

Processing and Degradation of Exogenous Prion Protein by CD11c⁺ Myeloid Dendritic Cells In Vitro

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The immune system plays an important role in facilitating the spread of prion infections from the periphery to the central nervous system. CD11c⁺ myeloid dendritic cells (DC) could, due to their subepithelial location and their migratory capacity, be early targets for prion infection and contribute to the spread of infection. In order to analyze mechanisms by which these cells may affect prion propagation, we studied in vitro the effect of exposing such DC to scrapie-infected GT1-1 cells, which produce the scrapie prion protein PrP^{Sc}. In this system, the DC efficiently engulfed the infected GT1-1 cells. Unexpectedly, PrP^{Sc}, which is generally resistant to protease digestion, was processed and rapidly degraded. Based on this observation we speculate that CD11c⁺ DC may play a dual role in prion infections: on one hand they may facilitate neuroinvasion by transfer of the infectious agent as suggested from in vivo studies, but on the other hand they may protect against the infection by causing an efficient degradation of PrP^{Sc}. Thus, the migrating and highly proteolytic CD11c⁺ myeloid DC may affect the balance between propagation and clearance of PrP^{Sc} in the organism.

Prions are the cause of severe neurodegenerative diseases in a number of animals as well as in humans. These proteinaceous infectious particles are devoid of nucleic acids, and their only known components are modified isoforms, PrP^{Sc}, of a normal cellular glycoprotein denoted PrP^C (34). A biochemical hallmark of PrP^{Sc} is that it contains a protease-resistant core, denoted PrP27-30. In contrast to most other infectious agents, it is generally believed that prions are nonimmunogenic (33, 41). Nevertheless, the immune system plays an important and paradoxical role in the transmission of prion infections from the periphery to the central nervous system. Nonspecific immunosuppression tends to decrease (31), whereas nonspecific stimulation of the immune system tends to increase the spread (7) of peripherally inoculated prions. For instance, immunodeficient SCID mice are relatively resistant to peripheral inoculation of scrapie (3, 10, 16, 19, 30), but susceptibility can be restored by reconstitution with bone marrow cells from wild type mice (10).

In the immune system, migrating CD11c⁺ dendritic cells (DC) of myeloid origin are important in the uptake and transport of antigens from subepithelial sites to lymphatic tissues (20, 42). Recently, this type of DC has been implicated in the spread of PrP^{Sc} from the gut to lymphoid tissue (12), and, further, from lymphoid tissues to the central nervous system (1). The CD11c⁺ DC are distinct from the nonmigrating follicular DC (FDC), which reside in the center of lymphoid organs. FDC have previously been suggested to facilitate the spread of prion infections to the nervous system by serving as a source for their replication (3, 24, 27). Thus, PrP^C, a prereq-

uisite for prion replication, and/or PrP^{Sc} have been detected in FDC-containing regions of both human and murine lymphoid tissues (3, 16, 17, 27). Tumor necrosis factor- α -deficient mice, which fail to develop mature FDC, as well as mice treated with the soluble lymphotoxin- β receptor-immunoglobulin fusion protein, which eliminates functional FDC, show prolonged incubation time after peripheral administration of scrapie (21, 27). The mechanisms by which DC of the various phenotypes are involved in propagation of prions to the nervous system remain, however, to be clarified.

The present study was undertaken with the aim to determine how CD11c⁺ DC can affect PrP^{Sc} when encountering a scrapie-infected cell. In an effort to mimic the complex cell-cell interactions that take place in vivo, we used PrP^{Sc}-producing cells rather than purified PrP^{Sc} rods (35). For this purpose, we exposed primary cultures of mouse CD11c⁺ DC to scrapie-infected neuronal GT1-1 cells (ScGT1-1 cells). These cells produce abundant levels of both PrP^C and PrP^{Sc} (37). We here report that ScGT1-1 cells underwent cell death and were phagocytosed by DC under coculture conditions. This was, surprisingly, accompanied by a gradual appearance of the three glycoforms of PrP27-30 and later by the total disappearance of PrP^{Sc} from the culture. Given the general resistance of PrP^{Sc} to degradation by a variety of physical and/or chemical means, we find this process striking.

