JOURNAL OF VIROLOGY, Apr. 2005, p. 5174–5184 Vol. 79, No. 8
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.8.5174–5184.2005

Comparison of Inflammatory and Acute-Phase Responses in the Brain and Peripheral Organs of the ME7 Model of Prion Disease

Colm Cunningham,†* David C. Wilcockson,† Delphine Boche, and V. Hugh Perry

CNS Inflammation Group, Southampton Neuroscience Group, School of Biological Sciences, Southampton, Hampshire, United Kingdom

Received 27 August 2004/Accepted 24 November 2004

Chronic neurodegenerative diseases such as prion disease and Alzheimer's disease (AD) are reported to be associated with microglial activation and increased brain and serum cytokines and acute-phase proteins (APPs). Unlike AD, prion disease is also associated with a peripheral component in that the presumed causative agent, PrP^{Sc}, also accumulates in the spleen and other lymphoreticular organs. It is unclear whether the reported systemic acute-phase response represents a systemic inflammatory response to prion disease or merely reflects central nervous system (CNS) inflammation. For this study, we investigated whether intracerebrally initiated prion disease (ME7 model) provokes splenic, hepatic, or brain inflammatory and acute-phase responses. We detected no significant elevation of proinflammatory cytokines or activation of macrophages in the spleens of these animals, despite clear PrP^{Sc} deposition. Similarly, at 19 weeks we detected no significant elevation of transcripts for the APPs serum amyloid A, complement C3, pentraxin 3, and α_2-antiplasmin in the liver, despite CNS neurodegeneration and splenic PrP^{Sc} deposition at this time. However, despite the low CNS expression levels of proinflammatory cytokines, there was robust expression of these APPs in degenerating brains. These findings suggest that PrP^{Sc} is not a stimulus for splenic macrophages and that neither peripheral PrP^{Sc} deposition nor CNS neurodegeneration is sufficient to produce a systemic acute-phase response. We also propose that serum cytokine and APP measurements are not useful during preclinical disease. Possible consequences of the clear chronic elevation of APPs in the CNS are discussed.

The prion diseases (transmissible spongiform encephalopathies) share similarities with other neurodegenerative conditions, such as amyotrophic lateral sclerosis and Alzheimer's, Parkinson's, and Huntington's diseases, in that they are characterized by the deposition of insoluble protein plaques, neurodegeneration along neuroanatomical pathways, and marked astrocytosis and microglial activation (19, 41, 48). However, unlike these diseases (1), prion diseases also have a non-central nervous system (CNS) component in that there is an accumulation of PrP^{Sc} plaques in the spleen and other lymphoreticular organs (34). There are reports that proinflammatory cytokines and acute-phase proteins (APPs) are elevated in the sera of Creutzfeldt-Jakob disease (CJD) patients (15, 30, 51). It is not clear, however, whether such cytokine and APP synthesis is a general feature of the disease. It is also unknown whether the synthesis of cytokines and APPs is a result of inflammatory activity occurring in the brain, as has been proposed for Alzheimer's disease (AD) patients (30), a systemic response to non-CNS aspects of prion disease pathology, such as PrP^{Sc} accumulation in the spleen, or indeed merely a consequence of an undetected coincident infection. This relationship is examined in the present study.

We have previously shown that during ME7-induced prion disease, there is an atypical CNS inflammatory response (38) characterized by microglial activation in regions of synaptic loss rather than in areas of PrP^{Sc} deposition (17) and dominated by the anti-inflammatory cytokine transforming growth factor beta 1 (TGF-β1) rather than the proinflammatory cytokines interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and IL-6 (5, 16, 52). Microglial cells are of the macrophage lineage (39) but remain in a down-regulated state in the healthy adult brain, as judged by the low expression levels of cell surface and endosomal markers (23, 57). Tingible body macrophages are known to phagocytose PrP^{Sc} in the spleen white pulp (26), and it would be interesting to know whether these macrophages show a similar anti-inflammatory phenotype as their counterparts in the brain. For the present study, we investigated the degree to which spleen macrophage populations are activated by the presence of PrP^{Sc} deposits.

In peripheral tissues, infection or injury results in the secretion of cytokines, including IL-1β, TNF-α, and IL-6, which circulate to the liver and induce the synthesis of APPs, collectively termed the acute-phase response (APR) (49). APPs are a diverse group of molecules that include complement proteins, antiproteases, clotting factors, and pentraxins such as serum amyloid P component (SAP) and C-reactive protein (CRP). In general, the APPs function to isolate and neutralize pathogens and proteases, opsonize and clear debris, and attenuate the local inflammatory response in order to effect a return to homeostasis and to minimize damage to healthy tissue (49). Although the synthesis of APPs is generally associated with the liver, both hepatic and CNS expression of APP mRNA has been shown after acute CNS inflammation (53). There have been some reports of acute-phase protein expression in the CNS during prion disease (11, 15), but no systematic analyses of these proteins have been performed.

Thus, the present study was aimed to determine whether PrP^{Sc} deposition in the spleen provokes local macrophage ac-

* Corresponding author. Mailing address: CNS Inflammation Group, School of Biological Sciences, Bassett Crescent East, Southampton, Hampshire SO16 7PX, United Kingdom. Phone: (44) 023 80597642. Fax: (44) 023 80592711. E-mail: C.Cunningham@soton.ac.uk.

† C.C. and D.C.W. contributed equally to this work.

tivation and/or cytokine synthesis and to assess whether prion disease provokes a hepatic acute-phase response. Splenic macrophage activation and splenic-hepatic cytokine and acute-phase gene transcription were assessed in ME7 prion-diseased and normal brain homogenate (NBH)-injected animals at 19 weeks postinoculation. This particular time point was chosen in order to fulfill the following important criteria for the validity of our conclusions that are not met by other time points of the disease: (i) clear PrP^{Sc} deposition is apparent in the spleen, (ii) robust neurodegeneration is present, and (iii) animals have not yet progressed to terminal disease, whereupon urinary incontinence (9) causes inflammation of the urogenital area that is sufficient to provoke a hepatic acute-phase response that is not caused by either neurodegeneration or PrP^{Sc} deposition per se. Finally, we also aimed to perform a systematic analysis of the time course of CNS expression of acute-phase proteins and inflammatory cytokines during neurodegeneration.

MATERIALS AND METHODS

Animals and stereotaxic surgery. Male C57BL/6J mice were obtained from Harlan-Olac Ltd. (Bicester, United Kingdom), housed in groups of five with standard light and temperature regimens, and fed pelleted food and water ad libitum. They were anesthetized intraperitoneally with 2,2,2-tribromoethanol (Avertin) and positioned in a stereotaxic frame, and 1 μl of a 10% (wt/vol) ME7-infected C57BL/6J brain homogenate, made in phosphate-buffered saline (PBS), was injected into the right dorsal hippocampus (coordinates from bregma: anterior-posterior, −2.0 mm; lateral, −1.6 mm; depth, −1.5 mm) via a 10-μl Hamilton syringe. Control animals were injected with 10% (wt/vol) NBH, derived from a naive C57BL/6J mouse, in PBS. ME7 in this mouse strain has an incubation period of approximately 165 days, and animals show the first clinical signs at approximately 133 days. All procedures were performed in accordance with a United Kingdom Home Office license.

Tissue preparation for prion time course studies. Twelve, 15, 18, 20, and 23 weeks after ME7 injection, animals (three, four, or five at each time point) were anesthetized with sodium pentobarbitone and then transcardially perfused with heparinized saline. Thick coronal sections (approximately 2 mm) were taken at the level of the hippocampus, and the hippocampus and thalamus was quickly removed for total RNA extraction, immediately frozen in liquid nitrogen, and stored at −80°C. NBH tissues were treated in exactly the same way, but only the 12- and 23-week time points were examined. Three ME7- and three NBH-treated animals were also killed by terminal anesthesia immediately prior to the appearance of overt clinical symptoms at 19 weeks postinoculation. These animals were perfused as follows. During perfusion with heparinized saline, parts of the liver and spleen were removed for total RNA extraction as described above. Further spleen tissue was removed and frozen in OCT embedding medium (Sakura, Zoeterwoude, The Netherlands) over isopentane for immunocytochemistry for CD68 and other macrophage markers, and perfusion was then continued with 10% formalin in order to fix the brain and remaining spleen tissue for PrP^{Sc} detection. Spleens were postfixed in the same fixative overnight and then embedded in paraffin.

LPS-challenged mice. At 19 weeks postinoculation, ME7 ($n = 5$)- and NBH ($n = 5$)-treated mice were injected intraperitoneally with 10 μg of bacterial endotoxin (lipopolysaccharide [LPS]). These animals were anesthetized and perfused with heparinized saline 6 h after the injection of LPS. Spleen and liver samples were removed and stored as described above for RNA extraction and analysis.

