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Research Paper

Toxic Effects of Intracerebral PrP Antibody Administration During the Course of BSE Infection in Mice

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KEY WORDS

prion, PrP, BSE, mice, passive immunization, intracerebral injection, anti PrP antibody, neurotoxicity

SUPPLEMENTAL MATERIAL

Supplementary Methods

Recognition of native PrP in FACS by the 4H11 antibody

4H11 binding capacity to native cell-surface PrPc was tested by flow cytometry measurements on infected and non-infected N2a cells overexpressing murine PrP (kindly provided by S. Lehmann[38]). Confluent cells (3-day culture) were washed with PBS, incubated at 37°C for 5 min with cell dissociation buffer (Sigma) before splitting into equal samples of 50 µl containing $5x10^5$ cells. These samples were incubated for 20 min at room temperature with 0.5 µg of primary antibody (either 4H11 IgG2a, positive control anti-PrP IgG2a antibodies SAF34 or BAR214, or isotypic control antibody NSP-11 (anti-substance P)). After washes, cells were reacted with the secondary antibody, phycoerythrin-conjugated goat anti-mouse IgG 1:100 (Beckman Coulter), for 20 min at room temperature in the dark. On completion of washing, samples were resuspended in 1 ml of PBS and immediately analyzed in a flow cytometer (Epics XL, Beckman Coulter) equipped with a 488-nm argon laser, previously calibrated with flow check beads (Beckman Coulter).

In vitro evaluation of therapeutic effect of the 4H11 antibody

The prion-infected (strain RML) mouse neuroblastoma cell line 3F4-ScN2a has been described[39]. Cells were treated in duplicate for 5 days with different concentrations of either 4H11 or the $F(ab')_2$ fragment. A non-related IgG2a (5 µg/ml) antibody and untreated cells served as a controls. The medium was changed every other day with addition of fresh antibodies . After 5 days, cells were lysed for 10 min in lysis buffer (10 mM Tris-HCl, pH 7.5; 100 mM NaCl; 10 mM EDTA; 0.5% Triton X-100; 0.5% Na-deoxycholate) and subjected to proteinase K (PK) digestion for 30 min at 37° C with a PK concentration of 20 µg/ml lysate. PK treatment was terminated by addition of Pefabloc protease inhibitor (Roche; 1 mM). Following precipitation with 5 volumes of methanol, an aliquot of resuspended samples was analysed in immunoblot as described[39]. The antibody 4H11 was used for the

detection of PrP^{Sc} specific bands.

Supplementary Results.

Recognition of native PrP by the 4H11 antibody

Binding of different anti-PrP IgG2a to native murine PrP^{C} at the surface of non infected cells was compared. The binding capacity of the 4H11 was at least similar or even better than those of two other anti-PrP antibodies, SAF34 (anti-octarepeat) and BAR214 (conformational epitope) previously described to inhibit PrP^{Sc} replication in infected cell line models[10] (Supplementary Figure 1a).

Anti-PrP 4H11 IgG2a binding capacity to cell-surface PrP was tested by flow cytometry measurements on prion-infected and non-infected N2a cells. A higher staining intensity of the 4H11 to the surface of non-infected N2a cells than on infected ones was observed (Supplementary Figure 1b).

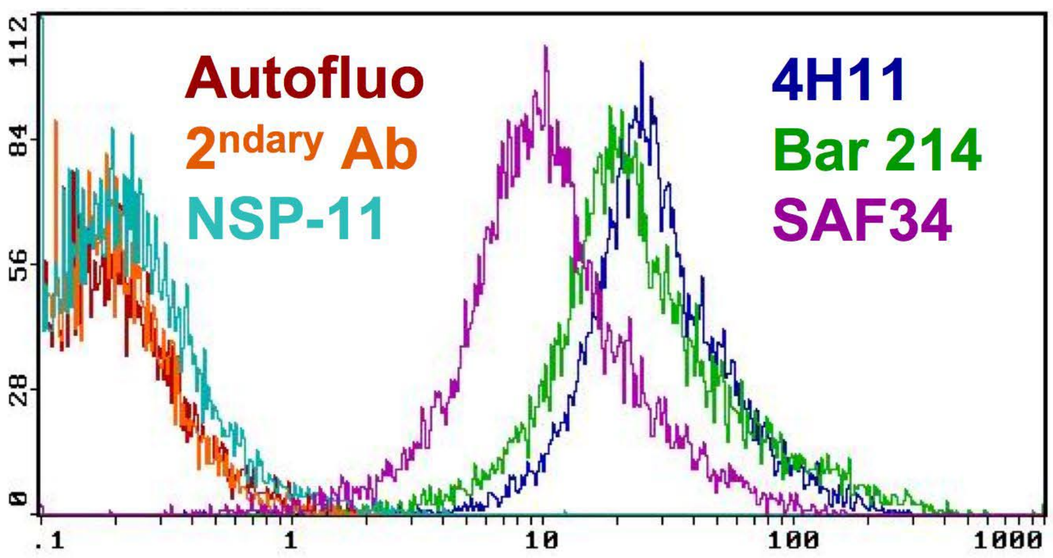
Therapeutical effect of the 4H11 antibody on ScN2a cell

