Virology 423 (2012) 205–213

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Reaction of complement factors varies with prion strains in vitro and in vivo

Rie Hasebe [a,b,*], Gregory J. Raymond [a], Motohiro Horiuchi [b], Byron Caughey [a]

[a] Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institute of Health, 903 South 4th street, Hamilton, MT 59840, USA

[b] Laboratory of Veterinary Hygiene, Graduate School of Veterinary Medicine, Hokkaido University, Nishi 9, Kita 18, Kita-ku, Sapporo 060-0818, Japan

ARTICLE INFO

Article history:
Received 29 August 2011
Returned to author for revision 21 September 2011
Accepted 21 November 2011
Available online 3 January 2012

Keywords:
Prion
Scrapie
Complement factors
Strain difference

ABSTRACT

Roles of complement factors in prion infection of the central nervous system remain unclear. In this study, we assessed the strain-dependent reactivity of complement factors in prion infections of Neuro2a (N2a) cells and mouse brains. N2a cells persistently infected with either Chandler or 22L scrapie strains were cultured in the presence of normal mouse serum (NMS), followed by staining with phosphatidylserine binding protein and early apoptosis marker Annexin V. The proportion of Annexin V positive cells was increased both in Chandler- and 22L-infected cells. Preincubation of NMS with anti-C1q, C3 and/or C9 antibodies reduced Annexin V positive cells in Chandler-infected cells, while only anti-C3 antibodies were effective on 22L-infected cells. The immunohistochemistry showed that deposition of C1q and C3 was different between Chandler- and 22L-infected mouse brains. These results indicate that the reactivity of complement factors differs between prion strains both in vitro and in vivo.

Introduction

The complement system plays key roles in the immune system including regulation of immune reactions and the elimination of phagocytosed antigens, immune complexes, tumor cells and apoptotic cells. Complement factors also have multiple functions for synapse remodeling (Stevens et al., 2007), neurogenesis (Shinjyo et al., 2009), cell survival (Dashiell et al., 2000; Soane et al., 1999, 2001) and cell death (Ren et al., 2008). Complement factors also seem to be involved in pathogenesis of neurodegenerative disease such as Alzheimer's disease (AD). Previous studies showed that β-amyloid, the major constituent of senile plaques, binds C1q and induces complement activation, which may promote either neuroprotection or neurotoxicity (Guan et al., 1994; Sarvari et al., 2003; Webster et al., 1997).

Prion diseases are fatal neurodegenerative disorders including scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, chronic wasting disease in cervids and Creutzfeldt–Jakob disease in humans. These diseases are characterized in the central nervous system (CNS) by deposition of abnormal forms of prion protein (e.g. PrP^{Sc}), vacuolation of neural tissue, astrocytosis and microglial activation. Previous studies using C1q, factor B/C2 or C3 depleted mice (Klein et al., 2001; Mabbott et al., 2001) have implicated the involvement of these complement factors in the spread of prions from peripheral tissues to CNS. Klein et al. (2001) and Zabel et al. (2007) showed that complement receptor CD21/35 on follicular dendritic cells has an important role in lymphoid prion accumulation and neuroinvasion of prion. Flores-Langarica et al. (2009) demonstrated that C1q is involved in PrP^{Sc} uptake into conventional dendritic cells, which have an important role in the prion propagation from the peripheral tissue to the CNS. Direct binding of C1q to amyloid fibrils, beta-oligomers prepared from human or mouse recombinant PrP and purified PrP^{Sc}, resulting in activation of the classical complement pathway, has been demonstrated in vitro, suggesting that prion infection induces complement activation (Blanquet-Grossard et al., 2005; Dumestre-Perard et al., 2007; Erlich et al., 2010; Mitchell et al., 2007; Sim et al., 2007; Sjoberg et al., 2008).