RNA extraction. Total RNA was extracted from brain, spleen, and liver samples by the use of RNeasy mini columns (QIAGEN, Crawley, United Kingdom) according to the manufacturer's instructions. Contaminating genomic DNAs were degraded during extraction by use of the QIAGEN DNase I enzyme. Typical yields were 8, 50, and 40 μg per 10 mg of tissue for brains, spleens, and livers, respectively. RNAs were stored at −80°C until assay.

Taqman RT-PCR. All equipment and reagents were supplied by Applied Biosystems Ltd. (Warrington, United Kingdom) unless otherwise stated. Assays for the absolute quantification of serum amyloid A (SAA), α_2-antiplasmin (α_2-AP), and complement C3 were performed as previously described (53). Assays for pentraxin 3 (PTX3), IL-1β, IL-6, TNF-α, TGF-β1, macrosialin, and scavenger receptor types A I and II (SRA-I and SRA-II, respectively) were designed by using the published sequences of their genes in Primer Express software. When possible, probes were designed to cross an intron such that they were cDNA specific. Table 1 lists the sequences of the primers and probes used for each assay. All primer pairs were checked for specificity by standard reverse transcription-PCRs (RT-PCRs) using Promega PCR reagents (Southampton, United Kingdom) followed by gel electrophoresis. Each primer pair produced a discrete band of the expected amplicon size (not shown).

For Taqman PCRs, cDNAs were generated from the total RNA by the use of Taqman Gold RT reagents. Two hundred nanograms of total RNA was reverse transcribed in a 10-μl reaction volume. One microliter of the RT reaction mix (equivalent to 20 ng of RNA) was subsequently used for PCR, which was performed as previously described (53). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured in each sample by use of an Applied Biosystems rodent GAPDH Taqman kit.

For absolute quantification of the SAA, α_2-AP, C3, and PTX3 transcripts, standard curves were made from in vitro transcripts of each gene, as previously optimized in our laboratory and described elsewhere (59). Acute-phase protein mRNA levels were thus expressed as numbers of copies per nanogram of total RNA (as determined by normalization with GAPDH). This method is significantly more time-consuming than relative quantification, and the advantage of absolute quantification was judged as insufficient to merit its continuance in subsequent assay designs. Thus, cytokine and scavenger receptor mRNA expression in prion-diseased and healthy animals was assessed by relative quantification. Briefly, an intracerebral (i.c.) challenge with 2.5 μg of LPS, known to up-regulate all target transcripts in the mouse brain, was performed, and tissues were harvested after 6 h. The total RNA was isolated, and 1 μg was used to synthesize cDNAs. A standard curve was made with serial one-in-five dilutions of these cDNAs, with the undiluted standard being assigned an arbitrary value from which all other values followed. Plotting these values against the cycle threshold (C_T) values produced in the quantitative PCR allowed us to construct a linear standard curve from which relative concentration values could be calculated from the C_T values of unknown samples. Thus, all data for these quantifications are expressed in relative units (arbitrary units). Like the data for acute-phase genes, these data were normalized to GAPDH expression. The principles of quantitative PCR and methods for relative quantitation have been described in detail elsewhere (10).

Immunocytochemistry. Paraffin-embedded formalin-fixed tissues from both brains and spleens were rehydrated and immunolabeled for PrP^{Sc} as follows. Sections were autoclaved in distilled water for 15 min at 121°C to destroy PrP^{Sc}. After being washed in PBS, the sections were placed in 90% formic acid for 5 min and then washed in distilled water and PBS. To avoid nonspecific binding that may occur with monoclonal mouse antibodies, we added a mouse-on-mouse blocking solution. The primary antibody 6H4 (1:4,000; Prionics, Zurich, Switzerland) was left overnight before being incubated with biotinylated anti-mouse immunoglobulin G (IgG; 1:250). Sections were then incubated with ABC (Vector Labs), and after washing, were reacted with hydrogen peroxide and diaminobenzidine (DAB) for approximately 2 min. The sections were counterstained with hematoxylin before being dehydrated and covered with coverslips. Neuronal loss in CA1 of the dorsal hippocampus was verified by hematoxylin staining of formalin-fixed tissues from ME7- and NBH-treated animals at 19 weeks postinoculation.

FA11 staining for CD68-positive red and white pulp macrophages in the spleen was performed as follows. Sections were dried in an oven at 37°C for 30 min before a 10-min fixation in alcohol at 4°C. Endogenous peroxidase activity was quenched by the use of 0.3% hydrogen peroxide in methanol. After a thorough washing, the slides were blocked with 10% normal rabbit serum before being stained with FA11 (Serotec, Oxford, United Kingdom) at a 1/20 ratio for 2 h at room temperature. A biotinylated rabbit anti-rat secondary antibody was added for 45 min, and the sections were washed again prior to the ABC peroxidase reaction. Sites of antibody binding were revealed by using DAB and hydrogen peroxide as substrates. The sections were counterstained with cresyl violet before being dehydrated and covered with coverslips. Sections were similarly stained for 3D6 (sialoadhesin-positive metallophil macrophages), 4C11 (follicular dendritic cells in germinal centers), MOMA-1 (unknown metallophil macrophage antigen), and ED31 (marginal-zone macrophages positive for the scavenger receptor MARCO). All of these antibodies were also raised in rats against mouse antigens and were applied at the following ratios: 1/400 (3D6), 1/200 (4C11), and 1/120 (ED31).

Statistics. Spleen and liver cytokine and acute-phase response gene transcription levels were compared by analysis of variance (ANOVA) with the Bonferroni post hoc test. Time course data were analyzed by ANOVA. Comparisons between NBH- and ME7-treated animals at 12 weeks postinjection were also performed as planned pair-wise comparisons by use of the t test. Animal groups

TABLE 1. Mouse scavenger receptor, cytokine, and APP Taqman primer and probe sequences

Target	Accession no.	Oligonucleotide	Sequence (5′-3′)	Amplicon size (bp)
CD68	NM009853	Forward primer	CAAGGTCCAGGGAGGTTGTG	75
		Reverse primer	CCAAAGGTAAGCTGTCCATAAGGA	
		Probe	CGGTACCCATCCCCACCTGTCTCTCTC	
SRA-I	L04274	Forward primer	GGATCAACCCCCCTTAAGACAG	69
		Reverse primer	GATCTCCACTCGGCCCTCA	
		Probe	CGACTGGTTGGTGGTAGTGGAGCCC	
SRA-II	L04274	Forward primer	GTTCCTGTGTCATGCCATGC	76
		Reverse primer	ATGTCAATGGAGGCCCCA	
		Probe	TGGCTTCTTGTAACTTCTTCTAACCCTGGGTG	
C3	J00367	Forward primer	CCATGTATTCCATCATTACTCCCAA	72
		Reverse primer	CGTGGGCCTCCAGTCAGA	
		Probe	CCTACGGCTGGAGAGCGAAGAGACC	
PTX3	X83601	Forward primer	ACAACGAAATAGACAATGGACTTCAT	62
		Reverse primer	CTGGCGGCAGTCGCA	
		Probe	CCACCGAGGACCCCACGCC	
SAA	M13522	Forward primer	GCCATGGAGGGTTTTTTTCATT	80
		Reverse primer	CCTTTGGGCAGCATCATAGTTC	
		Probe	CACATGTCTCCAGCCCCTTGGAAAGC	
SAP	X16899	Forward primer	GTCTTCACCAGCCTTCTTTCAGA	146
		Reverse primer	TCAGATTCTCTGGGGAACACAA	
		Probe	CTTCCTCTTGAGGTCTGTCTGACAAAAGGC	
α_2-AP	Y12312	Forward primer	GCGGTTCACAGTGTCGGT	66
		Reverse primer	TCCAGCAGGAACCATCGAA	
		Probe	ACATGATGCACGCGGTGTCATATCCT	
IL-1β	M15131	Forward primer	GCCACCCACCCTGCA	69
		Reverse primer	ACCGCTTTTCCATCTTCTTCTT	
		Probe	TGGAGAGTCTGGATCCCAAGCAATACCC	
TNFα	M11731	Forward primer	CTCCAGGCGGTGCCTATG	149
		Reverse primer	GGGCCATAGAACTGATGAGAGG	
		Probe	TCAGCCTCTTCTCATTCCTGCTTGTGG	
IL-6	NM031168	Forward primer	TCCAGAAACCGCTATGAAGTTC	72
		Reverse primer	CACCAGCATCAGTCCCAAGA	
		Probe	CTCTGCAAGAGACTTCCATCCAGTTGCC	
TGF-β1	AJ009862	Forward primer	CGTGGAAATCAACGGGATCA	84
		Reverse primer	GGCCATGAGGAGCAGGAA	
		Probe	ACCTGGGCACCATCCATGACATGA	

in the peripheral LPS challenge groups were compared by ANOVA, and data were treated as significant at the 95% confidence interval.

