Early and Progressive Accumulation of Reactive Microglia in the Huntington Disease Brain

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Abstract. Microglia may contribute to cell death in neurodegenerative diseases. We studied the activation of microglia in affected regions of Huntington disease (HD) brain by localizing thymosin β-4 (Tβ4), which is increased in reactive microglia. Activated microglia appeared in the neostriatum, cortex, and globus pallidus and the adjoining white matter of the HD brain, but not in control brain. In the striatum and cortex, reactive microglia occurred in all grades of pathology, accumulated with increasing grade, and grew in density in relation to degree of neuronal loss. The predominant morphology of activated microglia differed in the striatum and cortex. Processes of reactive microglia were conspicuous in low-grade HD, suggesting an early microglia response to changes in neuropol and axons and in the grade 2 and grade 3 cortex, were aligned with the apical dendrites of pyramidal neurons. Some reactive microglia contacted pyramidal neurons with huntingtin-positive nuclear inclusions. The early and proximate association of activated microglia with degenerating neurons in the HD brain implicates a role for activated microglia in HD pathogenesis.

Key Words: Human brain; Huntington; Huntington disease; Mutant huntingtin; Nuclear inclusions; Reactive microglia; Thymosin β-4.

INTRODUCTION

Huntington disease (HD) neuropathology is characterized by a loss of neurons in the striatum and the cortex (1). There is a gradient of neuronal depletion that progresses medial to lateral and dorsal to ventral within the striatum (2). In the cortex, pyramidal neurons are depleted in layers II, V, and VI (3). Morphometric studies of the HD brain indicate that there is also a marked increase in reactive astrocytes and oligodendroglia in the striatum (2, 4, 5) and in oligodendroglia in the cortex (3, 4). Changes in microglia in the HD brain are less clear. In a morphometric study of cresyl violet-stained sections of the prefrontal cortex, Sotrel et al (3) noted that “the very small total number of microglial cells, demonstrated across all layers in all grades of HD as well as in normal controls, rendered the results of the statistical analysis meaningless.” In our observations of hematoxylin and eosin-stained paraffin-embedded sections of the HD striatum, microglia cells appear sparse in all grades of HD when the cells are identified by the conventional criteria of dark, elongated nuclei (M. DiFiglia and J.P. Vonsattel, unpublished observations).

Microglia appear in the brain following a variety of insults including infection, ischemia, and trauma (6).

These cells can respond rapidly to subtle changes (frequently within hours) by entering an activated state. The distinct and graded morphological changes in microglial cells from resting cells to macrophages were described in early studies of human brain (7–9). A primary role of activated microglia is to remove dying neurons and other cells by phagocytosis. Reactive microglia express increased MHC class I and II antigens, and secrete proteases and pro-inflammatory cytokines (interleukin 1β and tumor necrosis factor alpha [TNFα]), which participate in the immune inflammatory response. Cytokines also may stimulate neuronal signaling cascades involved with cell death. Thus, microglia can contribute to neurodegeneration through multiple pathways. A neurotrophic role for reactive microglia has also been suggested. In the injured striatum, activated microglia secrete brain derived neurotrophic factor and glial cell line-derived neurotrophic factor and can induce sprouting of dopaminergic axons (10).

Neurodegenerative disorders including Alzheimer disease (AD), Parkinson disease, Pick’s disease, and Creutzfeldt-Jakob disease (prion disease) are associated with the activation of microglia (11–14). Some studies have implicated microglia as potential mediators in the neurodegenerative process (15–19). Injection of β-amyloid into the striatum induces activation of microglia and microglial expression of inducible nitric oxide synthase, a known neurotoxic agent (20). In vitro studies suggest that microglia stimulated by treatment with fragments of β-amyloid or prion proteins release neurotoxic substances (21). Microglia may contain quinolinic acid (22), an excitatory amino acid that can induce neurotoxicity.

In view of their potential importance in other neurodegenerative disorders, we investigated the localization of microglia in control and HD brain by immunohistochemistry with 2 antibodies made against thymosin β-4.
microglia (26).

