Cellular mechanisms of prion propagation

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Title of project or programme

Cellular mechanisms of prion propagation

Principal Investigators of project/programme grant

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- United Kingdom

Source of funding information

Medical Research Council

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2125863.38

Start date of award

01-04-2005

Total duration of award in months

60

The project/programme is most relevant to

- Prion disease

Keywords

Research abstract in English

The objectives of this programme are (a) to identify cellular susceptibility factors required for the propagation of prions, (b) to characterise the mode of prion spread in the lymphoreticular system of

mice.

(a) Cell lines have been recognized as a very useful tool for prion detection and assay, since the cell-based amplification of prions increases sensitivity levels by about 3-4 orders of magnitude as compared to conventional protein assays. To date, the number of cell lines in which prions can be propagated long term is very limited. In addition the majority of prion-susceptible cell lines are permissive only to one or two mouse-adapted prion strains, but not to ovine, bovine or human prions. Understanding the cellular factors mediating prion propagation is of major importance to the field. To identify cellular susceptibility factors we selected revertants from highly susceptible cells that showed about 100-fold lower rates of prion propagation. We are using Affymetrix microarrays to identify differentially expressed genes between highly susceptible cells and revertants. To validate potential gene candidates we are using state-of the art gene intervention methods in a tetracycline (Tet)-inducible fashion to exclude confounding clonal effects.

(b) In most transmissible spongiform encephalopathies (TSE) prions accumulate in the lymphoreticular system (LRS) long before they are detectable in the central nervous system. A considerable body of evidence demonstrates that the transfer of infectivity between hematopoietic and non-hematopoietic cells is essential for neuroinvasion of prions. However, the molecular mode by which prions are transferred between different cells is ill-defined. Recently in-vitro studies demonstrated that prions are released into the cell supernatant in form of exosomes, a secretion process that has been well characterised in hematopoietic cells. We could demonstrate the release of infectivity from scrapie-infected cells to spatially separated non-infected cells via the external medium using the Scrapie-cell assay, a quantitative cell-based infectivity assay that we developed. We are now testing whether infectivity is released by antigen-presenting cells from the LRS of scrapie-infected mice. A variety of potentially infectious cells, like B-and T-cells, dendritic cells, macrophages and follicular dendritic cells are isolated by immunolabling (MACS separation) and infectious titres and the release of infectivity are determined subsequently. Although the biological function of exosomes has not yet been elucidates, novel findings suggest a role in antigen presentation and immune response. It has been demonstrated that exosomes from dendritic cells (DC) that were loaded with tumour-proteins from melanomas ex vivo triggered a T-cell response when re-injected into tumour-bearing mice. In analogy we will test whether exosomes isolated from DC of scrapie-infected mice trigger a T cell response in vitro and in vivo. If this is the case the therapeutic effect of DC-derived exosomes will be tested in vivo by injecting purified exosomes into scrapie-infected animals and incubation times will be determined in parallel to mock-treated mice.

Lay Summary