RESULTS

PrP^{Sc} deposition in the spleen does not cause activation of local macrophages. A clear deposition of PrP^{Sc} was apparent in the white pulp of the spleens of prion-infected animals at 19 weeks postinoculation, as visualized by 6H4 staining (Fig. 1d). Approximately one-third of all white pulp areas examined showed clear PrP^{Sc} deposition. NBH-injected animals showed no positive staining with 6H4 (Fig. 1a), although deposits of lipofuscin and iron were visible in the spleens of both animal groups, presumably as a feature of aging in this tissue. These have previously been described as being present in the spleen (58). However, there was no obvious activation of local macrophages in response to PrP^{Sc} deposition, as visualized by the use of markers for spleen macrophage populations. Macrophage activation in the region of PrP deposition, the white pulp, was assessed by use of the FA11 antibody against the type D scavenger receptor CD68. Although this marker is not specific for white pulp macrophages, its location in the white pulp and its function as a phagocytic receptor make it an appropriate marker for assessments of this macrophage population, which is known to phagocytose the PrP^{Sc} generated in follicular dendritic cells. This antibody showed no clear difference in patterns of staining between animal groups, with clear staining in tingible bodies (Fig. 1b and e) and proximal to the central arteriole (not shown). FA11 staining also showed essentially uniform staining of the red pulp in both animal groups. 4C11 staining for follicular dendritic cells in germinal centers of the white pulp showed a pattern that was clearly distinguishable from that of FA11 staining (Fig. 1c and f) but did not appear different between prion-diseased and healthy animals. Other splenic macrophage populations were also assessed. The metallophil macrophages appeared similar by both sialoadhesin (3D6) (Fig. 1g and j) and MOMA-1 (Fig. 1i and l) staining. Staining for scavenger receptor A (types I and II) in the marginal-zone macrophages was unsuccessful in this tissue due to a polymorphism in C57BL/6J mice that alters the expression of the epitope to which the 2F8 antibody binds (18). However, there was no apparent up-regulation of the scavenger receptor MARCO in this population, as judged by ED31 staining (Fig. 1h and k). Since the antibodies used for this study required different tissue preparations, it was not possible to perform double staining for macrophage markers and PrP^{Sc}, but given the frequency of PrP^{Sc} deposits (one in three white pulp areas), it was judged that macrophage responses to

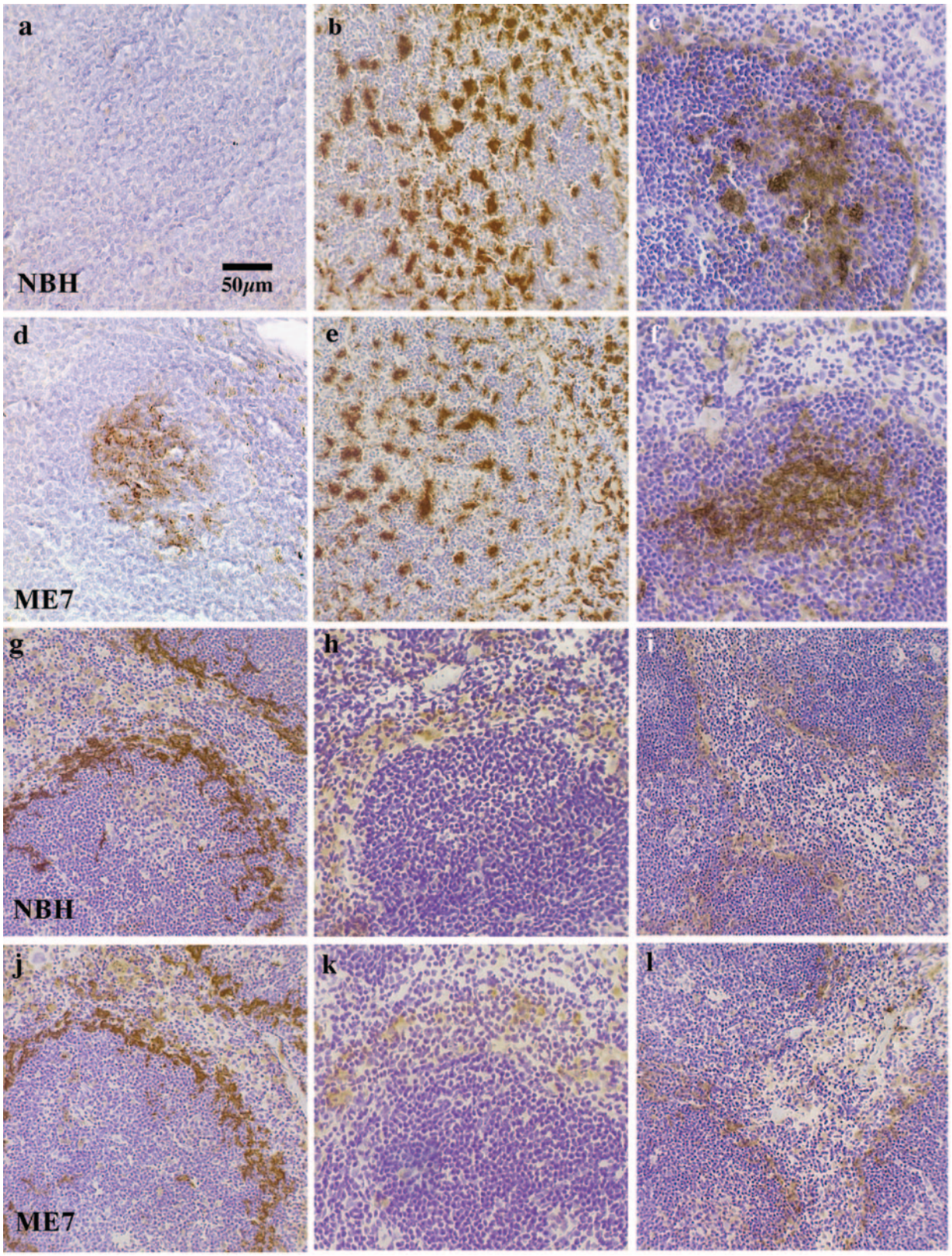

FIG. 1. PrP^{Sc} deposition in the spleen does not activate local macrophages. PrP^{Sc} immunolabeling was performed with the spleen white pulp from NBH-treated (a) and ME7-injected (d) animals at 19 weeks postinjection. White pulp macrophages in healthy (b) and ME7-infected (e) spleens were stained with FA11. Staining for follicular dendritic cells in white pulp germinal centers of healthy (c) and ME7-injected animals (f) was done with 4C11. Metallophil macrophages were stained with antibodies against sialoadhesin (3D6) (g and j) and MOMA-1 (i and l). Staining with ED31 for the scavenger receptor MARCO was performed with spleens of NBH-treated (h) and ME7-injected (k) animals.

these deposits, if ongoing, should be apparent throughout the spleen tissue. Such responses were not apparent.

Semiquantitative Taqman RT-PCRs of isolated RNAs from spleens of healthy and prion-diseased animals revealed a decreased expression of both scavenger receptors SRA-I (Fig. 2a) and SRA-II (Fig. 2b) in ME7-injected animals compared to NBH-treated controls ($P < 0.005$ by ANOVA with the Bonferroni post hoc test). No up-regulation of the phagocytic marker CD68 was observed when compared by ANOVA ($P = 0.79$). Similar analyses of the expression of the proinflammatory cytokines IL-1β ($P = 0.69$) and TNF-α ($P = 0.24$) revealed no significant up-regulation, while IL-6 mRNA was not detectable in prion-diseased or healthy animals, although it was clearly up-regulated after an LPS challenge (Fig. 2d, e, and f). Conversely, there was a twofold up-regulation of TGF-β1 gene expression (Fig. 2g) that was statistically significant ($P = 0.0051$ by ANOVA with the Bonferroni-Dunn post hoc test). There was a robust synthesis of proinflammatory cytokines after LPS challenges to both healthy and prion-diseased animals. These macrophages did not appear to be primed in the manner that we have previously described for brain microglia, in that they did not produce exaggerated responses to a peripheral challenge with LPS, i.e., there was no difference between cytokine production after ME7-LPS and NBH-LPS challenges.