PBS, frozen on blocks of dry ice, and stored at buffered saline (PBS) overnight, cryoprotected in 30% sucrose/histochemistry was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), frozen on blocks of dry ice, and stored at

Blocks of interest were removed from fresh brain with a post-mortem interval ranging from 8 to 48 h. Tissue for immunohistochemistry was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight, cryoprotected in 30% sucrose/PBS, frozen on blocks of dry ice, and stored at −80°C. Tissue for protein analysis was rapidly frozen in liquid nitrogen vapor. All of the HD cases were examined for extent of neurodegeneration and assigned a grade from 1 (minimal) to 4 (extensive) according to the method of Vonsattel et al (2). A total of 6 control cases were used in this study and 13 adult onset HD cases including 3 HD grade 1, 5 HD grade 2, 4 HD grade 3, and 1 HD grade 4 (Table). The mean age is 71 yr for control cases and 61 yr for HD cases. These cases were free from AD and other neurological disorders and most of the cases were used previously for the immunohistochemical localization of huntingtin (27, 28). The postmortem brains of an Alzheimer patient (age 80) and a control (age 71) fixed in PLP (periodate-lysine-paraformaldehyde) were also used.

MATERIALS AND METHODS

Brain Tissue

Brains were supplied from the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA) and the Massachusetts General Hospital Neuropharmacology Laboratory. Blocks of interest were removed from fresh brain with a post-mortem interval ranging from 8 to 48 h. Tissue for immunohistochemistry was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight, cryoprotected in 30% sucrose/PBS, frozen on blocks of dry ice, and stored at −80°C. Tissue for protein analysis was rapidly frozen in liquid nitrogen vapor. All of the HD cases were examined for extent of neurodegeneration and assigned a grade from 1 (minimal) to 4 (extensive) according to the method of Vonsattel et al (2). A total of 6 control cases were used in this study and 13 adult onset HD cases including 3 HD grade 1, 5 HD grade 2, 4 HD grade 3, and 1 HD grade 4 (Table). The mean age is 71 yr for control cases and 61 yr for HD cases. These cases were free from AD and other neurological disorders and most of the cases were used previously for the immunohistochemical localization of huntingtin (27, 28). The postmortem brains of an Alzheimer patient (age 80) and a control (age 71) fixed in PLP (periodate-lysine-paraformaldehyde) were also used.

TABLE

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0 = only resident microglia; + = scattered, regional reactive microglia–layer 6 cortex, medial caudate, dorsal putamen; +++ = evenly distributed reactive microglia, <15 per 0.4 mm²; +++ = 15–25 reactive microglia per 0.4 mm²; ++++ = >25 reactive microglia per 0.4 mm²; na = not available.

(Tß4), an actin-binding protein (23, 24), which is markedly elevated in the cell bodies and processes of activated microglia (25), and with an antibody (CR3/43) that recognizes human β-chain MHC class II antigens in brain microglia (26).

Primary Antibodies

Two rabbit polyclonal antibodies to Tß4 were used in this study. One was made against amino acids 16–43 of Xenopus Tß4 (Tß4,) and has been used in immunohistochemical studies of the developing Xenopus nervous system (29). The other antibody (Tß4) was made against amino acids 16–43 of human thymosin β4 (30), which differs from Xenopus in 4 amino acids. A cysteine was added to the amino terminus of the synthetic peptide for conjugation to a carrier protein, thyroglobulin. Rabbits were immunized using the conjugated peptide (Immuno-biology Laboratory, Fujioka, Japan). The antiserum (IgG fraction) was affinity-purified through a peptide affinity column. After washing with 0.25 M NaCl, the final fraction was obtained under 0.1 M glycine-HCl buffer (pH 2.0) and neutralized. Protein concentration of the purified antiserum for Xenopus and human Tß4 was 0.87 mg–1.0 mg/ml and were diluted 1:1,000 or 1:500 respectively for immunohistochemistry and 1:1,000 or 1:2,000 respectively for Western blot analysis. Other antibodies used were monoclonal antibody CR3/43 (DAKO, Carpinteria, CA), which recognizes MHC class II antigens and labels human reactive microglia in the brain (26), diluted 1:50, anti-huntingtin antisera (Ab 1 [27, 31]) diluted to 1 μg/ml for immunohistochemistry, and monoclonal anti-G-actin clone AC-40 (Sigma, St. Louis, MO) diluted 1:500 for Western blot. Ab1 was made in rabbit against amino acids 1–17 of huntingtin, and recognizes intraneuronal nuclear inclusions and dystrophic neurites in the HD cortex and striatum (27).