In order to evaluate the potential of 4H11 antibody and $F(ab')_2$ fragments thereof to interfere with prion propagation, we employed a well-established cell culture system. Persistently infected 3F4-ScN2a cells were treated for 5 days with varying concentrations of antibodies (0.1, 0.5, 1, 2.5 or 5 µg/ml). Untreated cells or cultures treated with a non-related IgG2a antibody were used as negative controls. Immunoblot analysis of proteinase K-treated cell lysates revealed that both the full IgG molecule and the $F(ab')_2$ fragment inhibited PrP^{Sc} propagation (Supplementary Figure S2). Whereas the negative controls (lanes 1 and 2) harboured high amounts of PrP^{Sc}, the signals were completely abolished in cells treated with higher concentrations of the antibodies(lanes 3, 4, 8, 9 and 10). With the lower doses of 4H11, the PrP^{Sc} signal was still significantly reduced (lanes 5-7), with $F(ab')_2$ a strong reduction was still found with 0.5 µg/ml (lane 11), whereas the signal intensity of cells treated with 0,1 µg/ml was comparable to the control cells (lane 12).

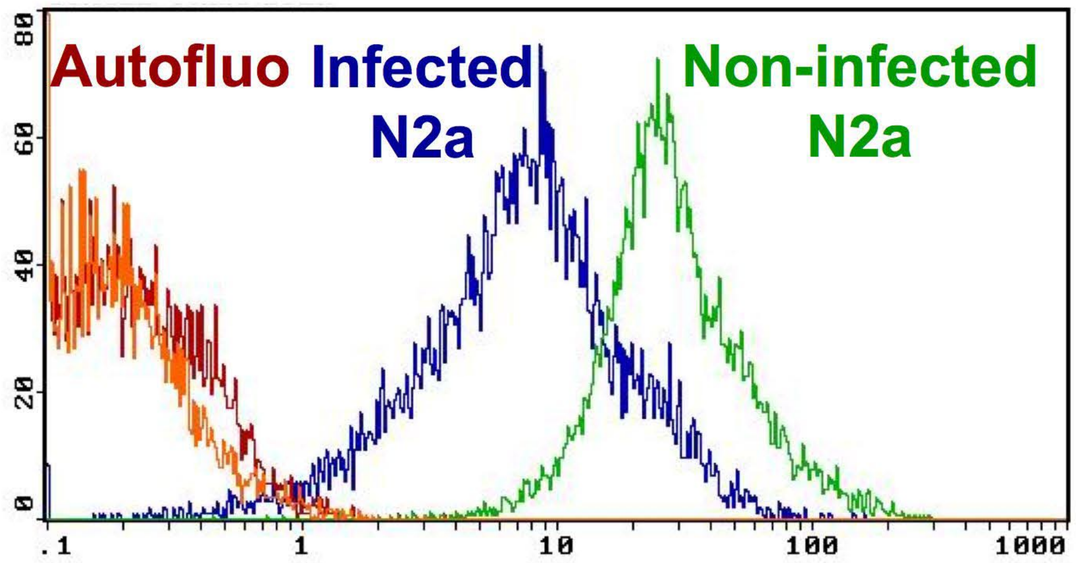
These experiment shows that both the full IgG 4H11 and the $F(ab')_2$ fragment can inhibit prion propagation, with an IC_{50} of 5 and 3 nM respectivly.

Supplementary Figure. S1.