Prion disease does not cause a peripheral APR. At 19 weeks postinoculation, there was no statistical difference in the mRNA expression levels of the SAA, α_2-AP, and C3 genes between the livers of ME7 prion-infected and NBH-treated mice (Fig. 3). PTX3 mRNA was invariably below the limits of the assay (not shown). Prion disease does not appear to cause any significant elevation of hepatic acute-phase protein gene transcription. The peripheral injection of LPS (20 μg intraperitoneally) induced a very robust hepatic elevation of the acute-phase genes SAA (4.25×10^6 copies of mRNA per ng of total RNA) and C3 (5×10^5 copies of mRNA per ng of total RNA). The time point at which this assessment was made (19 weeks postinoculation) encompasses clear PrP^{Sc} deposition in the spleen (Fig. 1d), considerable PrP^{Sc} deposition in the brain (Fig. 4b), and robust neurodegeneration in the hippocampus (Fig. 4d).

APP mRNA expression in the brain during prion disease progression. All brain APP mRNAs investigated were up-regulated throughout prion disease compared with mRNAs taken from NBH-injected control mice (Fig. 5). This trend was statistically significant over time for α_2-AP ($P < 0.01$), C3 ($P < 0.01$), and PTX3 ($P < 0.05$), and increases were evident at the earliest time point studied (12 weeks). At 12 weeks, increases in the mean values of α_2-AP and C3 were approximately sevenfold and fivefold, respectively ($P < 0.05$). All genes continued to have increased expression, with α_2-AP and C3 reaching 40- and 55-fold increases, respectively. α_2-AP expression peaked at week 18 and had declined slightly by week 23. C3 transcripts increased in an exponential fashion and were maximal in this experiment at 23 weeks. For PTX3 and SAA, the data were more variable than those for α_2-AP and C3. At its peak, the PTX3 mRNA had increased eightfold with respect to the controls. SAA never increased more than threefold over control levels and approached the lower limits of the assay. SAP was not detected in the brains of the animals in this study.

Cytokine mRNA expression in the brain during prion disease progression. Proinflammatory cytokine gene expression was measured by using the same cDNA samples as those assayed for APP transcripts. All three cytokine transcripts were expressed at markedly lower levels than when measured following an acute i.c. challenge with LPS (Fig. 5). Notwithstanding, there was a statistically significant increase in the expression of IL-1β and TNF-α (Fig. 6) (for IL-1β, $P < 0.01$; for TNF-α, $P < 0.001$ by ANOVA). Increases in IL-1β mRNA (increased approximately threefold compared to NBH-treated mice) were seen 18 weeks after infection, and the transcript levels decreased thereafter. TNF-α mRNA showed changes in expression at early time points of disease; at 12 weeks, an 8-fold increase was observed compared to control levels ($P < 0.05$), and a progressive rise in transcript numbers was seen throughout the disease, reaching an approximately 40-fold increase at week 23 compared with NBH-treated controls at this time point, although this was also >50-fold less than that induced by i.c. LPS. IL-6 mRNA was strongly induced by the LPS challenge but was barely detectable in prion-diseased and control brains and was below the lower limits of quantitation of the assay (data not shown).

DISCUSSION

In the present study, we have addressed a number of questions about peripheral and CNS macrophage and acute-phase responses to chronic neurodegeneration and deposition of PrP^{Sc}. We demonstrated that PrP^{Sc} accumulation in the spleen white pulp does not cause a significant activation of local macrophage populations on the basis of cytokine and phagocytic marker expression at both the mRNA and protein levels. Similarly, neither the deposition of PrP^{Sc} nor extensive chronic CNS neurodegeneration results in the induction of APP gene expression in the liver, a marker of systemic inflammatory activity. We also showed that in the brain, increasing amounts of APP mRNA are synthesized throughout disease progression, despite the low expression levels of the typical proinflammatory cytokines IL-1β, TNF-α, and IL-6.

Spleen and brain macrophage response to PrP^{Sc}. We have shown that spleen macrophages remain inactivated by the presence of PrP^{Sc} deposits. Despite the i.c. injection of an ME7-infected homogenate, PrP^{Sc} deposition can clearly be shown in the spleens of infected animals. This splenic deposition occurs slowly and in an atraumatic fashion, unlike the acute intraperitoneal injection of PrP^{Sc} peptides or homogenate, but is known to increase approximately 200-fold between 2 and 20 weeks after injection (43, 50). As such, the time point of 19 weeks post-i.c. inoculation coincides with the peak of splenic PrP^{Sc} accumulation and provides a useful model system with which to assess peripheral macrophage responses to PrP^{Sc} in the absence of the inflammatory trauma that the direct injection of a homogenate may cause. PrP^{Sc} replication is known to occur in follicular dendritic cells in the white pulp, and extracellularly deposited protein is taken up by CD68-positive tingible body macrophages (27). It is not known which macrophage receptors are involved in the phagocytosis of PrP^{Sc}. The marginal-zone macrophages are a highly phagocytic population of macrophages that reside at sites of antigen entry into the spleen (31) and are known to respond to modified

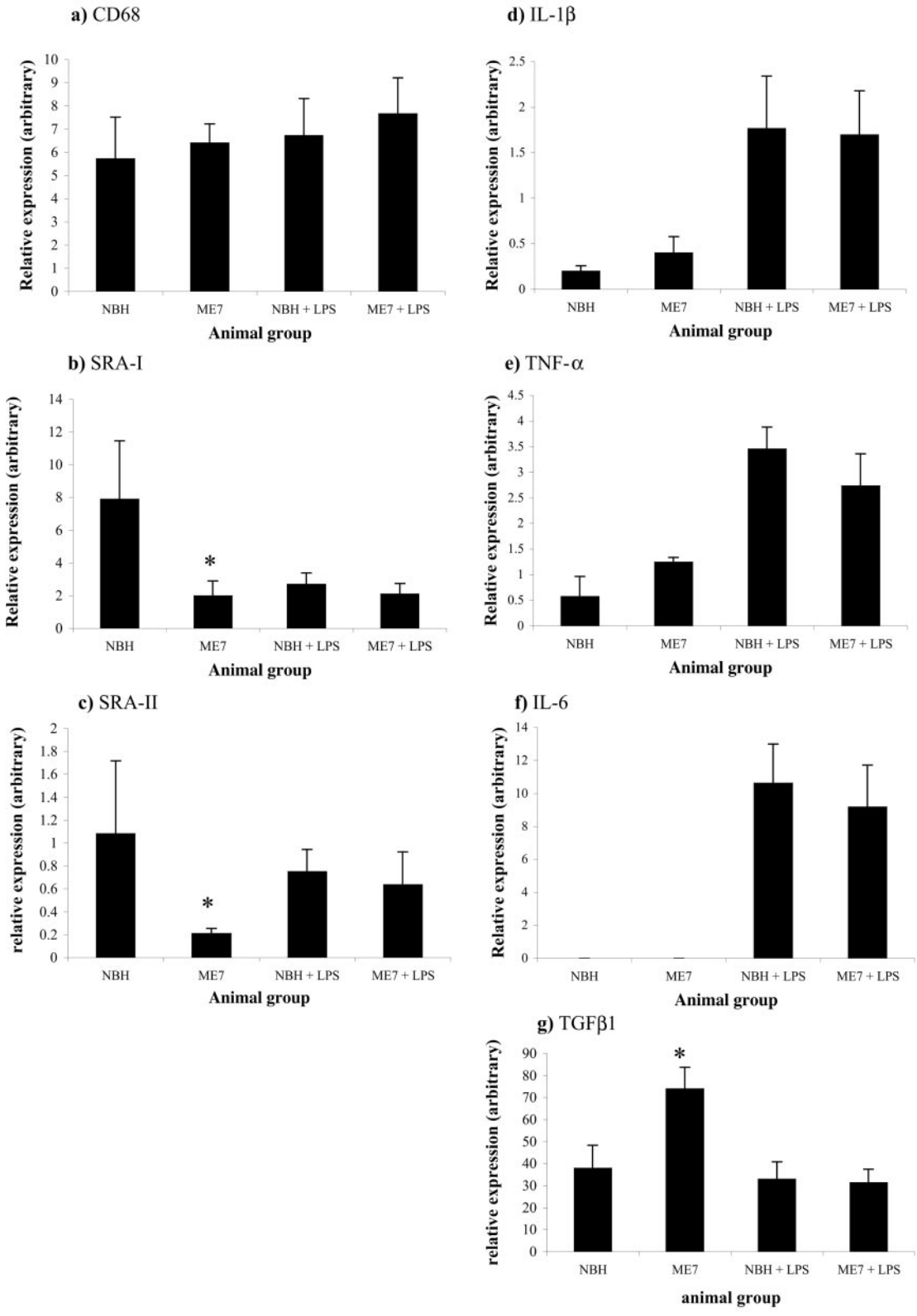

FIG. 2. PrP^{Sc} does not activate proinflammatory genes in the spleen. The graphs show the levels of transcription of the genes for CD68 (a), SRA-I (b), SRA-II (c), IL-1β (d), TNF-α (e), IL-6 (f), and TGF-β1 (g) in spleens of healthy and prion-infected animals at 19 weeks postinfection. The data were normalized to GAPDH expression and are expressed as relative concentrations in arbitrary units. Bars represent means ± standard errors of the means (SEM). *, P values were <0.005 for comparisons of TGF-β1, SRA-I, and SRA-II between the NBH- and ME7-treated groups (ANOVA with the Bonferroni-Dunn post hoc test; $n = 5$ for all groups). P values were >0.24 for all other comparisons between NBH- and ME7-treated mice.