Immunohistochemistry

Sections from the frontal cortex (Brodmann area 9), the caudate-putamen at the level of the anterior commissure, the globus
pallidus, and the cerebellum from control and HD brain and from temporal cortex of an AD brain were cut on a sliding microtome (E. Leitz Wetzlar) at a thickness of 40 μm and blocked immediately in 5% normal goat serum (NGS), 1% bovine serum albumin (BSA), 0.2% Triton X-100, and 1% hydrogen peroxide in PBS for 30 min. They were washed in PBS and incubated in primary antibody for 2 days at 4°C. Sections were serially incubated in biotinylated anti-rabbit IgG (Tβ4ab, Ab1) or anti-mouse IgG (CR3/43) (Vector Laboratories, Burlingame, CA) diluted 1:200, avidin-biotin-peroxidase complex (Vectastain kit, Vector), and DAB reagent kit (KPL) until desired intensity was reached. Sections were placed onto slides, dehydrated, and mounted with coverslips. Sections from control and HD or AD cases were processed together to control for differences in staining intensity. Some sections were counterstained with cresyl violet after the immunohistochemistry. Sections omitting the primary antibody or treated with antibody plus Tβ4 peptide (9 μg/ml) or an unrelated peptide (9 μg/ml) were included as controls. Some sections were co-incubated in anti-Tβ4, and Ab1.

Cross-linking G-actin and Tβ4

The method of Safer et al. (32) was followed. In brief, a solution containing 50 μM G-actin and 2 μM purified bovine Tβ4 (generous gift of D. Safer) was incubated at room temperature for 1 h with mixing in 3 mM triethanolamine hydrochloride, 0.2 mM ATP, 0.2 mM CaCl₂, 0.2 mM NaCl₂, pH 7.0 with 0.5 mM 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide and 0.5 mM N-hydroxyssuccinimide. The reaction was quenched by adding Tris-Cl pH 8.8 to a final concentration of 15 mM and 10 μl of this reaction mixture was analyzed by SDS-PAGE and Western blot.

Western Blot Analysis

Protein samples (20 μg) from control and HD cortical gray matter were run on 4%–20% gradient gels and 12.5% Tris-Tricine gels (BioRad, Hercules, CA) and transferred to 0.1 μm nitrocellulose. Some gels were pre-fixed with 0.4% glutaraldehyde prior to transfer to stabilize Tβ4-actin complexes (33). Blots were stained with Ponceau S (Sigma) to observe protein transfer, blocked in 5% non-fat dry milk (BioRad) in Tris-buffered saline with 0.1% Tween-20 (TBST) and incubated in primary antibody diluted in non-fat dry milk/TBST overnight at 4°C. Afterwards, blots were incubated in peroxidase labeled anti-rabbit IgG (Tβ4a,b) or anti-mouse IgG (actin) (Vector), which was diluted 1:10,000 in non-fat dry milk/TBST and then processed for enhanced chemiluminescence (ECL kit, Amersham, Piscataway, NJ) and exposed to Hyper-film (Amersham).

RESULTS

Immunohistochemistry

Control Brain: Immunoreactive Tβ4 was detected in cells that were morphologically identical to the resident microglia described in early studies by Del Rio-Hortega (9) and Spielmeyer (8), and in more recent studies using different immunohistochemical markers (6, 34) (Fig. 1). The faintly labeled cells in cortex and striatum had round or ovoid cell bodies, approximately 6–8 μm in diameter in the long dimension, and wispy, ramifying processes (Fig. 1a, c). Cells with globoid macrophage-like morphology were not identified. Resident microglia in the subcortical white matter were more intensely labeled than microglia in the cortical gray matter (not shown). In the cerebellar cortex, resident microglia were more robustly stained and had more variable morphologies than those in the cerebral cortex.

HD Brain: Labeled cells with morphologies identical to those of activated microglia as described in other studies (6, 8, 9) were identified by Tβ4 immunostaining within the striatum and the cortex of all HD cases. The activated cells were easily recognized because they were larger and much more intensely immunoreactive than resident microglia in control brains (Fig. 1b, d). Cell bodies of activated microglia were 12 to 30 μm compared to 6 to 8 μm for resident microglia and in the HD striatum were generally round or ovoid. Throughout the HD neostriatum, the processes of activated microglia were thicker, longer and more branched than resident microglia (Fig. 2a–c). In the medial caudate, a region of severe neuronal loss, Tβ4 labeled cells exhibited large amoeboïd-like cell bodies with no processes or with short stout extensions (Fig. 1b). In the cortex, the predominant reactive microglial cell was rod-shaped (Fig. 1d); globular cell bodies with radiating processes also occurred. A striking characteristic of the reactive cells in low-grade HD was the appearance of prominent large swellings in the processes (Fig. 3a, b). Isolated immunoreactive appendages of these reactive cells appeared in the neuropil of grade 1 and grade 2 cortex and striatum (Fig. 3a, b).

