資料５

説明資料（北本委員）

プリオン病における最近の話題のなかで、傑出しているのは1996年に起こった牛海綿状脳症（Bovine Spongiform Encephalopathy, BSE いわゆる狂牛病）騒ぎである。従来は、ヒトと動物の種差のため、羊のScrapieからはヒトに感染することはなかろうと思われていた。しかし、1980年初め羊や牛のくず肉（脊髄や脾臓などを含む）で作製された蛋白製剤（Meat and bone meals）から牛にBSEとして種の壁を乗り越えた。1986年始めてのBSEの報告から1993年には年間３万頭に及ぶ牛にBSEが報告され、徐々に沈静化に向かいつつあった1995年から1996年にかけて、英国を中心にヒトで新しいタイプのCJDが１０例報告されたのである。New variant CJD (nvCJDと略され、現在はnewをとって vCJDと略されている)は、ティーンエイジを含む若年者に認められ、従来にない臨床・病理を呈するCJDであった。ｖCJDの特徴を列挙すると、１）若年発病である　２）臨床経過が長い　３）特徴的な脳波（PSD）が認められない　４）病理像でアミロイド斑が多発する　５）異常プリオン蛋白が特殊である（タイプ４またはタイプ２＊と呼ばれる）６）全身のリンパ装置（扁桃、リンパ節、脾臓、など）に異常プリオン蛋白が沈着している。このように、vCJDは従来のどのCJDとも異なる新しいタイプのCJDであった。現時点で、英国を中心に１００名以上のvCJDの発生があり、今後も増えつづける可能性がある。

さて、BSEからヒトへ感染したと考えられるｖCJDであるが、もちろん決定的な証拠は証明されたわけではない。しかし、BSEとｖCJDの異常プリオン蛋白が同じような異常型プリオン蛋白をとり、同じような動物（野生型マウスやトランスジェニックマウス）への感染性を示すこと、BSEの多発している国にしかｖCJDが認められないことなど、学問的にはBSEとｖCJDの因果関係はほぼ確実であると考えられている。経口的接種でも、kuru病が感染可能であることはすでに報告されており、ｖCJDもBSEに感染した牛組織の経口接種がその原因と考えられている。牛組織のなかでは、英国は、SBO（Specific Bovine Offals）として、年齢が６ヶ月以上の牛の脳、脊髄、扁桃、胸腺、腸管のヒトへの食材とすることを禁止している（１９８９年）。また、実際にBSEの牛の感染性が証明されているのは、脳、脊髄、網膜が自然発病したBSEで証明され、大腸遠位部、後根神経節、骨髄が実験的に感染させた牛のBSEで証明されている。しかしながら、マウスへの感染実験では、感度の問題もあり、１９９０年からは、牛のSBOは全ての哺乳類と鳥類の餌とすることを禁じるようになった。（WHO Mannuals, １９９８）

検査法でのトピックは、2001年6月にNatureに報告された異常プリオン蛋白の増幅法である。Scrapieに感染した異常プリオン蛋白を鋳型として、正常プリオン蛋白を含む脳抽出物を添加、超音波処理をすることによってPCRで遺伝子を増幅するように、異常プリオン蛋白を増幅するという方法である。方法は斬新であり、増幅効果も97%が新たに添加した正常プリオン蛋白から構成されるという効果的なものである。これは鋳型とした約30倍の異常プリオン蛋白を生成したことになる。一方従来の感染性で検討する実験では、脳乳剤を1000, 000倍希釈してもまだ感染性を証明できるという感度の高いものであり、この試験管内の増幅法がこの感染性の感度までいたるかどうかは不明である。また、孤発性CJDの増幅にも成功したと記載されているが、実際に血液や、感染性の低い臓器での増幅が可能かどうか、またタイプの異なるvCJDの異常プリオン蛋白でも増幅可能かは、未知であるが、新しい試験管内の増幅法として期待をこめてここで紹介する。

次に2001年のJ. Biol. Chem. に報告された尿中のPrPScに関して検査法として報告しておく。CJD, Scrapie, BSEなどの尿を生理食塩水で透析するとProtease抵抗性のPrPScが証明可能であるという報告である。透析をして初めてProtease抵抗性となり、また病気の初期から検出できることから診断法として有効である可能性がある。しかしながら、感染性はむしろ否定的である。よって、著者らはUPrPScと呼び、感染性のある脳のPrPScとは区別している。結果から考えると前述した、試験管内でのプリオン蛋白の異常化と同様の現象と考えていたほうが良い。検査法としては有効であるが、むしろ従来から異常プリオン蛋白の定義として考えられていた性質が、感染性のないプリオン蛋白にも存在したということの方がインパクトが大きい。つまり、いくら免疫学的な異常プリオン蛋白の検出法が感度を上げても、感染性のないプリオン蛋白を定量しているかもしれないという危険性が出てきたわけである。

the advanced stages of scrapie animals become stuporous and manifest visual impairment, excessive salivation and wasting. The duration of the natural clinical course is usually less than four months.