MATERIALS AND METHODS

GT1-1 cell culture and scrapie infection. GT1-1-cells, a subtype of murine immortalized gonadotropin-releasing hormone neurons (26), were provided as a generous gift from Pamela Mellon (University of California, San Francisco). The cells were grown in 75-cm² cell culture flasks (Costar, Corning, N.Y.) with Dulbecco's modified Eagle's medium (4.5 g of glucose/liter) containing GLUTAMAX I supplemented with 5% heat-inactivated fetal bovine serum (FBS), 5% heat-inactivated horse serum, and penicillin-streptomycin (50 U/ml) (all from GibcoBRL, Paisley, United Kingdom). The cells were split once a week

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using $1\times$ trypsin-EDTA (GibcoBRL). Infection of the GT1-1 cells with mouse-adapted scrapie was performed in 24-well cell culture clusters (Costar). The GT1-1 cells were incubated with 0.1% homogenate of mouse brains infected with the Rocky Mountain Laboratory strain of scrapie (the homogenates were obtained as a generous gift from Stanley B. Prusiner, University of California, San Francisco) at 30°C in CO_2 -independent medium (GibcoBRL) supplemented with 5% FBS, 5% heat-inactivated horse serum, penicillin-streptomycin (50 U/ml), and 2 mM L-glutamine (GibcoBRL). After 4 days, the medium was changed, and the ScGT1-1 cells were transferred to an incubator with 5% CO_2 at 37°C . Western blotting confirmed the presence of protease-resistant PrP^{Sc} after 14 passages.

Differentiation and isolation of CD11c⁺ DC. Murine bone marrow-derived DC were obtained from femur and tibia bone marrow of C57BL/6 mice (obtained from B&K Universal AB, Sollentuna, Sweden, or the Microbiology and Tumor Biology Center, Karolinska Institutet) essentially as described by Inaba et al. (13). Briefly, after removal of small pieces of bone and debris, the cells were pelleted and resuspended in Dulbecco's modified Eagle's medium (4.5 g/liter of glucose) with GLUTAMAX I, 15% FBS, and penicillin-streptomycin (50 U/ml) to which recombinant murine granulocyte-macrophage colony-stimulating factor (granulocyte-macrophage CSF) (10 ng/ml) and murine interleukin-4 (10 ng/ml; PeproTech, Rocky Hill, N.Y.) were added. The cells were grown in six-well cell culture clusters (Costar) in 5% CO_2 at 37°C and replated after 7 days. For activation, lipopolysaccharide (LPS) (10 $\mu\text{g}/\text{ml}$; Sigma-Aldrich Chemie, Steinheim, Germany) was added to the cells overnight. In contrast to murine bone marrow macrophages, DC differentiated from bone marrow express CD11c. Thus, to obtain pure DC, the cells from the bone marrow cultures were affinity purified using MACS CD11c⁺ magnetic MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purity of the DC was assessed, and presence of contaminating macrophages was excluded, using monoclonal antibodies recognizing CD11c (BD Pharmingen, San Diego, Calif.), major histocompatibility complex (MHC) class II, and the macrophage marker F4/80 (Serotec Ltd., Oxford, United Kingdom) for flow cytometric analysis.

Cocultivation of DC with GT1-1 cells. Before exposure to DC, GT1-1 cells or ScGT1-1 cells were grown overnight in 10-mm-diameter tissue culture dishes (Corning) to a concentration of about 10^6 cells/dish, and the CD11c⁺-sorted murine bone marrow DC were added at a concentration of either 1×10^6 or 2×10^6 cells/dish, giving an approximate DC/GT1-1 cell ratio of 1:1 or 2:1. The cells were incubated for 24 to 96 h in AIM-V medium (GibcoBRL) supplemented with granulocyte-macrophage CSF (10 ng/ml; PeproTech). In one set of experiments, an anti-Fas ligand antibody (MFL3; BD Pharmingen) was added to the medium at a concentration of 10 $\mu\text{g}/\text{ml}$. In another set of experiments the DC were cultured with the GT1-1 or ScGT1-1 cells without cell-cell contact in a Transwell system (pore size, 0.4 μm ; Costar)

