheumatic fever, ankylosing spondylitis and rheumatoid arthritis.

The possibility arises that feeding of cattle with supplementary foods containing meat and bonemeal could have exposed these animals to such common soil and animal bacterial fragments.

Continuous exposure to such antigenic materials may have evoked autoimmune responses leading to a disease like BSE.

The two theories have different economic implications: the prion-virus hypothesis proposes that cows/sheep (BSE/scrapie) are infected by the prion-virus agent. Therefore such animals should be culled, the farmers compensated and meat production greatly curtailed but leading to huge financial costs, to the farmer, customer and taxpayer.

The autoimmune hypothesis by contrast proposes that neuronal damage is caused by immune processes similar to those found in experimental allergic encephalomyelitis (EAE) following exposure in the gut to bacterial protein sequences resembling myelin and other nervous tissues.

In this situation, the tissue damage is caused by self-proteins, namely antibacterial antibodies acting as autoantibodies. The affected animals are not infected and the treatment is to remove the offending cross-reactive antigenic bacterial fragments from the bowel flora.

Maternal transmission of BSE has occurred from dam to calf. However a similar situation occurs in human pathology in which pregnant women suffering from myasthenia gravis or thyrotoxicosis can transmit the disease via transplacental transfer of maternal IgG to their offspring.

After birth, these neonates with thyroid diseases progressively recover as the level of trans-placental maternal IgG autoantibodies subside over time.

The autoimmune hypothesis predicts that BSE affected animals should have elevated levels of antibodies to bacteria which carry antigens cross-reacting or resembling brain tissues.

The autoimmune hypothesis is a new theory that explains BSE by molecular mimicry between bacteria and brain tissues. However the theory does not conflict with the existing tenets of molecular biology as does the prion theory.

The theory could be readily tested by examining sera from BSE affected animals for antibodies to these bacteria cross-reacting with brain tissues.

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