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Scratch-wounding renders cultivated cells less permissive to prion infection ☆

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Abstract

Using permissive cell lines of epithelial or neuroglial origin, we found that scratch-wounding a small proportion of the recipient cells prior to prion exposure strongly reduced the cell culture's susceptibility to infection. We provide evidence suggesting that wound-triggered inhibition of prion infection was mediated by the release of nucleotides in the extracellular medium of injured cultures. While cell wounding or ATP treatment of unwounded target cells inhibited de novo infection, we found that they had no effect on steady-state infected cultures, indicating that these treatments affect the early stages of infection. These findings support the view that cells have the capacity to modulate their permissiveness to prion infection in response to external stimuli, such as a signalling molecule.

Keywords: Prion; Extracellular ATP; Scrapie; PrP; Cell model; Cell wounding

Prion diseases, or transmissible spongiform encephalopathies, include scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, and Creutzfeldt–Jakob disease in humans. This group of fatal neurodegenerative disorders is characterized by spongiform change, neuronal loss, and accumulation in the infected tissues of a detergent-insoluble and protease-resistant isoform of the cellular PrP protein [1,2]. The causative infectious agent, or prion, is thought to be an abnormal form of PrP, or a precursor of it [3]. Prions multiply by triggering the conversion of the normal cellular form of PrP into the abnormal conformer. While prion multiplication is absolutely dependent on the presence of the PrP protein [4,5], several convincing lines of evidence indicate that expression of PrP is not sufficient to confer permissiveness to any given cell type. Indeed, mouse tissues expressing high levels of PrP, either naturally or after genetic engineering, do not necessarily accumulate prion infectivity [6,7]. Strong cellular selectivity unrelated to PrP expression is also observed in cellular models of prion infection as very few PrP-expressing cell lines are able to replicate prions [8,9]. The current view is that prion multiplication requires a number of cellular and/or molecular factors, that may be restricted to a few cell types. However, these prion multiplication-controlling factors have yet to be identified.

In this study, we found that cellular mechanical injury affects the susceptibility of cultivated permissive cells to prion infection as scratch-wounding inhibits de novo infection of permissive target cells. We provide evidence that this inhibitory effect may be mediated by the release of nucleotides in the extracellular medium. These findings introduce the notion that an external biological

☆ *Abbreviations:* PrP, cellular prion protein; PrPres, abnormal prion protein resistant to proteinase K digestion; PK, proteinase K; PBS, phosphate-buffered saline; mAb, monoclonal antibody, dox, doxycycline; PIPLC, phosphatidylinositol-specific phospholipase C.

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stimulus, e.g., an injury or a signalling molecule, can modulate cell permissiveness to prion infection.

Materials and methods

Chemicals. ATP, UTP, and GTP were from Pharmacia, while ADP was from Roche. EGTA and apyrase with a low ATPase/ADPase ratio (grade VII) were from Sigma–Aldrich.

Cells. Rov cells [10] were maintained in α-minimal essential medium (α-MEM) containing 10% fetal calf serum, 100 U/ml penicillin, and 10 μg/ml streptomycin, and were split at a 1:4 dilution weekly. To induce the expression of the transfected ovine PrP protein, the cultures were treated with 1 μg/ml doxycycline (dox). MovS cells (the MovS6 clone) are mouse Schwann-like cells established from mice transgenic for the ovine PrP which constitutively express this protein [11]. These cells were cultured in a mixture of Dulbecco's modified Eagle's medium and F12 medium (3/4, 1/4, respectively) containing 10% foetal calf serum, 100 U/ml penicillin, and 10 μg/ml streptomycin, and were split at a 1:10 dilution weekly.

Experimental infection of wounded cell cultures by ovine prions. Confluent Rov or MovS cultures, grown in 12-well tissue culture plates, were wounded according to a geometrical pattern (30 horizontal and 30 vertical scratches) with a 19 G gauge needle. The protein content of wounded Rov cultures was assessed after immediate solubilization of wounded cultures in Triton/DOC lysis buffer. In some experiments, cultures were pretreated with 5 mM EGTA for 45 min in complete culture medium to disrupt cellular junctions. A few minutes after wounding, the cell culture medium was removed and replaced by fresh culture medium containing 0.25% of sonicated infectious brain homogenate (from tg338 transgenic mice terminally affected with sheep prions [12]). Two days later, the infectious medium was removed, the monolayers were rinsed once in phosphate-buffered saline (PBS), and the cells were left for 2 more days in regular culture medium before being passaged in one T-25 cm^2 flask. One week later, the cultures were rinsed with cold PBS, lysed at 4 °C in 1.5 ml Triton/DOC lysis buffer (50 mM Tris–HCl, pH 7.4; 0.5% Triton X-100, and 0.5% Na-Deoxycholate), clarified (2000 rpm, 1 min), and stored at −20 °C.