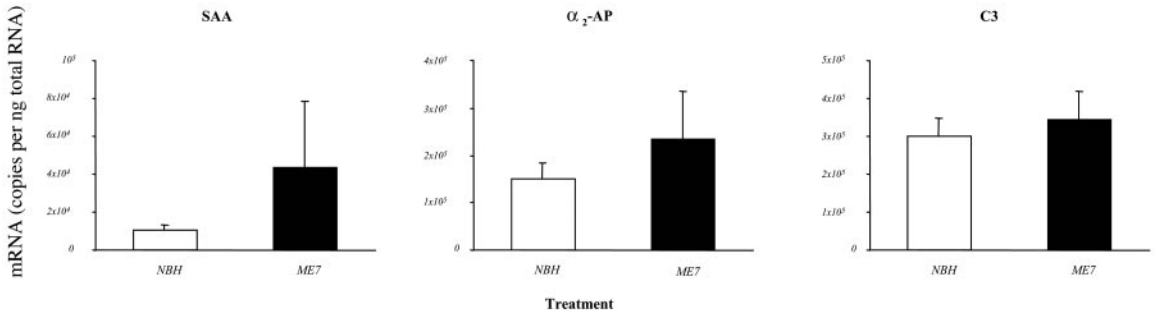

FIG. 3. ME7-induced prion disease does not evoke hepatic expression of APPs. A comparison of expression of mRNA levels for SAA, α_2-AP, and C3 in the livers of ME7 prion-infected mice and NBH-treated mice was performed at 19 weeks postinfection. There was no significant difference in the expression of any of the APPs, as measured by ANOVA with the Bonferroni-Dunn post hoc test. Open bars represent data for NBH-treated controls, and filled bars represent data for ME7 prion-infected mice. The data shown are absolute copy numbers of mRNA, normalized to GAPDH, and bars represent means ± SEM. For the NBH group, $n = 5$; for the ME7 group, $n = 3$.

proteins through receptors such as scavenger receptor types AI, AII, B, and MARCO (25). These receptors are also known to be involved in the phagocytosis of other fibrillar proteins, such as β-amyloid (3, 20, 21), and thus are possible candidates for the uptake of deposited PrP^{Sc}.

For the present study, we observed no up-regulation of CD68, a down-regulation of SRA-I and SRA-II mRNAs, no increase in immunocytochemical staining for CD68, MOMA-1, sialoadhesin, or MARCO, and no proinflammatory cytokine induction in the spleen. In addition, whatever the splenic response, it was not sufficient to provoke a hepatic acute-phase response. Conversely, we did observe an increase in the expression of the anti-inflammatory cytokine TGF-β1. This anti-inflammatory cytokine has been reported to down-regulate the expression of scavenger receptors and to hamper phagocytic function (2, 59) and thus is consistent with the scavenger receptor gene expression observed here and with the persistence of PrP^{Sc} in the spleen. Thus, the present data suggest that PrP^{Sc} accumulation in the spleen down-regulates macrophage responses, and they provide in vivo evidence that PrP^{Sc} is not inherently proinflammatory. Indeed, if there is any proinflammatory response to the deposition of PrP^{Sc} at any time during disease, it is clearly insufficient to effect PrP^{Sc} clearance. This failure to activate innate immunological pathways may be an important factor in the inefficiency of its clearance by these macrophages.

Currently, the only data suggesting that PrP^{Sc} is proinflammatory come from in vitro studies in which microglia no longer show a quiescent phenotype (8, 40). We have previously shown that the acute in vivo CNS responses to injected prion-infected and normal brain homogenates are indistinguishable (4), demonstrating that initial microglial activation is a nonspecific response to the injection of brain homogenate rather than to the PrP^{Sc} contained in the homogenate. Consistent with the present data, it has now also been shown that the coating of inert latex beads with a fibrillar prion peptide (106-126) actually hampers their phagocytosis by cultured microglia (14). Taking these data together, it seems unlikely that PrP^{Sc} is a stimulus for microglial activation in vivo. While extrapolating from our present data on splenic macrophages to brain microglia remains speculative, it should be noted that this is a difficult issue to address directly in the brain since activated microglia, degenerating neurons and synapses, and PrP^{Sc} coexist in the brain throughout disease. Our own observations on early prion pathology showed that the spatial distribution of activated microglia is more correlated with areas of synaptic degeneration than with regions of PrP^{Sc} staining (17). Earlier studies on murine prion disease also showed that microglial activation is more marked in areas of vacuolar degeneration than in areas of PrP^{Sc} deposition (54). These data reinforce the view that microglial activation in vivo is a response to the earliest events of neurodegeneration rather than to the deposition of PrP^{Sc}.

Systemic acute-phase response to prion disease. There have been some reports of acute-phase protein expression in serum and in the CNS during prion disease (11, 15), and it has been

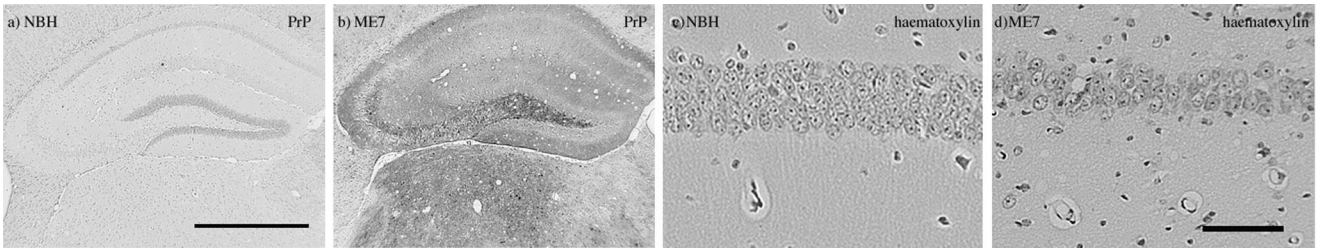

FIG. 4. Prion-associated pathology at 19 weeks postinoculation. PrP staining was performed by 6H4 immunolabeling of brains of NBH-treated (a) and ME7-infected (b) mice, with clear diffuse staining throughout the hippocampus and thalamus and more punctate staining in the dorsal thalamus and dentate gyrus/CA3. Hematoxylin-stained sections through the hippocampi of NBH-treated (c) and ME7-injected (d) animals show marked neurodegeneration of the pyramidal neurons of the hippocampal CA1. Bar = 1 mm (a) or 50 μm (d).

