Prospects For a Preclinical Diagnostic Test

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At present diagnosis of BSE in cattle and sheep scrapie routinely requires post-mortem confirmation which is traditionally performed by neurohistological examination for Spongiform Vacuolation. Detection of disease specific forms of the host protein PrP, called PrP^{Sc}, offers alternatives. Deposits can be detected immunohistochemically, by searching tissue extracts by electron microscopy for the fibrils (scrapie associated fibrils: SAF) which are comprised of PrP^{Sc}, or immunoblotting of tissue extracts for PrP^{Sc}. In this paper we examine the possibilities of using the immunochemical detection of PrP^{Sc} diagnostically. We have raised antibody reagents to PrP, used them to determine the kinetics of PrP^{Sc} deposition in murine models of the disease and to determine if PrP^{Sc} can be detected in ruminant tissues from BSE or scrapie infected animals.

Diagnosis based on the immunochemical detection of PrP^{Sc} relies on the development of antibody reagents of sufficient specificity and sensitivity to detect small amounts of PrP. In addition it is necessary to distinguish between the normal form of the protein (PrP^{c}) and the scrapie-specific form (PrP^{Sc}). This is dependent on the operational properties of the two PrP fractions. PrP^{c} is soluble when tissue homogenates are treated with detergents such as Sarkosyl, whereas PrP^{Sc} sediments upon ultracentrifugation. Furthermore PrP^{c} is sensitive to protease digestion (e.g. with Proteinase K) but PrP^{Sc} shows partial resistance to proteolytic degradation before solubilisation with denaturants (Somerville *et al.*, 1989).

Antibody reagents have been raised in rabbits to denatured PrP^{Sc} extracts from mouse scrapie models (Farquhar *et al.*, 1989). These reagents recognise all forms of PrP from rodents with high sensitivity and specificity, and also ruminant PrP but at lower avidity. Two monoclonal antibodies which recognise epitopes of ruminant PrP have been produced and in addition chicken antibodies have also been raised against recombinant PrP (Birkett, 1996). The affinity purified chicken anti-PrP antibody reagent recognises PrP on immunoblots with high sensitivity and specificity.

Using the rabbit antisera, PrP^{Sc} deposition has been examined in tissues from mice of different *Sinc* genotypes infected with a range of scrapie strains, including one derived from the mouse passage of BSE, inoculated by either the intracerebral or intraperitoneal route. The results show that PrP^{Sc} can be detected early after infection in lymphoid and other tissues in some models of the disease but not until near the clinical end point of the disease in other models. PrP^{Sc} could also be detected in brain at similar times as peripheral tissues after intracerebral injection but later after intraperitoneal injection. Differences in the time at which PrP^{Sc} could first be detected depended primarily on the strain of scrapie agent with which the mouse was infected but also on the *Sinc* genotype of the mouse and route of infection (Farquhar, 1996, Fraquhar *et al.*, 1994).

The chicken antibody detected PrP in ruminant brain and spleen with high specificity and sensitivity. PrP^{Sc} has been detected in brains of sheep killed when showing clinical signs of natural scrapie or a range of experimental sources of infection. Brains from cattle with BSE were also positive. PrP^{Sc} was also found in spleen extracts from sheep with natural scrapie but in spleen extracts from only some experimental sources. Spleen extracts from cattle with BSE were negative in all cases.

These results show that the deposition of PrP^{Sc} in peripheral tissues of scrapie-affected sheep occurs in some cases but not others. Similarly, infectivity has been found in spleens and other peripheral organs of sheep with natural scrapie (Hadlow *et al.*, 1982) but not in non-CNS organs from cattle infected with BSE (except distal ileum and retina) (Fraser & Foster, 1993). Data from experimental models indicate that control of PrP deposition is a characteristic of the interaction between the infecting agent and the host.

Selection of particular tissues in which PrP is deposited and when it occurs may be a similar property to the targeting of lesions in the brain (Bruce *et al.*, 1989). Lesions (and presumably infectivity) may be localized to only small areas of the brain or to selected organs.

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Diagnosis using PrP can presumably only be made by sampling those tissues (or areas of the brain) where infection or its sequelae occur.

In experimental cases, deposition of PrP in peripheral tissues may be of diagnostic value but only when it has been ascertained that PrP is deposited in the tested organ in the model of the disease under study. With field scrapie in sheep it would be necessary to show that within an individual flock proven cases of disease with similar PrP genotype do have PrP^{Sc} deposited in the selected organ. The data indicate that any PrP deposition in spleen of BSE infected cattle, like infectivity, is not detectable and therefore of no potential diagnostic value. The absence of infectivity in other peripheral organs of BSE infected cattle (except distal ileum) suggests little hope for the detection of PrP^{Sc} in non-CNS organs in these cases. By contrast detection of PrP^{Sc} in lymphoid organs of some sheep with natural scrapie may have diagnostic value when the parameters controlling its deposition are better understood.

References

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NOTICE

THE RULE to be observed in this stable at all times, toward the cattle, young and old, is that of patience and kindness. A man's usefulness in a herd ceases at once when he loses his temper and bestows rough usage. Men must be patient. Cattle are not reasoning beings. Remember that this is the Home of Mothers. Treat each cow as a mother should be treated. The giving of milk is a function of motherhood; rough treatment lessens the flow. That injures me as well as the cow. Always keep these ideas in mind in dealing with my cattle.

W. D. HOARD
Founder of Hoard's Dairyman
In 1965

The above notice has been prominently displayed in the Hoard's Dairyman dairy barn for many years.