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Studies of cathepsin B and L activities and poly(ADP-ribose) production in scrapie–infected mouse neuroblastoma cells

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Cultured cell lines infected with prions produce the disease-associated isoform of the prion protein (PrP^{Sc}). Comparisons between prion-infected and uninfected cells have the potential to demonstrate scrapie-specific alteration in cellular metabolism, pathological consequences of prion propagation and compensatory mechanism of the cell. The traditional method of deriving scrapie-infected cell lines has resulted in selection or cloning artifacts that invalidated comparison between the scrapie–infected and uninfected cells. In order to avoid artifacts from post-infection cloning, N2a sublines highly susceptible to prion infection were isolated. Cultures producing sufficient quantities of PrP^{Sc} were established using these susceptible sublines without further post-infection subcloning. This strategy of using newly infected cells minimises the risk of clonal artifacts and enables valid comparisons of infected and uninfected cells.
Cathepsins are lysosomal proteases, which, besides their function in the non-selective degradation of proteins, are also involved in a variety of physiological and pathological phenomena. Two major lysosomal cysteine proteases, cathepsins B and L, were analysed by fluorometric cytochemical and biochemical methods in both uninfected N2a cells and persistently scrapie-infected neuroblastoma cells as well as in newly infected highly susceptible sublines and compared with uninfected parental cells. Prion infection was consistently associated with statistically significant twofold increases in cathepsin B and L activities in both cell systems. Viewed together with published data describing the blockade of PrP^{Sc} formation in infected cell cultures by cysteine proteinase inhibitors, the hypothesis is now posited that lysosomal proteases may be actively involved in an autocatalytic mechanism of formation of PrP^{Sc} or provide selective advantage for prion replication.
Oxidative stress has been suggested to be a possible pathological mechanism in prion disease. One of the immediate early responses of most eukaryotic cells to oxidative and other type of DNA damage is the covalent post-translational modification of nuclear proteins with poly(ADP-ribose). *In-situ* poly(ADP-ribose) production in scrapie-infected and uninfected cells was analysed by immunofluorescence. No spontaneous poly(ADP-ribose) production was detected either in newly infected highly susceptible sublines or in uninfected parental N2a cells. Neither was there any clear difference in poly(ADP-ribose) production in acutely H_2O_2-treated infected cells compared with uninfected counterparts. Apparently the level of

prion-induced oxidative stress in this cell culture system is too low to allow detection of poly(ADP-ribose) formation by the method used.