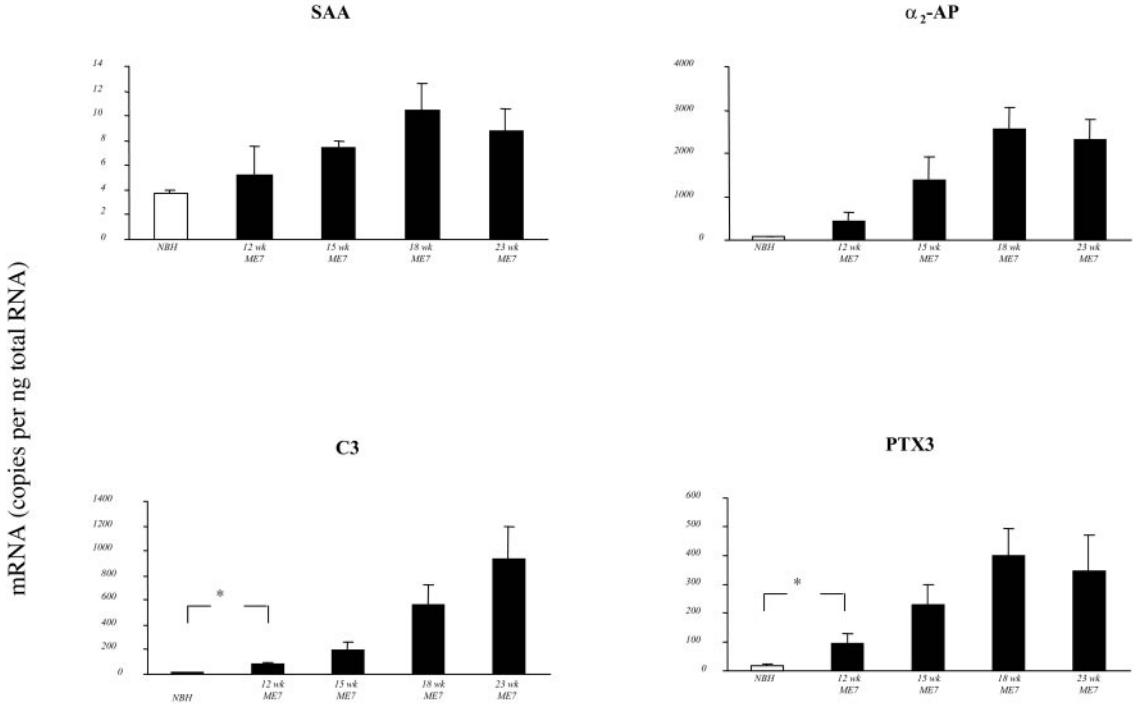

FIG. 5. Temporal expression of APP genes in the brains of ME7 prion-infected mice. At time points throughout prion disease progression, brain samples containing the hippocampus and dorsal thalamus were assayed for APP mRNAs by quantitative RT-PCRs. All gene transcripts showed a trend of increased expression during disease progression compared with NBH-treated controls (for α_2-AP, $P < 0.01$; for C3, $P < 0.01$; for PTX3, $P < 0.05$ [all by ANOVA]). Open bars represent data for NBH-treated controls, and filled bars represent data for ME7 prion-infected mice. The data shown are absolute copy numbers of mRNA normalized to GAPDH expression and are presented as means ± SEM. *, $P < 0.05$ by the *t* test. For the NBH group, $n = 4$; for the ME7 group at 12 weeks, $n = 3$; for the ME7 group at 15 weeks, $n = 3$; for the ME7 group at 18 weeks, $n = 4$; for the ME7 group at 23 weeks, $n = 5$.

suggested for AD that ongoing CNS neurodegeneration is sufficient to provoke a systemic acute-phase response (30). Acute CNS inflammation has been shown to evoke a hepatic acute-phase response (12), although acute neurodegeneration induced by NMDA (*N*-methyl-D-aspartate) does not (53). Thus, it would be interesting to know whether chronic CNS neurodegeneration evokes this response. Coe et al. reported elevated serum acute-phase proteins in animals with prion disease, but only in those with terminal disease (15). We chose the period immediately preceding terminal signs to study this issue, since the urinary incontinence that accompanies terminal disease (9, 24) causes urogenital inflammation with consequent activation of an APR that is not a direct result of neurodegeneration or PrP^{Sc} deposition. Despite the robust neurodegeneration shown in this study at 19 weeks, there was no significant hepatic elevation of transcripts for the APPs. It was previously shown that neither proinflammatory cytokines nor α_1-antichymotrypsin is expressed in the livers or spleens of prion-diseased animals (11), and the present data show that this is true for a range of APRs. However, a systemic challenge with LPS resulted in marked liver transcription of these genes. In view of the present results, we propose that interpreting serum changes in APPs during CJD as an early indicator of prion disease pathology is unlikely to be informative since APPs are not induced by neurodegeneration and since elevated APP levels in sera are more likely to reflect ongoing undetected peripheral inflammation rather than being products of the disease per se.

Chronic CNS synthesis of APPs and cytokines. We showed here that chronic neurodegeneration evokes an APR in the brain. Moreover, the levels of APP mRNAs were comparable to those previously reported following excitotoxic injury or proinflammatory challenges in the striatum (53). While the proinflammatory cytokines IL-1β, TNF-α, and IL-6 are the principal regulators of the acute-phase response (35, 44, 45), their expression in the ME7 model of prion disease is not marked. We have previously failed to find statistically significant increases in IL-1β, TNF-α, and IL-6 by either PCRs or enzyme-linked immunosorbent assays while reporting significant increases in mRNA and protein for the anti-inflammatory cytokine TGF-β1 (16, 52). These results have been repeated here with the Taqman RT-PCR method, which showed consistently low levels of IL-1β and IL-6 expression and larger increases in TNF-α than previously detected. However, the TNF-α protein is not detectable by an enzyme-linked immunosorbent assay during ME7-induced prion disease (16). The levels of transcripts for proinflammatory cytokines are also extremely low in the ME7/CV model of prion disease (7) and in transgenic models of AD (47) and are 50-fold lower than those induced by an i.c. challenge with LPS. While it is possible that these low levels of cytokines induce APP expression, it is also possible that other mediators are responsible for this reg-

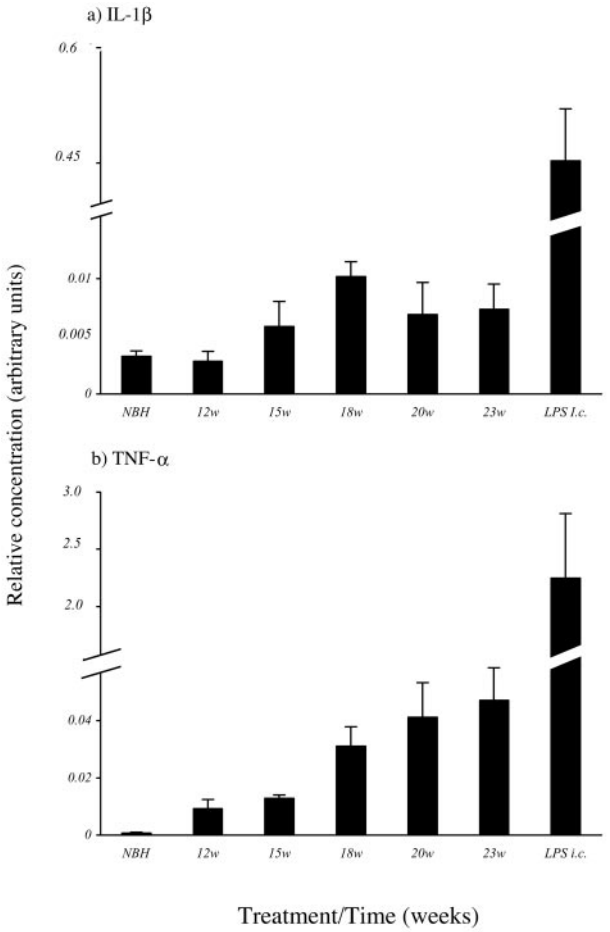

FIG. 6. Temporal expression of proinflammatory cytokines in the brains of ME7 prion-infected mice. At time points throughout disease progression, brain samples containing the hippocampus and dorsal thalamus were assayed for cytokine mRNAs by quantitative RT-PCRs. IL-1β (a) and TNF-α (b) transcripts showed statistically significantly increased expression in ME7 prion-infected mouse brains ($P < 0.01$ and 0.001, respectively, by ANOVA). Broken bars show cytokine gene expression following an acute i.c. challenge with 2.5 μg of LPS. The data were normalized to GAPDH expression and are expressed as relative concentrations in arbitrary units. Bars represent means ± SEM. *, $P < 0.05$ by the *t* test. For the NBH group and the ME7 group at 18 and 20 weeks, $n = 4$; for the ME7 group at 12 and 15 weeks and the group receiving LPS i.c, $n = 3$; for the ME7 group at 23 weeks, $n = 5$.

ulation. Proteins such as IL-11, oncostatin M, cardiotropin-1, and leukemia inhibitory factor have been reported to induce the expression of acute-phase proteins that, in some cases, cannot be induced by IL-6 (13, 29, 46).

The proinflammatory cytokines IL-1β, IL-6, and TNF-α are not essential for prion disease progression (32, 42), but acute-phase proteins may have some influence. The systemic circulation of APPs following injury or infection typically attenuates local inflammation and mediates a return to homeostasis (44). However, unresolved inflammatory events may lead to the chronic overproduction of APPs, with deleterious effects. Few studies have been undertaken on this subject with regards to prion disease. It has been shown, after i.c. inoculations, that the progression to the terminal stages of scrapie is delayed only marginally in complement C3-deficient mice (28). In a transgenic mouse model of AD, the inhibition of C3 caused a substantial increase in amyloid deposition and overt neurodegeneration (56). The latter study suggests an amyloid-clearing and consequently neuroprotective role for C3.

