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Role of the draining lymph node in scrapie agent transmission from the skin

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Abstract

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases that affect humans and animals. Diseases include scrapie in sheep and Creutzfeldt–Jakob disease in humans. Following peripheral exposure, TSE agents usually accumulate on follicular dendritic cells (FDCs) in lymphoid tissues before neuroinvasion. Studies in mice have shown that TSE exposure through scarified skin is an effective means of transmission. Following inoculation by this route TSE agent accumulation upon FDCs is likewise essential for the subsequent transmission of disease to the brain. However, which lymphoid tissues are crucial for TSE pathogenesis following inoculation via the skin was not known. Mice were therefore created that lacked the draining inguinal lymph node (ILN), but had functional FDCs in remaining lymphoid tissues such as the spleen. These mice were inoculated with the scrapie agent by skin scarification to allow the role of draining ILN in scrapie pathogenesis to be determined. We show that following inoculation with the scrapie agent by skin scarification, disease susceptibility was dramatically reduced in mice lacking the draining ILN. These data demonstrate that following inoculation by skin scarification, scrapie agent accumulation upon FDCs in the draining lymph node is critical for the efficient transmission of disease to the brain.

Keywords: Transmissible spongiform encephalopathy; Prion disease; Scrapie; Skin; Lymph node; Follicular dendritic cell

1. Introduction

Transmissible spongiform encephalopathies (TSEs), or 'prion diseases', are sub-acute neurodegenerative diseases that affect humans and both domestic and free-ranging animals. The neuropathological features within the central nervous system (CNS) characteristically include spongiform pathology, neuronal loss, glial activation and amyloidal aggregations of an abnormally folded host protein. There is little evidence of pathology in other tissues. The nature of the TSE agent is unknown, but an abnormal, relatively proteinase-resistant isoform (PrP^{Sc}) of the host cellular prion protein (PrP^{c}), co-purifies with infectivity in diseased tissues and is a useful biochemical marker [1]. The 'prion hypothesis' argues that PrP^{Sc} constitutes a major, or the sole component of infectious agent and facilitates conversion of PrP^{c} to PrP^{Sc} [2].

Many TSE agents, including natural sheep scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) in mule deer and elk, and kuru and variant Creutzfeldt–Jakob disease (vCJD) in humans, are acquired by peripheral exposure. Although the main route of transmission of BSE to cattle and other species (e.g.: humans) is considered to be oral (ingestion), other routes of TSE transmission have been identified. In humans accidental iatrogenic transmissions have occurred through transplantation of sporadic CJD contaminated tissues or tissue products, or transfusion of vCJD-contaminated blood [3,4]. Neurosurgical instruments contaminated with TSE agents also have the potential to transmit disease [5]. Therefore the wide tissue tropism of the vCJD agent [6], and potential for large numbers of sub-clinical vCJD cases [7] implies that a serious risk of iatrogenic transmission exists. Studies in mice have also highlighted that skin scarification is an effective means of TSE transmission [8]. Thus, it is possible that some natural TSE cases might be transmitted through skin lesions during close contact, such as from an infected mother to offspring through skin lesions at birth or via the unhealed umbilical cord. Likewise, consumption of coarse feed contaminated with TSE agents

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might also scarify the mucosa of the mouth or oesophagus aiding transmission.

Within the lymphoid tissues of patients with vCJD [6], mule deer fawns with CWD [9], some sheep with natural scrapie [10] and rodents inoculated with scrapie [11] early PrP^{Sc} accumulation takes place in the germinal centres upon follicular dendritic cells (FDCs). In the absence of FDCs, TSE agent accumulation in lymphoid tissues and the subsequent spread of disease to the CNS (termed *neuroinvasion*) are both impaired [12–16]. Neuroinvasion from lymphoid tissues proceeds along both sympathetic nerves and fibres of the vagus nerve [17,18].

Following inoculation via skin scarification, the scrapie agent accumulates first in the draining lymph node, and subsequently spreads to non-draining lymph nodes and the spleen [16,19]. TSE agent accumulation upon FDCs in lymphoid tissues is likewise critical for efficient transmission of disease from the skin to the CNS [8,16,20], but which lymphoid tissues are crucial for neuroinvasion is not known. Therefore, in the current study mice deficient in the draining lymph node were utilized to address the following questions: First, whether TSE-susceptibility is reduced in the absence the draining lymph node? Second, whether neuroinvasion occurs from multiple sites such that the lack of the draining lymph node can be compensated for by the presence of another such as the spleen?