The number of activated microglia in the striatum and cortex correlated with the extent and progression of neuronal loss (Table). Grade 2 and grade 3 brains, which show greater cell loss compared to grade 1 brains (2), had more reactive microglia than grade 1 cases. In the striatum of grade 1 brains, collections of reactive cells were conspicuous mainly in the medial caudate, dorsal putamen, and in the cell bridges interdigitating with fiber bundles of the internal capsule. In the grade 2 and grade 3 striatum, reactive microglia showed declining medial to lateral (Fig. 2a–c) and dorsal to ventral gradients. In the grade 1 cortex, patches of reactive microglia appeared in layers 5 and 6 and in the subcortical white matter. Reactive cells were more prevalent and widespread throughout all layers of cortex in grade 2 and grade 3 brains (not shown). Reactive microglia were also markedly accumulated in the globus pallidus of grade 2 and grade 3 HD brains (not shown). There was no difference between the inner and outer pallidum in the extent of activated microglia.

Reactive microglia were present in the subcortical white matter and internal capsule of all HD brains and their presence increased with increasing grade of striatal pathology similar to gray matter regions. Activated cells in the internal capsule were aligned in parallel with fibers (Fig. 2d, e).
Fig. 1. Immunoreactive Tβ4 in controls, C14 and C11, (a and c) and HD brains of grade 3 patients A19 and A21, (b and d). Resident microglia (arrows) in the medial caudate (a) and the cortex (c) of controls are faintly labeled and have small cell bodies and fine, branched processes. Reactive microglia in medial caudate (b) and cortex (d) of HD brain (arrows) are enlarged and intensely immunoreactive. The labeled cells in the medial HD caudate have a macrophage-like morphology and those in the cortex are rod-shaped. Tβ4, antibody was used. Scale bar: a–d = 25 μm.

The processes and cell bodies of reactive microglia were closely associated with neurons (Fig. 3). In the cortex, the rod-shaped microglia processes, which extended 200–300 μm from the cell body, were oriented in parallel with the longitudinal plane of pyramidal cell somata and dendrites and had thickenings at regions in contact with neurons (Figs. 3c, 5b). Cell bodies of reactive microglia rimmed the surfaces of neuronal cell bodies (Fig. 4). Most of the pyramidal neurons contacted by perineuronal reactive microglia were normal in size and morphology but some neurons were shrunken and undergoing phagocytosis. Double labeling with anti-huntingtin antisera revealed that some of the pyramidal cells in contact with reactive microglia had nuclear inclusions (Fig. 4c, d).

The morphology and number of labeled microglia in the HD cerebellum, which is relatively unaffected by the HD mutation compared to the cortex and striatum (1), was generally not different from control brain. One grade 3 HD brain, which had extensive microglia activation in the neocortex, had some rod-shaped activated microglia in the molecular layer of the cerebellum.

The Tβ4, antisera revealed the same immunoreactivity of activated microglia in the HD brain as Tβ4, antisera (Fig. 5a, b), although the extent and intensity of labeling of microglia processes appeared to be lower. Tβ4 immunoreactivity was blocked by co-incubation of Tβ4, antibody with the Tβ4, peptide antigen and co-incubation of Tβ4, antibody with purified Tβ4 (not shown). Staining
Fig. 2. Immunoreactive Tβ4 in neostriatum and internal capsule. a, b, and c show medial caudate, lateral caudate, and putamen, respectively, of HD grade 3 patient A21. The density of reactive microglia from medial to lateral neostriatum correlates with the decreasing gradient of neuronal loss described by Vonsattel et al (2). Activated microglia immunoreactive for Tβ4 in the internal capsule at the level of neostriatum of control patient C14 (d) and HD grade 3 patient A22 (e). Note the marked increased Tβ4 immunoreactivity in white matter of HD brain compared to control. Tβ4 antibody was used. Scale bars: a–c = 50 μm; d, e = 25 μm.

was unaffected when the antibodies were pre-incubated with an unrelated peptide.