Immunofluorescence. Cells grown on 10-mm-diameter tissue culture dishes (Costar) were fixed in 10% formalin (Merck KGaA, Darmstadt, Germany) for 30 min, permeabilized with 0.1% Triton X-100 (Sigma, St Louis, Mo.) in phosphate-buffered saline (PBS) for 5 min, blocked with 5% bovine serum albumin (Sigma) in PBS for 40 min, and then incubated with the antibodies. The following primary monoclonal antibodies were used: recombinant Fab D13 (40) (provided as a generous gift from Stanley B. Prusiner), 1.4 $\mu\text{g}/\text{ml}$; anti-neuron-specific class III β -tubulin (TUJ1; BABC0, Richmond, Calif.), 2 $\mu\text{g}/\text{ml}$; and anti-MHC class II purified from supernatant of the hybridoma M5/114.15.2 (also available from BD Pharmingen), 10 $\mu\text{g}/\text{ml}$. All antibodies were diluted in PBS containing 5% bovine serum albumin. The cells were incubated with the primary antibodies overnight at 4°C followed by incubation with secondary antibodies for 40 min at room temperature. The secondary antibodies used were Alexa Fluor 488-conjugated goat anti-human immunoglobulin G (IgG) (Molecular Probes, Leiden, The Netherlands), 4 $\mu\text{g}/\text{ml}$, for detection of Fab D13; Cy2-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, Pa.), 6.5 $\mu\text{g}/\text{ml}$, for detection of TUJ1; and Texas Red-conjugated donkey anti-rat IgG (Jackson ImmunoResearch), 30 $\mu\text{g}/\text{ml}$, for detection of M5/114.15.2. The cells were rinsed in PBS with 1% NH_4Cl (Sigma) and mounted in antifading solution (5% *n*-propyl gallate, 100 mM Tris-HCl [pH 9], 70% glycerol).

Western immunoblotting. After removal of the medium, the cells in the cultures were lysed in 100 μl of lysis buffer (10 mM Tris-HCl [pH 8], 150 mM NaCl, 0.5% sodium deoxycholate, 0.5% Triton X-100) on ice. In samples that later were not treated with proteinase K (PK) (see below) the following protease inhibitors were added to the lysis buffer: pepstatin A, leupeptin, and aprotinin (1 $\mu\text{g}/\text{ml}$ each; Sigma) and phenylmethylsulfonyl fluoride (0.2 mM; Sigma). Insoluble debris was removed by centrifugation at $16,000\times g$, and the protein content was determined by using the Bio-Rad Bradford protein assay (Bio-Rad) and spectroscopy (Ultrospec plus; Pharmacia LKB, Cambridge, United Kingdom) at 595

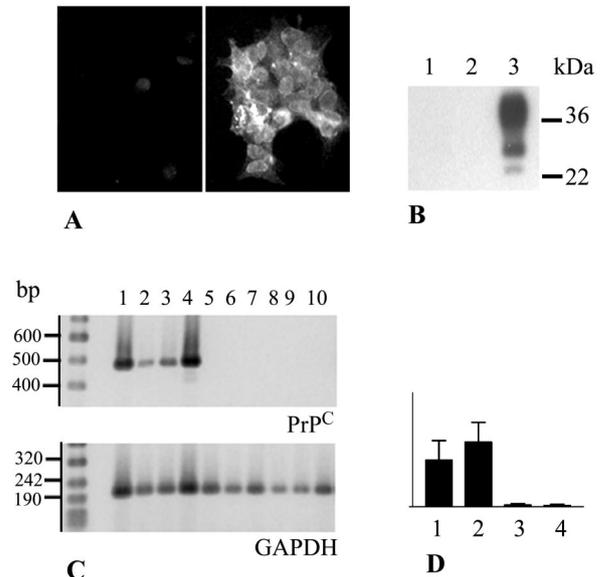


FIG. 1. PrP^C and PrP^C-mRNA in GT1-1 cells and CD11c⁺ DC. (A) PrP^C immunofluorescence in LPS-activated DC (left) (negative) and control GT1-1 cells (right) (positive) using Fab D13. (B) Western blot, using Fab D13, of lysates from DC (lane 1), DC activated with LPS (lane 2), and GT1-1 cells (lane 3). (C) RT-PCR showing transcription of mRNA encoding PrP^C (upper gel) and GAPDH (lower gel) in GT1-1 cells (lanes 1 and 2), and ScGT1-1 cells (lanes 3 and 4), DC (lanes 5, 6 and 7), and DC activated with LPS (lanes 8, 9, and 10). (D) PrP^C mRNA standardized to GAPDH. Bars 1 to 4 show results for GT1-1, ScGT1-1, DC, and DC activated with LPS, respectively.