The pentraxins SAP, CRP, and pentraxin 3 are structurally related proteins involved in the osponization of pathogens for phagocytosis and the activation of complement (55). Although we did not find SAP transcripts in the brains of these mice, we did show PTX3 up-regulation. PTX3 can induce complement activation by an interaction with C1q (6) or can block this interaction (37). As a potential opsonin or producer of bystander damage, PTX3 may be beneficial or deleterious in ME7. This issue has not been studied.

Increased protease activity may be a necessary host response to the extracellular protein deposition observed during prion disease and AD but may also cause the lysis of healthy tissue. Antiproteases may similarly have a beneficial or deleterious role. The overexpression of α_1-ACT, a gene product which is also up-regulated during murine prion disease (11), increases amyloid loads in mice that also overexpress amyloid precursor proteins, which is associated with increased synaptic loss (36). This suggests that protease activation is a necessary part of the host response to extracellular protein deposition. In the present study, we also found evidence for elevated α_2-AP, and it will be interesting to see whether this reflects a general disturbance of the plasmin cascade, since increased proteolytic activity may also have beneficial or deleterious roles in these disease processes. Plasminogen has previously been shown to interact directly with PrP^{Sc} (33), and the aberrant prion protein has also been shown to activate plasminogen activity (22).

Conclusion. The present results show that PrP^{Sc} is not a stimulus for splenic macrophages and suggest that PrP^{Sc} is not inherently proinflammatory. The data also indicate that neither the splenic accumulation of PrP^{Sc} nor chronic neurodegeneration is sufficient to provoke a systemic acute-phase response. These findings suggest that measures of serum cytokines and acute-phase proteins are not useful as surrogate markers of prion disease. In contrast, acute-phase reactants are found in the brains of prion-diseased animals, despite a minimal expression of the proinflammatory cytokines. Further studies on these acute-phase reactants may provide insights into disease pathogenesis.

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust and by the MRC.

We thank Luisa Martines-Pomares and Nick Platt for antibodies and helpful discussions of spleen macrophages.

REFERENCES

1. **Arai, H., V. M. Lee, M. L. Messinger, B. D. Greenberg, D. E. Lowery, and J. Q. Trojanowski.** 1991. Expression patterns of beta-amyloid precursor protein (beta-APP) in neural and nonneural human tissues from Alzheimer's disease and control subjects. Ann. Neurol. **30:**686–693.
2. **Argmann, C. A., C. H. Van Den Diepstraten, C. G. Sawyez, J. Y. Edwards, R. A. Hegele, B. M. Wolfe, and M. W. Huff.** 2001. Transforming growth factor-beta1 inhibits macrophage cholesteryl ester accumulation induced by native and oxidized VLDL remnants. Arterioscler. Thromb. Vasc. Biol. **21:**2011–2018.
3. **Bamberger, M. E., M. E. Harris, D. R. McDonald, J. Husemann, and G. E. Landreth.** 2003. A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. J. Neurosci. **23:**2665–2674.
4. **Betmouni, S., and V. H. Perry.** 1999. The acute inflammatory response in CNS following injection of prion brain homogenate or normal brain homogenate. Neuropathol. Appl. Neurobiol. **25:**20–28.