2. Materials and methods

2.1. Mice and bone-marrow grafting

Lymphotoxin (LT)$\alpha^{-/-}$ mice [21] and LT$\beta^{-/-}$ mice [22] were obtained from B & K Universal Ltd. (Hull, UK) and were maintained on a C57BL/6 background. Age- and sex-matched C57BL/6 mice were used as immunocompetent wild-type (WT) controls. Bone-marrow from the femurs and tibias of WT mice was prepared as a single-cell suspension (3×10^7–4×10^7 viable cells/ml) in HBSS (Invitrogen, Paisley, UK). Recipient adult (6–8 weeks old) LT$\alpha^{-/-}$ mice, LT$\beta^{-/-}$ mice and WT mice were γ-irradiated (950 rad) and 24 h later reconstituted with 0.1 ml bone-marrow by injection into the tail vein. Recipient mice were used in subsequent experiments 35 days after bone-marrow grafting. All protocols using experimental rodents were approved by the Institute's Protocols and Ethics Committee and carried out according to the strict regulations of the UK Home Office 'Animals (scientific procedures) Act 1986'.

2.2. Scrapie agent inoculation

Mice were inoculated with the ME7 scrapie agent strain by skin scarification of the medial surface of the left thigh. Briefly, prior to scarification approximately 1 cm^2 area of hair covering the site of scarification was trimmed using curved scissors and then removed completely with an electric razor. Twenty-four hours later a 23-gauge needle was used to create a 5 mm long abrasion in the epidermal layers of the skin at the scarification site. Then using a 26-gauge needle one droplet ($\sim$6 μl) of ME7 scrapie agent inoculum from a 1.0% (w/v) terminal scrapie mouse brain homogenate in physiological saline was applied to the abrasion and worked into the site using sweeping strokes. The scarification site was then sealed with OpSite (Smith & Nephew Medical Ltd., Hull, UK) and allowed to dry before the animals were returned to their final holding cages. Where indicated, separate groups of WT mice, LT$\alpha^{-/-}$ mice and LT$\beta^{-/-}$ mice were inoculated by intracranial injection with 20 μl of a 1.0% (w/v) scrapie mouse brain homogenate (containing approximately $1 \times 10^{4.5}$ ID_{50} units). Following inoculation, all animals were coded, assessed weekly for signs of clinical disease and killed at a standard clinical end-point [23]. Scrapie diagnosis was confirmed by histopathological assessment of TSE vacuolation in the brain.

2.3. Immunohistochemical and immunofluorescent analysis

Spleens were removed and snap-frozen at the temperature of liquid nitrogen. Serial frozen sections (10 μm in thickness) were cut on a cryostat. Follicular dendritic cells were visualized by staining with mAb 7G6 to detect CD21/CD35 or mAb 8C12 to detect CD35 (BD Biosciences PharMingen, Oxford, UK). B lymphocytes were detected using mAb B220 to detect CD45R (Caltag, Towcester, UK). Complement component (C) C4 was detected using mAb FDC-M2 (AMS Biotechnology, Oxon, UK). For fluorescent microscopy, following the addition of primary antibodies, species-specific secondary antibodies coupled to Alexa Fluor® 488 (green) or Alexa Fluor® 594 (red) dyes (Invitrogen) were used. Sections were mounted in fluorescent mounting medium (Dako, Ely, UK) and examined using a Zeiss LSM5 confocal microscope (Zeiss, Welwyn Garden City, UK). For light microscopy, following the addition of primary antibody, biotin-conjugated secondary antibodies (Stratech, Soham, UK) were applied followed by alkaline phosphatase coupled to the avidin–biotin complex using Vector Red as a substrate (Vector Laboratories, Peterborough, UK). Sections were subsequently counterstained with hematoxylin to distinguish cell nuclei.

For the detection of PrP in the brain, tissues were fixed in periodate–lysine–paraformaldehyde and embedded in paraffin-wax. Sections (thickness, 6 μm) were deparaffinized, and pretreated to enhance PrP immunostaining by hydrated autoclaving (15 min, 121 °C, hydration), and subsequent immersion in formic acid (98%) for 5 min [24]. Sections were then stained with the PrP-specific pAb 1B3 [25]. Glial fibrillary acid protein (GFAP) was detected on adjacent brain sections using rabbit GFAP-specific antiserum (Dako). Immunolabelling was detected using either horse radish peroxidase coupled to the avidin–biotin complex (Vector Laboratories) with diaminobenzidine (DAB) as a substrate, or alkaline phosphatase coupled to the avidin–biotin complex using Vector Red as a substrate. Sections were subsequently counterstained with hematoxylin to distinguish cell nuclei.

2.4. Immunoblot detection of PrP^{Sc}

Spleen fragments (approximately 20 mg) or inguinal lymph nodes (ILNs) were prepared as 10% (w/v) tissue homogenates and PrP^{Sc} enriched by sodium phosphotungstic acid (NaPTA)