nm according to the manufacturer's instructions. The lysate was then split into two parts. One part was treated with PK (10 $\mu\text{g}/\text{ml}$; Boehringer Mannheim, Mannheim, Germany) at 37°C for 30 min and the reaction was stopped by incubation with 3 mM phenylmethylsulfonyl fluoride (Sigma). The remaining lysate was not PK-treated. The samples were boiled in sodium dodecyl sulfate (SDS) sample buffer and investigated by Western blotting. The media sampled from the cultures were spun at $1,500\times g$ for 10 min, and the supernatants were removed. Fifty microliters of lysis buffer was added to the pellet in each centrifuge tube, after which the lysates were boiled in SDS sample buffer. In one set of experiment these lysates were analyzed separately, but in all other experiments they were added to the lysates from the cells in the culture. The removed supernatants were precipitated in 80% ice-cold acetone, centrifuged at 4°C at $3,500\times g$ for 30 min, dissolved in SDS sample buffer, and boiled.

The samples were loaded on 12% Tris-glycine precast gels and electrophoresed at 125 V, according to the method of Laemmli (18) (Fig. 1B; see also Fig. 4) or NuPAGE 12% Bis-Tris gels with MOPS (morpholinepropanesulfonic acid) SDS running buffer at 200 V according to the manufacturer's instructions (Invitrogen, Paisley, United Kingdom) (see Fig. 3B through E). Proteins were transferred to Immobilon-P^{SO} transfer membranes (Millipore, Bedford, Mass.) at 35 V for 3 h, blocked in 5% nonfat dry milk (Bio-Rad), and incubated with recombinant Fabs D13, D18, or R1 (all obtained from Stanley B. Prusiner), 1 $\mu\text{g}/\text{ml}$ each, followed by the secondary goat anti-human F(ab)₂ peroxidase-conjugated antibody (Pierce, Rockford, Ill.), 0.16 $\mu\text{g}/\text{ml}$. Detection was performed with enhanced chemiluminescence (ECLplus; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom). Optical densities of the bands were determined using a Gel Doc 2000 with the software Quantity One (version 4.2.2; Bio-Rad). The membranes were incubated in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl [pH 6.7]) for 30 min at 50°C , rinsed, and blocked with 5% nonfat dry milk before exposure to the antibody TUJ1, 0.5 $\mu\text{g}/\text{ml}$. As secondary antibody, a peroxidase-conjugated goat anti-mouse immunoglobulin (0.2 $\mu\text{g}/\text{ml}$; DAKO, Glostrup, Denmark) was used.

RNA analysis of PrP^C. Total RNA was extracted in duplicates or triplicates from the cells using an RNeasy Mini kit (Qiagen GmbH, Hilden, Germany). RNA content was determined by UV spectroscopy (Ultrospec plus). RNA (1 μg) was treated with 1.0 U of DNase I, amplification grade (DNase; GibcoBRL)

according to the manufacturer's instructions. Total DNase-treated RNA was reverse transcribed to cDNA by random priming using Superscript II (Gibco-BRL) in a 20- μ l reaction mixture according to the manufacturer's instructions.

Specific amplification of PrP^C and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts was subsequently performed according to the following protocol. Each PCR mixture contained 1 μ l of template; 100 ng of each primer; 1 \times Titanium *Taq* DNA polymerase (Clontech Laboratories Inc, Palo Alto, Calif.); 1 \times Titanium *Taq* PCR buffer (Clontech); and a 10 mM concentration (each) of dGTP, dATP, dTTP, and dCTP (GibcoBRL). With the use of a GeneAmp PCR system 9700 (P-E Biosystems, Foster City, Calif.) the PrP^C-PCR was run for 40 cycles: denaturation at 94°C for 30 s and annealing and extension at 70°C for 1 30 mins. The GAPDH-PCR was run for 20 or 25 cycles: denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s. The primer sets used for these assays were as follows: PrP (forward), 5'-AATCAGTGGAAACAAGCCCAAG-3'; PrP (reverse), 5'-ATCCCACGATCAGGAAGATG-3'; GAPDH (forward), 5'-CCATGGAGAAGGCCGGGG-3'; GAPDH (reverse), 5'-CAAAGTTGTCATGGATGACC-3' (GibcoBRL). The primers for PrP^C and GAPDH were designed to generate fragments of 478 and 195 bp, respectively; GAPDH primer sequences were taken from the work of Der et al. (6). The PCR products were electrophoresed in 2% agarose (Sigma) gels in 1 \times TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA [pH 8.3]) (Bio-Rad, Hercules, Calif.) followed by staining with SYBR gold nucleic acid gel stain (Molecular Probes) and were visualized with a UV transilluminator (Gel Doc 2000; Bio-Rad).