To further assess microglia activation in the HD brain, some tissue sections were treated with monoclonal antibody CR3/43, which recognizes human MHC class II antigens in reactive microglia (14, 26). Antibody CR3/43, which recognizes reactive microglia in human brain, labeled cells morphologically identical to those seen with Tβ4 antibody, although the intensity and density of labeled cells was somewhat less than that detected with Tβ4 antibody (Fig. 5c).

Reactive microglia have been described in AD brain with other antisera. To determine whether Tβ4 antisera recognize activated microglia in the AD brain, sections from the temporal cortex of an AD brain and an age-matched control brain were examined with Tβ4 antisera and with monoclonal antibody CR3/43. As expected, activated microglia, including those with rod shapes were detected with all 3 antisera in temporal cortex of AD brain, whereas only resident microglia were seen in the control brain (Fig. 6).

Western Blots

Western blots of protein extracts from control and HD brain showed that the Tβ4 and Tβ4a antisera recognized a prominent band at about 41 kD (Fig. 7a) or, in some
Fig. 3. Morphology of reactive microglia in HD brain. a: Grade 1 HD brain from patient A13, cortex layer 6: the first reactive microglia to appear in grade 1 cortex have prominent swellings (arrow) in the processes and some are associated with blood vessel walls (cell at upper left). b: Grade 2 HD dorsal putamen from patient A17. Tb4 immunoreactive structures (arrow) occur throughout the neuropil in a region of early neuronal loss. c: Rod-shaped microglia in layer 5 cortex of grade 3 HD brain from patient A21. The cell body (large arrow) has characteristic fusiform shape and extends a long vertically oriented process toward the dorsal surface of the brain in parallel with the orientation of pyramidal cells (arrowheads) which are revealed by counterstain with cresyl violet. Thickening of microglia processes (small arrows) at points of contact with neurons. Tb4, antibody was used. Scale bars: a, b = 25 μm; c = 25 μm.

cases, a series of 3 to 5 bands that migrated between 35 and 48 kD (Fig. 7b, top panel). The 41-kD band or the multiple bands were significantly reduced when Tβ4 antisera were incubated with Tβ4 peptide or purified bovine Tβ4 and were unaffected when co-incubated with an unrelated peptide (Fig. 7b). No signal was detected in brain at 5 kD, which is the approximate size predicted for the full length 43 amino acid Tβ4 peptide alone, even with Tricine gels which enhance the detection of low molecular weight proteins, or when gels were fixed with glutaraldehyde prior to protein transfer to stabilize Tβ4 (33). To determine whether the higher molecular weight bands seen in brain at around 41 kD with Tβ4 antisera were complexes of Tβ4 bound to actin, we cross-linked purified Tβ4 (generous gift of Dr. D. Safer, University of Pennsylvania) and G-actin (see methods), and analyzed by Western blot the cross-linked proteins alongside brain protein extracts. Results showed that both Tβ4 antisera recognized a cross-linked actin-Tβ4 complex of about 41 kD—the same size as the Tβ4 immunoreactive band seen in brain (Fig. 7c). Both Tβ4 antisera also recognized purified unbound Tβ4, which ran as a broad band at about 5 kD. This band was blocked in the presence of purified bovine Tβ4 on Western blot (Fig 7d). An antisera to G-actin also detected the same actin-Tβ4 complex at 41 kD and unbound actin at about 42 kD (results not shown). These results showed that although the Tβ4 antisera were capable of recognizing purified Tβ4 in vitro, the Tβ4 immunoreactivity detected in control and HD brain was a complex formed with monomeric G-actin.