In keeping with other TSEs the neuropathological triad of spongiform change, neuronal loss and astroglial proliferation occurs in scrapie. Vacuolation of the neuronal cytoplasm is a marked and pathognomonic feature, being particularly evident in the brainstem and the ventral and lateral horns of the spinal cord. Cerebral amyloidosis is seen in just over half of natural cases of scrapie. Another characteristic feature of scrapie and other TSEs is the presence of rod-shaped structures seen on electron-microscopy and known as scrapie-associated fibrils (see Figure 28). The SAFs are fibrillar forms of amyloid – the same amyloid which is contained in PrP plaques. Although there is no currently available clinical diagnostic test for the disease, a recent study has identified the presence of abnormal PrP in tonsillar tissue from sheep presumed to be infected with scrapie, long before the occurrence of clinical signs. When validated this would raise the possibility of tonsillar biopsy as a clinical diagnostic, and possibly presymptomatic, test.

3.2 Bovine spongiform encephalopathy

Bovine spongiform encephalopathy was first reported in British cattle in November 1986. Most cases were infected as calves; the modal age of disease occurrence is five years (range 29 months to 18 years) and the average incubation period 60 months. Current evidence suggests that the disease originated from the use of feed supplements containing MBM contaminated by a TSE agent. The stringency of the rendering procedure, by which animal materials were processed to produce MBM, changed during the 1970s and early 1980s and decreased use of hydrocarbon solvents and the adoption of lower temperatures may have resulted in increased survival of the infective agent. These changes were adopted in response to a fall in the value of tallow (the fat-rich fraction of the process whose yield is increased by using solvent), a rise in the cost of energy and a need to replace old plant with safer systems not using potentially explosive and carcinogenic solvents. Epidemiological evidence suggests that sheep scrapie, endemic in Great Britain, was the likely source of the infective agent that initiated the BSE epidemic. However, experiments indicate that BSE is associated with a single major strain of infective agent and although over 20 different scrapie strains are recognised, to date none appear to match that seen in BSE. This has led to the further hypothesis that BSE may have been an uncommon sporadic and/or hereditary disease of cattle that was dramatically amplified as a result of infected bovine material entering the modified rendering process. Whatever the origin of the agent responsible for BSE it is likely that the recycling of infected cattle through the rendering process in the 1980s was responsible for fuelling the large and explosive epidemic. It is of note that BSE has been experimentally transmitted via the oral route to cattle by as little as 1g of BSE-affected bovine brain.

The British Government made BSE notifiable in June 1988 and shortly afterwards a statutory ban on the feeding of ruminant-derived protein to ruminants was introduced. In November 1989 a ban was introduced on the use of certain specified 'high risk' bovine offals (SBO) for human consumption (brain, spinal cord, tonsils, thymus, spleen and intestines from animals >6 months old). The selection of which offals should be included in the SBO ban was based on the evidence of infectivity of tissues from scrapie-infected sheep. BSE infectivity has now been demonstrated in the brain, spinal cord and retina of naturally affected cattle and also in the distal ileum, dorsal root ganglia and bone marrow of those infected experimentally. However, a wide range of tissues from clinically affected cases of BSE have shown no detectable infectivity using the

mouse bioassay (which has potential limitations, in particular its sensitivity due to the 'species barrier' - see later), and these include muscle, milk and a range of lymphoreticular tissues. In September 1990 the use of SBO was further restricted, being prohibited for use in feed for all animals and birds. At the end of 1992 BSE reached its peak incidence in the UK but thereafter declined rapidly, almost certainly in response to the statutory measures (see Figure 29). However, new cases of BSE were being observed in cattle that were born after the implementation of the feed ban. It has been suggested that most of these cases occurred because of the continued use of feed rations produced before the ban; cross-contamination of cattle feed by feed containing MBM intended only for pigs or poultry; and an incomplete compliance with the SBO ban. Further measures were instituted to address these particular issues and following the announcement of a possible link between BSE and a nvCJD in March 1996, cattle >30 months old and heads from all bovines over six months old were excluded from all food or feed chains.

Although the pattern of the epidemic remains consistent with the hypothesis that the vast majority of cases arose through infection with contaminated feed, it remains possible that other routes of transmission may occur infrequently, in particular maternal transmission from dam to calf. A study to assess maternal transmission suggests that this may occur at a low rate (estimated to be responsible for only about 1% of cattle expressing disease) but also tentatively suggests that genetic factors may influence susceptibility.