RESULTS

Expression of PrP^C in DC versus GT1-1 cells. In order to study the effect of DC on PrP molecules derived from GT1-1 cells, it was important to determine whether the former cells express any endogenous PrP^C. To analyze the presence of PrP^C in DC, we first used immunofluorescence microscopy with the recombinant PrP Fab D13 (Fig. 1A). The present CD11c⁺ antibody affinity-purified DC, which displayed all morphological and phenotypic characteristics of DC, contained no PrP^C either before (Fig. 1B) or after (Fig. 1A and B) 12 h of activation with LPS. In contrast, control GT1-1 cells showed marked PrP^C immunolabeling at the cell surface (Fig. 1A). This result was confirmed by Western blots developed with the Fab D13. Lysates from the GT1-1 cells contained, as expected (37), large amounts of PrP^C, while no PrP^C could be detected in lysates from either immature or LPS-activated DC (Fig. 1B). To further investigate the level at which PrP expression is repressed in DC, we studied PrP mRNA levels using reverse transcription (RT)-PCR. An amplified product of 478 bp was evident in the GT1-1 cells after RT-PCR, but was not seen in the DC (Fig. 1C and D). Thus, neither PrP^C nor PrP mRNA is expressed in mouse CD11c⁺ DC in vitro, even after activation with LPS. This result facilitates analyses of the fate of GT1-1 cell-derived PrP^C and PrP^{Sc} delivered to the DC.

Processing of PrP^C and PrP^{Sc} by DC in culture. We next seeded immature CD11c⁺ DC onto ScGT1-1 cells growing in 10-mm-diameter dishes. Within 24 to 48 h, the DC adhered to and caused shrinkage of the ScGT1-1 cells as observed by phase-contrast microscopy and immunofluorescence (Fig. 2). To differentiate between the two cell types in the coculture, we used two cell-specific markers: MHC class II, which is expressed in DC, and neuron specific β -tubulin (TUJ1) that is expressed in GT1-1 cells. MHC class II immunopositive DC surrounded clusters of small shrunken β -tubulin immunopositive ScGT1-1 cells (Fig. 2B). The degenerated cells were subsequently engulfed by the DC in a manner similar to what has been described for the capture of various killed tumor cells by DC (28). DC were the only cell type to remain after 4 days of

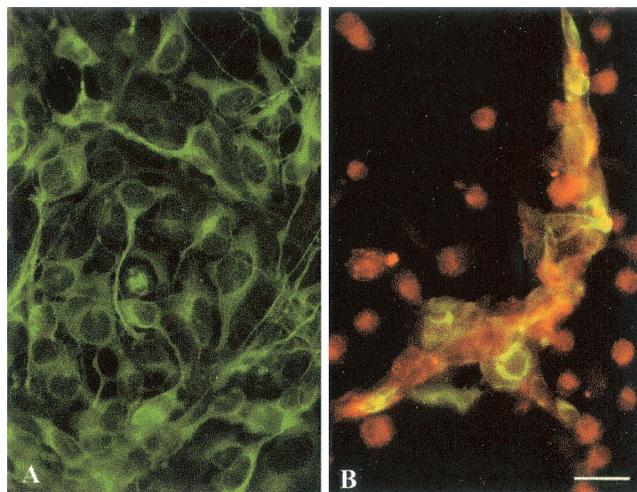


FIG. 2. Coculture of ScGT1-1 cells and DC. (A) ScGT1-1 cells, immunolabeled with anti- β -tubulin antibodies (green); (B) ScGT1-1 cells (green to yellowish) exposed to DC, ratio 1:1, immunolabeled with anti-MHC class II antibodies (red), for 48 h. Note the extensive loss of GT1-1 cells. Remnants are surrounded by clusters of DC. Scale bar, 25 μ m.

