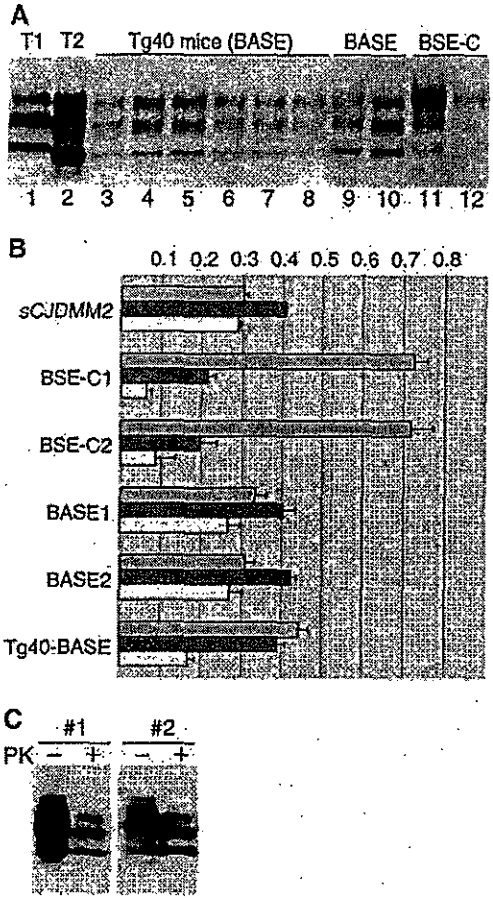

FIG. 1. Immunoblots and glycoform ratios of PK-resistant PrP^{Sc} from sCJD-affected, BASE strain-infected Tg(HuPrP) mice, the BASE strain inocula, and BSE-C and of PK-resistant PrP^{Sc} from the spleens of BASE strain-infected Tg(HuPrP) mice. (A) Immunoblot of PK-resistant PrP^{Sc} in the brain. Lanes 1 and 2, type 1 (sCJDMM1) (T1) and type 2 (sCJDMM2) (T2) sCJD, respectively; lanes 3 to 6, Tg(HuPrP) (Tg40) mice infected with BASE isolate 1 inoculum; lanes 7 to 8, Tg40 mice infected with BASE isolate 2 inoculum; lane 9, BASE isolate 1; lane 10, BASE isolate 2; lanes 11 and 12, two BSE-C isolates. All brain homogenates were treated with 100 μg/ml of PK for 30 min at 37°C and processed for immunoblot analysis with MAb 8H4. Five microliters of 10% brain homogenate was loaded for lanes 3 to 10. (B) Glycoform ratios of PK-resistant PrP^{Sc} in the brain. The upper (diglycosylated) (blue), middle (mostly monoglycosylated) (red), and lower (unglycosylated) (yellow) bands of PK-resistant PrP^{Sc} from BASE strain-infected Tg40 mice, the BASE strain, and BSE-C were quantified after optical scanning of duplicate immunoblots for panel A. Error bars indicate standard deviations. (C) PK-resistant PrP^{Sc} in the spleen. Ten milligrams of spleen tissue each from two of the BASE strain-infected Tg(HuPrP) (Tg40) mice (#1 and #2) was homogenized, PrP^{Sc} enriched by NaPTA precipitation, and either treated (+) or not treated (−) with 100 μg/ml of PK for 30 min at 37°C, followed by electrophoresis in a 10 to 20% Tris-Tricine SDS-polyacrylamide gradient gel and immunoblot analysis with MAb 8H4.

Histoblot analysis with MAb 3F4 showed a very distinct and selective distribution of PrP^{Sc} (Fig. 3A to D). Particular nuclei or groups of adjacent periventricular nuclei in the thalamus, hypothalamus, and brain stem were intensely immunostained for PrP^{Sc} (Fig. 3B to D). In contrast, PrP^{Sc} appeared to be overall less intense in the cerebral and cerebellar cortices (Fig. 3A to D). Immunohistochemical staining of paraffin-embedded brain tissue with 3F4 revealed PrP deposits in 5 of the 11 BASE strain-infected Tg40 mice examined. PrP^{Sc} deposits that stained intensely in the histoblots consisted of relatively large and well-circumscribed granules (Fig. 3E and G). Fine granular or small plaque-like aggregate patterns were occasionally seen in inferior regions of the cerebral cortex and in the thalamus (Fig. 3I and data not shown). In contrast, widespread, mostly fine-granular staining was detected in the cerebral cortex of symptomatic Tg40 mice inoculated with sCJDMM1 brain homogenate (Fig. 3J).

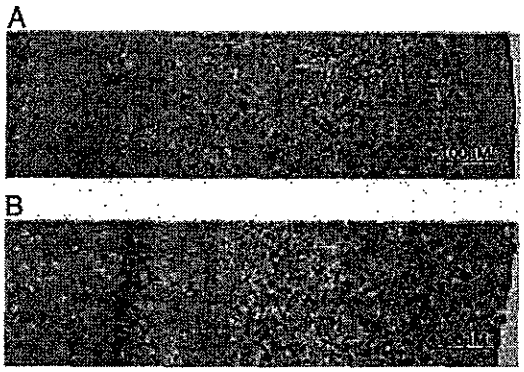

FIG. 2. Histopathology (with H&E) of BASE strain-infected and sCJDMM1-infected Tg(HuPrP) mice. (A) No consistent pathology was detected in the cerebral cortex as well as subcortical brain regions of symptomatic and immunoblot-positive BASE strain-infected Tg(HuPrP) (Tg40) mice. (B) In contrast, Tg40 mice inoculated with sCJDMM1 brain homogenate showed widespread spongiform degeneration.

The histopathological features of the BASE strain-inoculated Tg40 mice were quite different from those observed following inoculation with brain homogenates from the two forms of sCJD, sCJDMM1 and sCJDMM2. The sCJDMM1-inoculated Tg40 mice had widespread spongiform degeneration in the cerebrum (Fig. 2B) and moderate apoptosis of neuronal cells without spongiform degeneration in the cerebellum (13). Widespread spongiform degeneration was also seen in Tg40 mice inoculated with sCJDMM2 brain homogenate (data not shown).

DISCUSSION

We have shown that 60% of our Tg40 mice (in an inbred FVB background) that express normal levels of human PrP-129M became infected 20 to 22 months after i.c. inoculation with 0.3 mg of brain tissue from the two BASE isolates, suggesting a titer of approximately 3 50% infective dose units per milligram of brain tissue in the Tg40 line. An approximately 20% attack rate has been reported for the Tg650 line (in a mixed 129/Sv × C57BL/6 background) after i.c. inoculation with 2 mg brain tissues from BSE-C-infected cattle (2). It is noteworthy that the Tg650 mice express human PrP-129M at five to eight times the normal level, and high PrP levels are known to increase prion transmissibility (9, 17, 22). Inefficient BSE-C transmissions (0 to 30%) in Tg mouse lines of other genetic backgrounds expressing human PrP-129M at one or two times the normal level have also been reported by different groups (1, 4). Although it is difficult to compare results from different mouse lines, these findings suggest that the BASE strain has higher transmissibility than BSE-C does for human-