The appearance of a number of novel TSEs, causally linked with BSE, in domestic and captive animals raises the question of whether BSE occurs, or will occur, in further animal species. Particular concern has been expressed regarding the possibility of BSE in sheep, pigs and poultry. BSE has been experimentally transmitted to sheep by feeding as little as 0.5g of infected bovine brain and it is known that some sheep were fed MBM until this was practice was banned in 1988. In this regard the lack of evidence of a BSE-related epidemic of sheep scrapie is reassuring, but concern was sufficient to lead to the ban for human consumption of ovine brain and spinal cord from sheep over six months of age in the UK and over one year in France as part of a risk reduction strategy.

Pigs, but not chickens, have been shown to be susceptible to BSE by parenteral inoculation of infected bovine brain homogenate. However, challenging pigs with a very large oral dose of BSE-infected brain failed to produce disease, at least up to 6.5 years post-challenge.

By the end of 1996, over 168 000 confirmed cases of BSE had been reported in the UK. Relatively small numbers of cases have also been reported in native-born cattle in Switzerland, the Republic of Ireland, France, Belgium, Portugal and the Netherlands. A few cases have also been reported in Germany, Italy, Oman, Canada, Denmark, and the Falkland Islands, but solely in animals imported from the UK (source: cases reported to the Office International des Epizooties by member countries).

The duration of the clinical course of BSE is typically one or two months, but ranges from seven days to 14 months. The most commonly observed signs are apprehension, hyperaesthesia and ataxia, but affected animals may also show a decreased milk yield and loss of condition (see Figures 30 and 31). There is no effective treatment and the disease always progresses to death in the affected animal. A number of other bovine conditions can mimic the illness phenotype of BSE, *e.g.* magnesium deficiency ('staggers'), and currently no practical and reliable laboratory diagnostic disease marker has been reported, emphasising the importance of pathological diagnosis. Research is in progress to identify disease markers in CSF and urine.

Pathological changes (see Figures 32 - 35) are similar to scrapie in many respects with vacuolar lesions largely confined to the brainstem and accompanied by neuronal degeneration and an astrocytic reaction. Sparse cerebral amyloid plaques are seen in a small proportion of cases. In contrast to scrapie, greater diagnostic importance is attributed to the neuropil vacuolation than neuronal vacuolation.

3.3 Chronic wasting disease of mule and elk deer

Chronic wasting disease (CWD) is a TSE of mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*Odocoileus hemionus columbianus*) and Rocky Mountain elk (*Cervus elaphus* – see Figure 36). The disease has only occurred in limited areas in the western United States. It was first recognised as a clinical syndrome in 1967 and is typified by behavioural changes and chronic weight loss leading to death.

CWD has occurred in captive wildlife research facilities in northern Colorado and south-eastern Wyoming and has also recently been identified in commercial elk herds in South Dakota. Although cases of CWD have been seen in two zoological parks, the affected animals all originated from the above-mentioned research facilities. Soon after the recognition of the disease, animal movement from these facilities was stopped. CWD has also been confirmed in approximately 100 free-ranging deer and elk in a limited number of counties in northern Colorado and south-eastern Wyoming. Animals born in captivity and those born in the wild have been affected with the disease.

There is no clear evidence to suggest that CWD is caused by exposure to any other form of animal TSE. Furthermore, other ruminant species, including wild and domestic cattle, sheep and goats, have been housed in facilities in direct and indirect contact with CWD-affected deer and elk, but no cases of CWD have been detected in these animals. Although the exact origin and mode of transmission of CWD is unknown, epidemiological studies suggest that transmission may be lateral, and possibly maternal. Transmission via feed is not believed to occur, affected animals have been fed a variety of foodstuffs with no common ingredient of animal origin being identified. It is of note that painstaking attempts to eradicate CWD from captive facilities, including thorough decontamination and a 12-month period free of elk or deer, failed to prevent disease recurrence.

3.4 Transmissible mink encephalopathy

Transmissible mink encephalopathy (TME – see Figure 37) was first described in 1967 but had occurred on mink farms in Minnesota and Wisconsin as early as 1947. The disease occurs as outbreaks, in farmed mink only, and has been recognised in Idaho, Russia, Finland, Canada and Germany. The condition is rare and mortality high, with nearly all adult mink on an affected ranch succumbing to the disease during an outbreak. Evidence points to infected feed as the cause of TME and it has been suggested that scrapie is the likely contaminant. However, experimental transmission of scrapie to mink via the oral route has not been successful to date, although TME may be caused by a different scrapie strain that those used experimentally. The possibility of a bovine origin of TME has also been raised. Products from fallen or sick cattle ('downer cows') were said to have been fed to a colony of affected mink in the USA and that these animals had been fed a diet free of any ovine material. However, surveillance of cattle in the USA has not revealed a single