Detection of the normal and abnormal forms of PrP in cell lysates. To detect PrP or PrPres, proteins from cell lysates were methanol-precipitated or digested with proteinase K (PK), respectively, as described [10]. Aggregated PK-resistant PrP was collected by centrifugation at 13,000 rpm for 20 min at room temperature. Pellets from methanol precipitation or PK digestion were boiled in Laemmli buffer, subjected to SDS/PAGE electrophoresis, and transferred to nitrocellulose membranes. Blots were stained with anti-PrP ICSM18 mAbs [13] and revealed with an enhanced chemiluminescence detection system (ECL, Amersham Pharmacia). Signals were quantified with NIH image software.

Immunoprecipitation of cell surface PrP. Dox-induced Rov cells grown to confluence in 12-well tissue culture plates were scratch-wounded. Eighteen hours later, the cultures were placed on ice and anti-PrP monoclonal antibody 4F2 (ascites, 1/50) [14] was added for 1 h in regular culture medium. The monolayers were then extensively washed in PBS buffer, lysed with lysis buffer, and the cellular extracts were clarified (2000 rpm, 1 min). The antibody–PrP complexes were adsorbed with 30 μl protein A–Sepharose beads (Invitrogen), incubated on a wheel at 4 °C for 1 h, and washed three times with lysis buffer. The immunoprecipitated polypeptides were analysed by Western blot.

Nucleotides and apyrase treatments. Confluent Rov cells were wounded in the presence of apyrase (3 U/ml in complete culture medium) or were treated with either ATP or UTP (100 μM). Nucleotides- and wounded, apyrase-treated Rov cultures were then challenged with sheep prions by adding the infectious, sonicated, brain homogenate to the culture medium.

Wounding of persistently infected cultures. Chronically infected Rov cells were grown to confluence in 12-well tissue culture plates and were scratch-wounded as indicated before. Twenty-four hours later, each well was amplified in one T-25 cm^2 flask. One week later, the cultures were rinsed with cold PBS and lysed at 4 °C in 1.5 ml lysis buffer.

Dye transfer. Intercellular coupling was determined using the microinjection dye transfer method as described [15]. Rov cells were plated in 35-mm Petri dishes and microinjections were performed the day after seeding. Single cells were microinjected for 1 s with 5%(w/v) lucifer yellow CH (diluted in 0.33 M lithium chloride) using capillaries driven by a micromanipulator (IMT2-SYF, Tokyo, Japan) coupled to a pressure control unit (Eppendorf model 5242, Hamburg, Germany). All manipulations were performed under a microscope equipped with epifluorescence (Olympus, Rungis, France). The glass capillaries (Clark Electromedical Instruments) were prepared by an automatic horizontal puller (Narishige, Tokyo, Japan). Ten minutes after microinjection, cells were fixed with 4% formaldehyde in PBS and the numbers of dye-coupled cells were determined.

Results

Cell wounding inhibits prion transmission to exposed cells

Epithelial Rov cells expressing a stably transfected ovine PrP gene are one of the few cell systems permissive to sheep prion multiplication [10]. Upon exposure to infectious inoculum, Rov cells become infected and accumulate abnormal PrP at high levels. Effective infection of Rov cells can routinely be assessed by immunodetection of de novo produced abnormal PrP as early as one passage post-exposure. While studying the effect of various cellular stresses on cell permissiveness to prion infection, we noticed that wounding Rov cultures could result in significant impairment of prion transmission. To investigate further this intriguing observation, PrP-expressing Rov cells grown as confluent monolayers were scratch-wounded with a sterile needle according to a simple geometrical pattern. The total cell loss represented about 10% (see Materials and methods) so that a large majority of the cells remained physically unaffected by the mechanical injury. Shortly after wounding (i.e., within a few min), cultures were subjected to prion infection by exposure to fresh medium containing the infectious brain homogenate. The inoculum was removed, infected cells were passaged and grown for 1 week before measuring abnormal PrP content. PrPres levels were found to be consistently lower in wounded cultures, as shown by a representative experiment in Fig. 1A. Quantification showed that wounding resulted in a 3-fold inhibition of PrPres levels ($64 \pm 3\%$ inhibition, $n = 12$, see Fig. 4). As the levels of Rov-derived PrPres reflect the level of prion infection [16], these data indicated that wounding had a strong inhibitory effect on prion transmission to Rov cells. To determine whether or not this observation was a unique feature of rabbit epithelial Rov cells, we used mouse glial, Schwann-like MovS cells, another cell system permissive for sheep prions [11]. Wounding MovS cultures prior to exposure to