S1. a

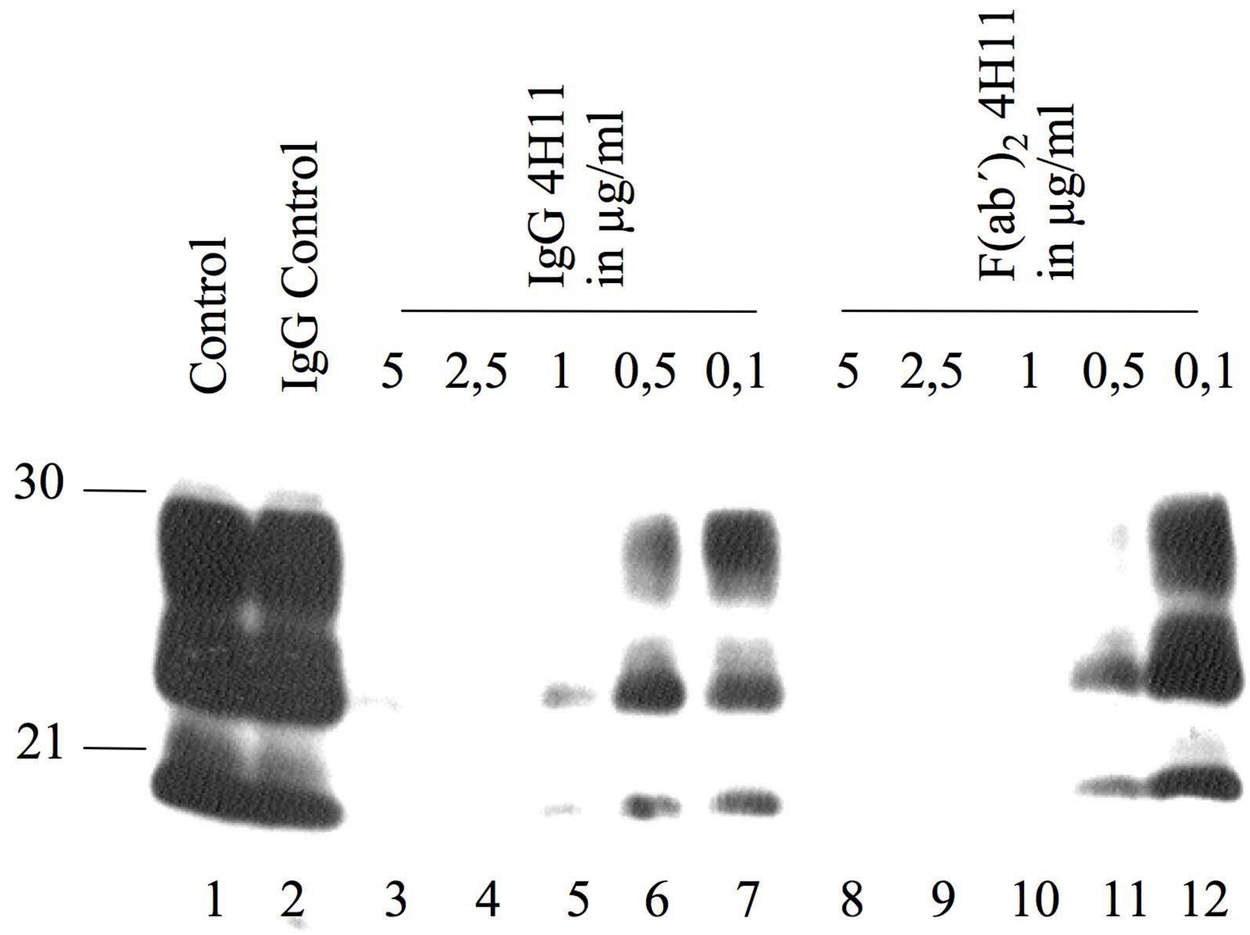


S1. b



Supplementary Figure. S1. Recognition of native PrP by the 4H11 antibody.
S1.a - Comparative flow cytometry staining intensities using different anti-PrP IgG2a antibodies on living non-infected N2a cells. Autofluorescence: red line; secondary antibody alone: orange line; isotypic control NSP-11: turquoise- blue line; SAF34: purple line; BAR214: green line; 4H11: dark blue line. S1.b - Comparative flow cytometry analysis of 4H11 binding capacity to non-infected and infected N2a cells (autofluorescence: red line; 4H11: green or blue line).

Supplementary Figure. S2.



Supplementary Figure. S2.

The protective effect of the 4H11 was first evaluated on Scrapie N2a cells. Chronically infected cells were incubated with different concentrations of the anti-PrP IgG (lanes 3-7) or $F(ab')_2$ (lanes 8-12). Controls include non treated cells (lane 1) and cells incubated with an irrelevant IgG (lane 2). At the end of the 5 days treatment, cells were harvested and PK digested to detect PrP^{Sc}.