5. **Betmouni, S., V. H. Perry, and J. L. Gordon.** 1996. Evidence for an early inflammatory response in the central nervous system of mice with scrapie. Neuroscience **74:**1–5.
6. **Bottazzi, B., V. Vouret-Craviari, A. Bastone, L. De Gioia, C. Matteucci, G. Peri, F. Spreafico, M. Pausa, C. D'Ettorre, E. Gianazza, A. Tagliabue, M. Salmona, F. Tedesco, M. Introna, and A. Mantovani.** 1997. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. J. Biol. Chem. **272:**32817–32823.
7. **Brown, A. R., J. Webb, S. Rebus, R. Walker, A. Williams, and J. K. Fazakerley.** 2003. Inducible cytokine gene expression in the brain in the ME7/CV mouse model of scrapie is highly restricted, is at a strikingly low level relative to the degree of gliosis and occurs only late in disease. J. Gen. Virol. **84:**2605–2611.
8. **Brown, D. R., B. Schmidt, and H. A. Kretzschmar.** 1996. Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. Nature **380:**345–347.
9. **Bruce, M. E., R. G. Will, J. W. Ironside, I. McConnell, D. Drummond, A. Suttie, L. McCardle, A. Chree, J. Hope, C. Birkett, S. Cousens, H. Fraser, and C. J. Bostock.** 1997. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. Nature **389:**498–501.
10. **Bustin, S. A.** 2002. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J. Mol. Endocrinol. **29:**23–39.
11. **Campbell, I. L., M. Eddleston, P. Kemper, M. B. Oldstone, and M. V. Hobbs.** 1994. Activation of cerebral cytokine gene expression and its correlation with onset of reactive astrocyte and acute-phase response gene expression in scrapie. J. Virol. **68:**2383–2387.
12. **Campbell, S. J., P. M. Hughes, J. P. Iredale, D. C. Wilcockson, S. Waters, F. Docagne, V. H. Perry, and D. C. Anthony.** 2003. CINC-1 is an acute-phase protein induced by focal brain injury causing leukocyte mobilization and liver injury. FASEB J. **17:**1168–1170.
13. **Cichy, J., S. Rose-John, and J. Travis.** 1998. Oncostatin M, leukaemia-inhibitory factor and interleukin 6 trigger different effects on alpha1-proteinase inhibitor synthesis in human lung-derived epithelial cells. Biochem. J. **329:**335–339.
14. **Ciesielski-Treska, J., N. J. Grant, G. Ulrich, M. Corrotte, Y. Bailly, A. M. Haeberle, S. Chasserot-Golaz, and M. F. Bader.** 2004. Fibrillar prion peptide (106–126) and scrapie prion protein hamper phagocytosis in microglia. Glia **46:**101–115.
15. **Coe, J. E., R. E. Race, and M. J. Ross.** 2001. Serological evidence for an inflammatory response in murine scrapie. J. Infect. Dis. **183:**185–191.
16. **Cunningham, C., D. Boche, and V. H. Perry.** 2002. Transforming growth factor beta1, the dominant cytokine in murine prion disease: influence on inflammatory cytokine synthesis and alteration of vascular extracellular matrix. Neuropathol. Appl. Neurobiol. **28:**107–119.
17. **Cunningham, C., R. Deacon, H. Wells, D. Boche, S. Waters, C. P. Diniz, H. Scott, J. N. Rawlins, and V. H. Perry.** 2003. Synaptic changes characterize early behavioural changes in the ME7 model of murine prion disease. Eur. J. Neurosci. **17:**2147–2155.
18. **Daugherty, A., S. C. Whitman, A. E. Block, and D. L. Rateri.** 2000. Polymorphism of class A scavenger receptors in C57BL/6 mice. J. Lipid Res. **41:**1568–1577.
19. **DeArmond, S. J.** 1993. Alzheimer's disease and Creutzfeldt-Jakob disease: overlap of pathogenic mechanisms. Curr. Opin. Neurol. **6:**872–881.
20. **El Khoury, J., S. E. Hickman, C. A. Thomas, L. Cao, S. C. Silverstein, and J. D. Loike.** 1996. Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature **382:**716–719.
21. **El Khoury, J. B., K. J. Moore, T. K. Means, J. Leung, K. Terada, M. Toft, M. W. Freeman, and A. D. Luster.** 2003. CD36 mediates the innate host response to beta-amyloid. J. Exp. Med. **197:**1657–1666.
22. **Ellis, V., M. Daniels, R. Misra, and D. R. Brown.** 2002. Plasminogen activation is stimulated by prion protein and regulated in a copper-dependent manner. Biochemistry **41:**6891–6896.
23. **Gordon, S., L. Lawson, S. Rabinowitz, P. R. Crocker, L. Morris, and V. H. Perry.** 1992. Antigen markers of macrophage differentiation in murine tissues. Curr. Top. Microbiol. Immunol. **181:**1–37.
24. **Guenther, K., R. M. Deacon, V. H. Perry, and J. N. Rawlins.** 2001. Early behavioural changes in scrapie-affected mice and the influence of dapsone. Eur. J. Neurosci. **14:**401–409.
25. **Husemann, J., J. D. Loike, R. Anankov, M. Febbraio, and S. C. Silverstein.** 2002. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia **40:**195–205.
26. **Jeffrey, M., G. McGovern, C. M. Goodsir, K. L. Brown, and M. E. Bruce.** 2000. Sites of prion protein accumulation in scrapie-infected mouse spleen revealed by immuno-electron microscopy. J. Pathol. **191:**323–332.
27. **Jeffrey, M., G. McGovern, S. Martin, C. M. Goodsir, and K. L. Brown.** 2000. Cellular and sub-cellular localisation of PrP in the lymphoreticular system of mice and sheep. Arch. Virol. **2000**(Suppl.)**:**23–38.
28. **Klein, M. A., P. S. Kaeser, P. Schwarz, H. Weyd, I. Xenarios, R. M. Zinkernagel, M. C. Carroll, J. S. Verbeek, M. Botto, M. J. Walport, H. Molina, U. Kalinke, H. Acha-Orbea, and A. Aguzzi.** 2001. Complement facilitates early prion pathogenesis. Nat. Med. **7:**488–492.
29. **Kordula, T., R. E. Rydel, E. F. Brigham, F. Horn, P. C. Heinrich, and J. Travis.** 1998. Oncostatin M and the interleukin-6 and soluble interleukin-6 receptor complex regulate alpha1-antichymotrypsin expression in human cortical astrocytes. J. Biol. Chem. **273:**4112–4118.
30. **Licastro, F., S. Pedrini, L. Caputo, G. Annoni, L. J. Davis, C. Ferri, V. Casadei, and L. M. Grimaldi.** 2000. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? J. Neuroimmunol. **103:**97–102.
31. **Linehan, S. A., L. Martinez-Pomares, and S. Gordon.** 2000. Mannose receptor and scavenger receptor: two macrophage pattern recognition receptors with diverse functions in tissue homeostasis and host defense. Adv. Exp. Med. Biol. **479:**1–14.
32. **Mabbott, N. A., A. Williams, C. F. Farquhar, M. Pasparakis, G. Kollias, and M. E. Bruce.** 2000. Tumor necrosis factor alpha-deficient, but not interleukin-6-deficient, mice resist peripheral infection with scrapie. J. Virol. **74:**3338–3344.
33. **Maissen, M., C. Roeckl, M. Glatzel, W. Goldmann, and A. Aguzzi.** 2001. Plasminogen binds to disease-associated prion protein of multiple species. Lancet **357:**2026–2028.
34. **McBride, P. A., P. Eikelenboom, G. Kraal, H. Fraser, and M. E. Bruce.** 1992. PrP protein is associated with follicular dendritic cells of spleens and lymph nodes in uninfected and scrapie-infected mice. J. Pathol. **168:**413–418.
35. **Mortensen, R. F., J. Shapiro, B. F. Lin, S. Douches, and R. Neta.** 1988. Interaction of recombinant IL-1 and recombinant tumor necrosis factor in the induction of mouse acute phase proteins. J. Immunol. **140:**2260–2266.
36. **Mucke, L., G. Q. Yu, L. McConlogue, E. M. Rockenstein, C. R. Abraham, and E. Masliah.** 2000. Astroglial expression of human alpha(1)-antichymotrypsin enhances Alzheimer-like pathology in amyloid protein precursor transgenic mice. Am. J. Pathol. **157:**2003–2010.
37. **Nauta, A. J., B. Bottazzi, A. Mantovani, G. Salvatori, U. Kishore, W. J. Schwaeble, A. R. Gingras, S. Tzima, F. Vivanco, J. Egido, O. Tijsma, E. C. Hack, M. R. Daha, and A. Roos.** 2003. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur. J. Immunol. **33:**465–473.
38. **Perry, V. H., C. Cunningham, and D. Boche.** 2002. Atypical inflammation in the central nervous system in prion disease. Curr. Opin. Neurol. **15:**349–354.
39. **Perry, V. H., D. A. Hume, and S. Gordon.** 1985. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience **15:**313–326.
40. **Peyrin, J. M., C. I. Lasmezas, S. Haik, F. Tagliavini, M. Salmona, A. Williams, D. Richie, J. P. Deslys, and D. Dormont.** 1999. Microglial cells respond to amyloidogenic PrP peptide by the production of inflammatory cytokines. Neuroreport **10:**723–729.
41. **Price, D. L., D. R. Borchelt, and S. S. Sisodia.** 1993. Alzheimer disease and the prion disorders amyloid beta-protein and prion protein amyloidoses. Proc. Natl. Acad. Sci. USA **90:**6381–6384.
42. **Prinz, M., M. Heikenwalder, P. Schwarz, K. Takeda, S. Akira, and A. Aguzzi.** 2003. Prion pathogenesis in the absence of Toll-like receptor signalling. EMBO Rep. **4:**195–199.
43. **Race, R. E., and D. Ernst.** 1992. Detection of proteinase K-resistant prion protein and infectivity in mouse spleen by 2 weeks after scrapie agent inoculation. J. Gen. Virol. **73:**3319–3323.
44. **Ramadori, G., and B. Christ.** 1999. Cytokines and the hepatic acute-phase response. Semin. Liver Dis. **19:**141–155.
45. **Ramadori, G., J. Van Damme, H. Rieder, and K. H. Meyer zum Buschenfelde.** 1988. Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. Eur. J. Immunol. **18:**1259–1264.
46. **Richards, C. D., C. Langdon, D. Pennica, J. Gauldie, I. Vorberg, and S. A. Priola.** 1996. Murine cardiotrophin-1 stimulates the acute-phase response in rat hepatocytes and H35 hepatoma cells. J. Interferon Cytokine Res. **16:**69–75.
47. **Sly, L. M., R. F. Krzesicki, J. R. Brashler, A. E. Buhl, D. D. McKinley, D. B. Carter, and J. E. Chin.** 2001. Endogenous brain cytokine mRNA and inflammatory responses to lipopolysaccharide are elevated in the Tg2576 transgenic mouse model of Alzheimer's disease. Brain Res. Bull. **56:**581–588.
48. **Soto, C., D. Volkel, K. Zimmermann, I. Zerr, T. Lindner, M. Bodemer, S. Poser, and H. P. Schwarz.** 2003. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat. Rev. Neurosci. **4:**49–60.
49. **Steel, D., and S. Whitehead.** 1993. The acute phase response, p. 373. *In* E. Sim (ed.), Humoral factors. Oxford University Press, Oxford, United Kingdom.
50. **Tatzelt, J., D. F. Groth, M. Torchia, S. B. Prusiner, and S. J. DeArmond.** 1999. Kinetics of prion protein accumulation in the CNS of mice with experimental scrapie. J. Neuropathol. Exp. Neurol. **58:**1244–1249.
51. **Volkel, D., K. Zimmermann, I. Zerr, T. Lindner, M. Bodemer, S. Poser, and H. P. Schwarz.** 2001. C-reactive protein and IL-6: new marker proteins for the diagnosis of CJD in plasma? Transfusion **41:**1509–1514.
52. **Walsh, D. T., S. Betmouni, and V. H. Perry.** 2001. Absence of detectable

IL-1beta production in murine prion disease: a model of chronic neurodegeneration. J. Neuropathol. Exp. Neurol. **60:**173–182.
53. **Wilcockson, D. C., S. J. Campbell, D. C. Anthony, and V. H. Perry.** 2002. The systemic and local acute phase response following acute brain injury. J. Cereb. Blood Flow Metab. **22:**318–326.
54. **Williams, A. E., L. J. Lawson, V. H. Perry, and H. Fraser.** 1994. Characterization of the microglial response in murine scrapie. Neuropathol. Appl. Neurobiol. **20:**47–55.
55. **Wolbink, G. J., M. C. Brouwer, S. Buysmann, I. J. ten Berge, and C. E. Hack.** 1996. CRP-mediated activation of complement in vivo: assessment by measuring circulating complement-C-reactive protein complexes. J. Immunol. **157:**473–479.
56. **Wyss-Coray, T., F. Yan, A. H. Lin, J. D. Lambris, J. J. Alexander, R. J. Quigg, and E. Masliah.** 2002. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. Proc. Natl. Acad. Sci. USA **99:**10837–10842.
57. **Yamada, Y., T. Doi, T. Hamakubo, and T. Kodama.** 1998. Scavenger receptor family proteins: roles for atherosclerosis, host defence and disorders of the central nervous system. Cell Mol. Life Sci. **54:**628–640.
58. **Ye, X., and R. I. Carp.** 1995. The pathological changes in peripheral organs of scrapie-infected animals. Histol. Histopathol. **10:**995–1021.
59. **Zuckerman, S. H., C. Panousis, and G. Evans.** 2001. TGF-beta reduced binding of high-density lipoproteins in murine macrophages and macrophage-derived foam cells. Atherosclerosis **155:**79–85.