cocultivation in our system. Since the ScGT1-1 cells contain both isoforms of PrP, the present system enabled studies of potential interactions between the PrP isoforms during degradation of apoptotic ScGT1-1 cells in the PrP^C-negative DC. Surprisingly, Western blot analysis of the cell lysates revealed a progressive decrease and subsequent loss of the PK-resistant PrP27-30 bands, which correspond to N-terminally trimmed products of the full-length PrP^{Sc} (39) (Fig. 3A and B). With an increased DC/ScGT1-1 ratio the rate of disappearance of PK-resistant bands was increased (Fig. 3B). The disappearance of the PK-resistant bands was not accompanied by an increase in PrP^{Sc} in either the pellets or acetone-precipitated fractions (Fig. 3D) of cell medium removed after cocultivation, which indicates that the disappearance of PrP^{Sc} from the cells was not caused by its excretion to the extracellular medium. As expected, no PK-resistant bands were found in lysates from cocultures of DC and uninfected GT1-1 cells, which were also engulfed by the DC and displayed a progressive loss of PrP^C (Fig. 3C). An anti-Fas ligand antibody added to the medium during the cocultivation had no effect on the progressive decrease of the PK-resistant PrP27-30 bands or cell death (data not shown) indicating that the DC-induced killing of the ScGT1-1 cells was mediated by other mechanisms than the Fas/FasL pathway. However, experiments using a Transwell system showed that the effects was not due to factors released from the DC into the medium, since no effects on either the expression of PrP^C or the occurrence of PrP^{Sc} was seen in this system (Fig. 3E).

Taken together, these results suggest that DC degraded the PrP^{Sc} derived from ScGT1-1 cells. To analyze this degradation in further detail, we also examined non-PK-treated lysates of ScGT1-1 cells cocultured with DC (Fig. 4). Western blot analysis indicated an increase in a set of small PrP bands, identified as the three PrP27-30 species (arrows in Fig. 4), during the first days after the cells were mixed. Indeed, these low-molecular-weight PrP bands were electrophoretically indistinguishable

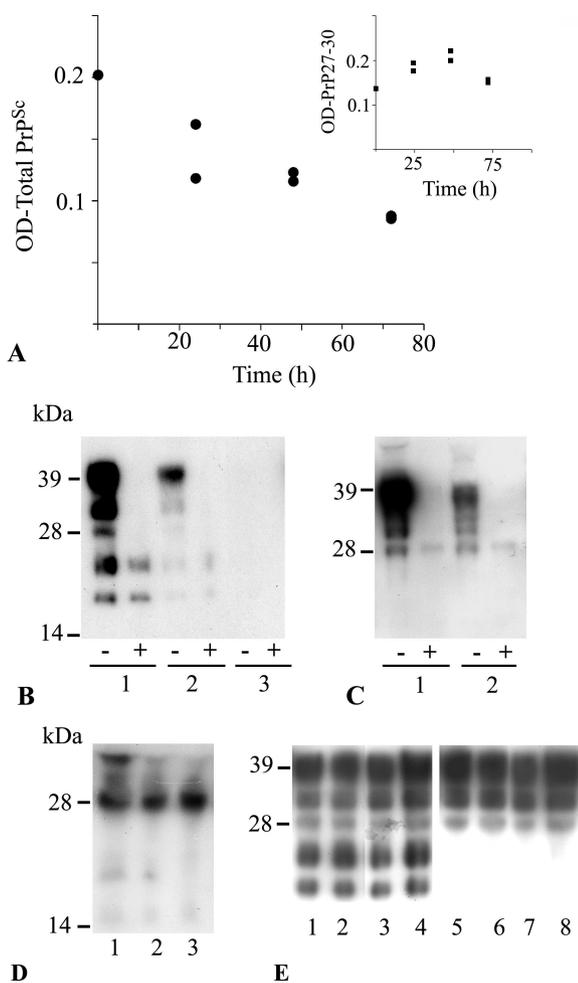


FIG. 3. Effect of DC on PrP^{Sc} derived from ScGT1-1 cells. (A) Intensity of protease-resistant prion protein in PK-treated (●) lysates at different times after exposure of ScGT1-1 cells to DC, ratio 1:1. The inset shows the intensity of the low-molecular-mass bands (sizes between 19 and 27 kDa) in non-PK-treated lysates, standardized to the intensity of β -tubulin, sampled at different time points after exposure of ScGT1-1 cells to DC; ratio 1:1. (B) Western blot, using Fab D13, of cell culture lysates from ScGT1-1 cells (lane 1); ScGT1-1 cells exposed to DC, ratio 1:1, for 72 h (lane 2); and ScGT1-1 cells exposed to DC, ratio 1:2, for 72 h (lane 3). (C) GT1-1 cells (lane 1) and GT1-1 cells exposed to DC, ratio 1:1, for 72 h (lane 2). Symbols: -, non-PK-treated lysates; +, PK-treated lysates. (D) precipitated proteins from the medium collected from cultures of ScGT1-1 cells (lane 1); ScGT1-1 cells exposed to DC, ratio 1:1, for 72 h (lane 2); or ScGT1-1 cells exposed to DC, ratio 1:2, for 72 h (lane 3). (E) Transwell experiment showing no difference in PrP expression between ScGT1-1 cells unexposed (lanes 1 and 2) and exposed (lanes 3 and 4) to DC, ratio 1:1, for 48 h. Exposure to DC did not affect PrP^C expression in uninfected GT1-1 cells (lanes 5 and 6 show unexposed GT1-1 cells, and lanes 7 and 8 show GT1-1 cells exposed to DC).