Klein et al. (2001) and Mabbott et al. (2001) suggested that complement factors seem to be less important in the CNS than the periphery, because depletion of either C1q, factor B/C2 or C3 did not affect the survival period of mice intracerebrally infected with Chandler and ME7 scrapie. Mabbott and Bruce (2004) showed that the incubation periods of C5 deficient mice infected with ME7 and 79A scrapie via intracerebral or peripheral route were similar to those of wild type mice. However, there still remains the possibility that complement factors are involved in neuropathogenesis of prion diseases. Association of complement factors with amyloid plaques of human prion disease was demonstrated by immunohistochemistry (Ishii et al., 1984; Kovacs et al., 2004). mRNA levels of C1q and C3 increase in the brains of mice intracerebrally infected with Chandler, 22L or ME7 strains in the pre-clinical phase of the disease, indicating that expression of complement factors is altered in the early stage of the neuropathogenesis in some prion strains (Dandoy-Dron et al., 1998; Hwang et al., 2009; Skinner et al., 2006).

* Corresponding author at: Laboratory of Veterinary Hygiene, Graduate School of Veterinary Medicine, Hokkaido University, Nishi 9, Kita 18, Kita-ku, Sapporo 060-0818, Japan. Fax: +81 11 706 5293.
E-mail address: r-hasebe@vetmed.hokudai.ac.jp (R. Hasebe).

0042-6822/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2011.11.017

In this study, we have further assessed the possible involvement of complement factors in the neuropathogenesis of prion disease using murine neuroblastoma (N2a) cells and mice infected with Chandler and 22L scrapie strains. Our data suggest that complement factors induce translocation of phosphatidylserine in the plasma membrane of prion-infected N2a cells and that the reaction of complement components varies with prion strain.

Results

Normal mouse serum treatment induces degenerative change in scrapie-infected N2a cells

To assess the possibility that complement factors react on scrapie-infected cells, we used N2a cells persistently infected with Chandler or 22L strains. For uninfected negative controls, we cured the scrapie infection in these cell lines using pentosan polysulfate (PPS). The cells were treated with normal mouse serum (NMS), heat-inactivated NMS (H-NMS) or fetal bovine serum (FBS) for 6, 12, 24 and 48 h (Fig. 1). NMS contains almost all murine complement components, whereas these factors are inactivated in H-NMS and absent in FBS. After these treatments, the cells were stained with Annexin V, a protein that labels phosphatidylserine in the outer leaflet of the plasma membrane as a marker of an early stage of apoptosis (Koopman et al., 1994). Time dependent increases in Annexin V-positive cells were observed only in the cultures treated with NMS, culminating in much higher percentages of positive cells in the Chandler- and 22L-infected N2a cultures (60–64%) than in the PPS-cured cultures (14–18%). These results indicated that NMS treatment induced degenerative change in Chandler- and 22L-infected N2a cells to an extent that was enhanced ~4-fold by scrapie infection. Less than 10% of the cells treated with H-NMS and FBS were Annexin V positive, indicating that the NMS factors mediating the increase in Annexin V positivity were heat sensitive, as is known to be true for complement factors. Moreover, a similarly low percentage of FBS-treated cells was Annexin V-positive, indicating that bovine serum factors cannot be substituted for murine factors in mediating the observed degenerative changes. To assess if cell death occurs in these cells or not, the cells were incubated with propidium iodide (PI) at 24 h after NMS treatment (Fig. 1D). Although PI uptake was slightly increased in the Chandler- and 22L-infected N2a cells compared to PPS-cured cells, less than 10% of the cells were positive for PI, suggesting that translocation of phosphatidylserine hardly resulted in cell death.

Different complement factors are involved in NMS-induced degenerative changes in Chandler- and 22L-infected N2a cells