DISCUSSION

This study found that there are numerous activated microglia in the HD brain. The immunoreactive cells had the morphology of activated microglia and were labeled by 2 antisera to the actin binding protein, thymosin β-4,
and antibody CR3/43 which recognizes microglia in the human brain (14, 26). The Tβ4 antisera did not appear to recognize globoide macrophages and these cells are not included in our definition of activated microglia. Very few globoide macrophages are seen in hematoxylin and eosin-stained sections of HD caudate (J.P. Vonsattel and M. DiFiglia, unpublished observations) possibly because the neuronal death is very slow. We found activated microglia more frequent with increasing grade of striatal pathology in both the striatum and cortex. These cells appeared early in the disease, in regions known to be the first affected such as the medial caudate, dorsal putamen, and cell bridges of the internal capsule. The distribution of reactive microglia to regions of neurodegeneration in HD suggests that the presence of these cells is related to the disease process and not to random pre-morbid events. In the HD cortex, there is a grade-dependent increase in activated microglia (this study) and in the formation of inclusions (35). This suggests that mutant huntingtin accumulation may influence the appearance of reactive microglia. Reactive microglia contact inclusion-bearing neurons in the HD brain, further supporting an association between the accumulation of mutant huntingtin and a microglia response. Furthermore, the activation of microglia by abnormal neuronal proteins has been recognized in other neurodegenerative diseases (21).
The presence of activated microglia in the HD striatum and cortex was somewhat unexpected since they were not mentioned in previous histopathological studies of the HD brain using conventional methods. With the appropriate antibody markers, immunohistochemistry provides a more sensitive and discriminating method of detecting different types of glial cells than histological methods (34). In their morphometric study, Rajkowska et al (4) found a significant increase in the number and size of non-neuronal cells in the HD cortex, although the type of cell was not identified. Our findings suggest that the non-neuronal cells in the latter study may have included reactive microglia. A marked proliferation of reactive astrocytes labeled with glial acidic fibrillary protein (GFAP) occurs in the HD striatum and is correlated with the gradient of striatal neurodegeneration (2). In contrast, astrocystosis is not seen in the HD cortex in regions of neuronal loss (3, 36), suggesting that microglia contribute to the main glial response in the HD cortex. An increase in the number of microglia but not astrocytes has been seen in the cortex of schizophrenic brain (34).

What features of mutant huntingtin expression trigger the widespread and chronic appearance of reactive microglia in the HD brain? Mutant huntingtin accumulates in the nucleus and cytoplasm of neurons and causes the formation of intranuclear inclusions and dystrophic neurites (27, 28, 35). All of the adult onset HD cases used in this study (except the HD grade 4 case) were shown in our previous work to exhibit an aberrant accumulation of mutant huntingtin in 1 or more subcellular neuronal...
Fig. 6. Reactive microglia in AD temporal cortex (top panel) and resident microglia in control temporal cortex (bottom panel). Reactive microglia, including rod-shaped cells (arrows) are detected in AD brain with Tβ4 (left), Tβ4h (middle), and CR3/43 (right) antisera. Scale bar = 25 μm for all images.

MICROGLIA IN HUNTINGTON DISEASE BRAIN

compartments (27, 28, 35). We showed that neurons with inclusions were contacted by reactive microglia, an indication that inclusions may be a stimulus for microglia activation. However, neurons with inclusions are relatively sparse in adult onset HD and account for only 5%–7% of neurons in the HD grade 2 and grade 3 cortex (27). Other forms of mutant huntingtin accumulation may be important in stimulating the microglia response. Dystrophic neurites containing ubiquitin (37) and mutant huntingtin (27) are prevalent in the cortex and striatum of low-grade HD brains (35) and may be the stimulus for the early appearance of Tβ4 immunoreactive swellings that develop in activated microglia. The N-terminal fragments of mutant huntingtin accumulate in degenerating neurites before they appear in nuclear inclusions (28) resulting in changes in the HD neuropil before the loss of neuronal cell bodies. The widespread appearance of activated microglia in the white matter of HD brain also suggests a response to axonal pathology and is consistent with the early loss of projection pathways (38, 39) and the profound reduction in the volume of white matter in the HD brain (40, 41). The time-course of microglial activation in the CNS is longer than that in the PNS (42) and is highly dependent on the type of insult. Excitotoxic injury, which is a model of HD pathogenesis, induces persistent and long-term microglial activation in the brain (43), in contrast to the more rapid and short-lived microglial response seen following stereotaxic lesions (44). Reactive microglia occur at both the site of excitotoxic or ischemic injury and along the axons that are distal from the injured neurons (45, 46). Fiber tract (fornix) transection also causes a persistent long-term (up to 1 yr) microglial activation (47). Therefore, axonal pathology rather than neuronal loss may trigger and sustain widespread microgliosis in the HD cortex and striatum.