from the three protease-resistant species that appear after proteolysis of PrP^{Sc} in the mouse system (compare lanes 1 in Fig. 3B). Exposing lysates to PK could mask this process by digesting the total amount of the full-length PrP^{Sc} into PrP²⁷⁻³⁰. In lysates from cocultures of ScGT1-1 cells and DC, low molecular weight PrP bands could be clearly visualized (the three lower bands in Fig. 3B), and their final loss was preceded

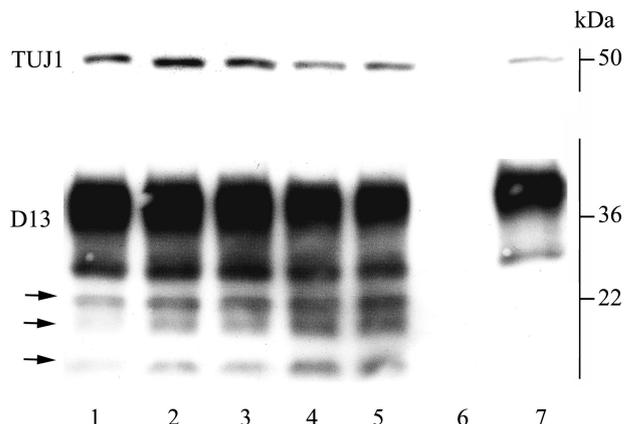


FIG. 4. Western blot of lysates from ScGT1-1 cells exposed to various amounts of DC for 48 h. Each lane represents the amount of proteins in an individual cell culture dish. Shown are ScGT1-1 cells (lane 1); ScGT1-1 cells exposed to DC, ratio 2:1 (lanes 2 and 3); ScGT1-1 cells exposed to DC, ratio 1:1 (lanes 4 and 5); control DC (lane 6); and control uninfected GT1-1 cells (lane 7). ScGT1-1 cells and GT1-1 cells were grown at 10^6 cells/dish (lanes 1 to 5 and 7), and 0.5×10^6 (lane 2 and 3) or 1×10^6 (lanes 4, 5, and 6) DC were added. The blot was first developed with D13 and then stripped and rehybridized to the neuron-specific β -tubulin antibody TUJ1 (upper line). The three glycoforms of PrP²⁷⁻³⁰ (arrows) increase, while β -tubulin decreases, with increasing amounts of DC.

by an increase in their amount (Fig. 3A [inset] and 4). These PrP bands appeared in lysates containing protease inhibitors to the same extent as in lysates without such inhibitors (data not shown). The proportions in intensity between the three bands did not undergo any changes. Since PrP²⁷⁻³⁰ products increased in parallel with a decrease in the marker for the GT1-1 cells, neuron-specific β -tubulin (Fig. 4), these data indicate an ongoing N-terminal trimming of the full-length PrP^{Sc} together with the ScGT1-1 cells induced by the DC. Exposure of the ScGT1-1 cells to LPS-activated DC, which have a lower phagocytic but a higher proteolytic capacity (14), had similar effects. As expected, lysates from cocultures of DC and control uninfected GT1-1 cells contained no PrP²⁷⁻³⁰ bands (Fig. 3C). The loss of PrP^C and PrP^{Sc} observed using the PrP Fab D13 (raised against PrP amino acids [aa] 96 to 106) was also found using Fabs D18 (raised against PrP aa 133 to 157) and R1 (raised against PrP aa 225 to 231) (data not shown). Interestingly, the fact that no PrP^C was detected in the lysates from the DC sampled 4 days after their coculture with ScGT1-1 cells shows that, similar to LPS, scrapie-infected cells do not induce PrP^C in the present DC.

Taken together, the results show that mouse bone marrow-derived CD11c⁺ DC cause degradation of PrP^{Sc} in vitro. Since neuron specific β -tubulin was degraded concomitantly with an increase in the partially degraded PrP^{Sc}, it is suggested that the degradation occurs subsequent to killing and digestion of the ScGT1-1 cells by the DC.