To further examine the role of murine complement factors in the NMS-induced degenerative changes in Chandler- and 22L-infected N2a cells, the NMS was pre-treated with anti-C1q, C3, C9 antibodies or mixture of these antibodies. Because all of the antibodies were derived from goats, we used normal goat serum (NGS) and anti-mouse IgG goat serum as controls. In Chandler-infected N2a (Fig. 2A), the pretreatment with individual anti-C1q, C3 or C9 antibodies reduced the proportion of Annexin V positive cells ($p<0.01$ vs pretreatment with NGS or anti-mouse IgG, Student's *t* test). The mixtures of anti-C1q + C3 and anti-C1q + C3 + C9 were even more effective than the individual anti-C1q, C3 or C9 antibodies ($p<0.01$, Student's *t* test), suggesting that multiple complement components are involved in the degeneration of Chandler-infected N2a cells. In contrast, in 22L-infected cells (Fig. 2B), the anti-C1q and anti-C9 antibodies were ineffective, while the anti-C3 antibody alone reduced the percentage of Annexin V positive cells as effectively as combinations of antibodies to C1q + C3, C3 + C9 and C1q + C3 + C9. These results suggested that only C3 was involved in the degenerative change of 22L-infected N2a cells. The involvement of multiple complement factors in the induction of Annexin V positivity in Chandler-infected N2a cultures (Fig. 2A) raised the possibility that membrane attack complex (MAC) formation occurs on the plasma membrane on these cells. To assess this possibility, we performed immunocytochemistry for MAC on Chandler-, 22L-infected (Fig. 2C) or PPS-cured N2a cells (data not shown). In Chandler-infected N2a cells, MAC was detected at 24 h after treatment, suggesting that MAC formation occurred on Chandler-infected N2a cells, but not on 22L-infected and PPS-cured N2a cells.

C1q colocalizes with PrP in Chandler-infected N2a cells

To examine if C1q is associated with PrP in scrapie-infected or PPS-cured N2a cells after exposure to NMS, we performed double staining of C1q (green) and PrP (red) under non-denaturing condition (Fig. 3). In Chandler-infected N2a cells, colocalization of C1q with PrP (yellow) was detected at 30 min and 1 h. In 22L-infected cells, colocalization was not detected at any time point and C1q staining seemed localized in the cytoplasm more than on the cell surface. C1q staining was hardly detected in PPS-cured cells at any time point. No C1q staining was detected in untreated cells (data not shown). These results suggest that PrP on Chandler-infected N2a cells was associated with C1q on the cell surface.

C3 colocalizes with PrP in 22L-infected N2a cells

Next we analyzed the association of C3 with PrP in scrapie-infected or PPS-cured N2a cells (Fig. 4). In 22L-infected cells, colocalization of C3 and PrP was detected in the cytoplasm and on the cell surface at 15 and 30 min after NMS treatment. Although C3 staining was observed rarely in Chandler-infected or PPS-cured N2a cells, no colocalization was detected. No staining was detected in untreated cells (data not shown). These results suggest that PrP on 22L-infected N2a cells was associated with C3 on the cell surface and in the cytoplasm.

Distribution of C1q and C3 in scrapie-infected mouse brain

We also analyzed the distributions of vacuolar lesions, PrP^{Sc}, C1q and C3 in Chandler-, 22L- or mock-infected mouse brains (Figs. 5 & 6). At 90 dpi (pre-clinical stage of the disease) in Chandler-infected mouse brains, mild vacuolar degeneration was observed in the thalamus. PrP^{Sc} was strongly detected in the thalamus, moderately in secondary motor cortex and mildly in retrosplenial agranular cortex, septum, CA1 of hippocampus, midbrain, pons and medulla of the cerebellum. The C1q distribution was almost consistent with PrP^{Sc} except in the secondary motor cortex. Mild deposition of C3 was detected in cerebral cortex, septum, stria medullaris of thalamus, midbrain and pons. Vacuolar lesions in the major part of thalamus lacked deposition of C3 (Fig. 6). In 22L-infected mouse brains at 90 dpi, mild vacuolar lesions and PrP^{Sc} were distributed similar to those in Chandler-infected mice. However, in contrast to the Chandler-infected mice, the dorsal part of thalamus lacked C1q immunoreactivity, despite the presence of vacuolar lesions and PrP^{Sc} (Fig. 6). Moreover, C3 staining was much more pronounced in the thalamus (Fig. 6) and also was distributed widely throughout other regions including the hypothalamus, stria terminalis, septum, cerebral cortex, midbain, pons, medulla oblongata and medulla of the cerebellum (Fig. 5). These results suggested that there were differences in complement activation between Chandler- and 22L-infected mouse brains at 90 dpi. At 133 and 166 dpi, C1q and C3 signals were widely distributed both in Chandler- and 22L-infected infected mouse brains and there was no substantial difference between the two scrapie strains (data not shown). Neither C1q nor C3 signal was detected in mock-infected mouse brains at any time points (Fig. 6).