The mechanisms involved in mutant huntingtin induced microglia activation are speculative but might involve multiple signaling pathways in the neuron (48). One pathway may involve the proteasome, which interacts with mutant huntingtin (49, 50) and is known to be involved in antigen presentation at the cell surface (51). There is also evidence in cultured cells that polyglutamine aggregates are secreted (52). As extracellular components, the huntingtin aggregates might activate microglia and be removed by phagocytosis. The activated microglia could also become neurotoxic. In vitro studies show that exogenously presented beta amyloid and prion peptides stimulate signal transduction cascades in microglia cells through the stimulation of CD40 receptors (53), the activation of tyrosine kinases Lyn and Syk, followed by PKC and ERK dependent pathways (21). This activation cascade causes expression and release of neurotoxic factors from the stimulated microglia cells (20, 21). Activated microglia also have neurotrophic properties under some experimental conditions (10). This could explain the regenerative changes that occur in some HD
Fig. 7. Biochemical characterization of Tβ4 antisera. a: Western blot of protein extracts from control brain (C18) show that both Tβ4 antisera strongly detect a protein that migrates at about 41 kD (arrow). b: Western blots with preadsorption controls demonstrate specificity of Tβ4 antisera for high molecular mass products. Protein extracts from grade 3 HD brain (A12) were probed with Tβ4A (top) or Tβ4D (bottom) antibodies alone (left column) or in the presence of appropriate blocking peptide (middle column), or an unrelated peptide (right column). Immunodetection of the 41 kD product is significantly reduced in the presence of antigen. Note that in the upper panel, bands of about 35 kD and 45 kD are detected with Tβ4, antibody and that these bands are also substantially reduced in the presence of the blocking peptide. c: Western blot probed with Tβ4, antibody of a sample obtained after cross-linking G-actin and purified bovine Tβ4, and of protein extracts from HD brain, which were run in neighboring lanes on SDS-PAGE. A product of similar molecular mass, about 41 kD (arrow) is seen in both lanes. d: Western blot probed with Tβ4A antibody shows unbound bovine Tβ4 running as a broad band (arrow, left) at the expected size of about 5 kD. The product is greatly reduced when the Tβ4A antibody is pre-incubated with purified Tβ4 (middle) but is unaffected when Tβ4A antibody is incubated with an unrelated peptide. Molecular mass standards in kilodaltons (kD) are on the left.

Our results show that antisera directed to epitopes in the actin-binding protein Tβ4 are excellent tools for detecting activated microglia in the human disease brain as they are in the injured rodent spinal cord (25). Actin regulation is important in cell growth and Tβ4 is the primary regulator of actin assembly through the inhibition of actin polymerization (24). Rapid growth of cells including activated microglia may require increased stabilization of unpolymerized actin (G-actin) by actin binding proteins such as Tβ4. Consistent with this idea, Tβ4 mRNA is highest in the rat brain during development and is decreased significantly after birth (56, 57). A 10-fold increase in Tβ4 mRNA occurs in PC 12 cells when they are differentiated with nerve growth factor (58). Carpintero et al (59) observed increased levels of Tβ4 mRNA associated with reactive gliosis in degenerating regions of rat forebrain after treatment with the excitotoxin kainic acid. The marked increase in Tβ4 immunoreactivity in reactive microglia in HD brain, especially in the processes of these cells at early stages of pathology, makes it a particularly useful indicator for the study of disease progression.

Previous studies showed that Tβ4 binds in a 1:1 molar ratio with unpolymerized actin monomers (G-actin) (23). In Western blots of brain, the Tβ4 antisera recognized a prominent protein product of about 41 kD that was similar in size to the complex formed when G-actin and Tβ4 were cross-linked in vitro (60; this study). The Tβ4 antisera recognized purified (unbound) Tβ4 at about 5 kD in Western blot but not in brain tissue. This suggests that most, if not all, of the Tβ4 immunoreactivity seen in resident and reactive microglia in brain by immunohistochemistry is Tβ4 bound to actin. These results are consistent with the critical role of Tβ4 in actin sequestration.

In summary, our results suggest that activated microglia are an important pathological correlate associated with neurodegeneration in the HD brain. Although the signaling pathways are unknown, it is likely that in addition to its direct effects in neurons mutant huntingtin aggregates contribute to neurotoxicity indirectly by stimulating the activation of microglia. Based on recent findings in Alzheimer disease, medications that attenuate the inflammatory response may be effective for slowing disease progression in HD. Non-steroidal anti-inflammatory drug use is associated with a reduced risk of AD (61) and with a significant reduction in activated microglia (identified using CR3/43 monoclonal antibody) and senile plaques (62). A better understanding of the neurotoxic and neuroprotective effects of activated microglia in HD is needed to assess the potential value of anti-inflammatory drug therapy in the disease.

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