DISCUSSION

The possibility that certain cells may degrade PrP^{Sc} has recently come into focus. For instance, prion-infected N2a cells can be cleared from PrP^{Sc} and prion infectivity by treatment

with PrP^C antibodies, and this clearance was suggested to be the result of an intracellular degradation of PrP^{Sc} in combination with hindered formation of new PrP^{Sc} (9, 32). Studies have also suggested that degradation of scrapie can be caused by macrophages (2, 5). Furthermore, vaccination with self-PrP peptides can reduce the amount of PrP^{Sc} in subcutaneous neuroblastoma produced by the transplantation of ScN2a cells in syngeneic A/J mice (38). Our study directly shows that post-mitotic cells with strong phagocytic and proteolytic capacities, such as DC, can efficiently degrade PrP^{Sc}. Taken together, our results suggest that exogenous PrP^{Sc} is digested in two steps. During the first days of coculture, full-length PrP^{Sc} is trimmed to PrP27-30 (Fig. 4). In analogy to other cultured cells, this trimming may occur in acidic organelles such as endosomes. In a second step, these PrP27-30 species are rendered progressively more sensitive to proteolysis, as shown by their complete degradation by proteinase K (Fig. 3B). The molecular mechanisms responsible for such a change remain to be determined, but we surmise that they may include the disaggregation of PrP^{Sc}. Existence of protease-sensitive PrP^{Sc} species has been previously demonstrated by Safar et al. (36). Thus, a balance could be envisioned between the formation and degradation of PrP^{Sc} at early time points after infection in a host, and DC may play a crucial role in this balance to regulate the outcome of the infection.

Although it has been reported that neither FDC nor CD11c⁺ DC may be absolutely required for neuroinvasion after inoculation of high doses of scrapie (29), and we now find that the latter cells can efficiently degrade PrP^{Sc}, CD11c⁺ DC may still have the capacity to spread prion infectivity in an organism. The time scale for degradation of PrP^{Sc}, observed in the present study, would allow PrP^{Sc}-containing DC to migrate from the periphery, where they may encounter the scrapie agent, to lymphoid organs. PrP^{Sc} has been found in lymphatic CD11c⁺ DC after intestinal scrapie inoculation in rats (12), and CD11c⁺ DC isolated from the spleen of scrapie-infected mice are able to transmit prions to the central nervous system (1).

Several possible mechanisms for involvement of CD11c⁺ DC in spread of scrapie to the nervous system exist. (i) Transport of scrapie to the spleen and lymph nodes may occur, thereby exposing nerve endings and/or FDC to the infectious agent (11). (ii) Processing of scrapie to smaller, more infectious particles may occur. We are now starting bioassays in mice to establish the rate at which prion infectivity is cleared from the cocultures and to see if infectivity increases transiently while PrP27-30 becomes progressively sensitive to proteases. Given the very small infectious titers found in cultured cells (4), these bioassays will require very long periods of time. CD11c⁺ DC could also assist the spread of prions by (iii) rapid induction of PrP conformational changes in the acidic endosomes in DC or (iv) release of so-called exosomes containing infectious material to surrounding cells. Considering the close association between axon terminals and DC described in the skin and liver (8, 22), and the finding of DC situated at an interface between the brain and the blood circulation in the choroid plexus and leptomeninges (23, 25), CD11c⁺ DC may also be in a position to present prion infectivity directly to the nervous system. Thus, it is speculated that CD11c⁺ DC may on the one hand facilitate neuroinvasion, as

suggested by the previous *in vivo* studies, but on the other hand be involved in an efficient degradation of PrP^{Sc}, as shown by our *in vitro* study. Such a dual function of phagocytic cells may also occur in the processing of other pathogens. For instance, CSF-1 dependent cells, which include populations of macrophages and DC, can protect against systemic infection with *L. monocytogenes* but facilitate their neuroinvasion (15).

In conclusion, the present study shows that *in vitro* the murine bone marrow-derived CD11c⁺ DC express neither PrP^C nor PrP^C mRNA either before or after exposure to LPS or PrP^{Sc}. On the other hand, we obtained direct evidence that these DC can cause a progressive and efficient degradation of PrP^{Sc} derived from scrapie-infected GT1-1 cells. Since CD11c⁺ DC may be exposed to prions early during an infection, the observed processing of PrP^{Sc} may play an important role in the balance between clearance and propagation of the prions in a host.

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