The β-amyloid peptide 1–42 (Aβ1–42), a major component of neuritic and core plaques found in Alzheimer’s disease, activates microglia to kill neurons. Selective modifications of amino acids near the N terminus of Aβ showed that residues 13–16, the HHQK domain, bind to microglial cells. This same cluster of basic amino acids is also known as a domain with high binding affinity for heparan sulfate. Both Aβ binding to microglia and Aβ induction of microglial killing of neurons were sensitive to heparitinase cleavage and to competition with heparan sulfate, suggesting membrane-associated heparan sulfate mediated plaque-microglia interactions through the HHQK domain. Importantly, small peptides containing HHQK inhibited Aβ1–42 cell binding as well as plaque induction of neurotoxicity in human microglia. In vivo experiments confirmed that the HHQK peptide reduces rat brain inflammation elicited after infusion of Aβ peptides or implantation of native plaque fragments. Strategies which exploit HHQK-like agents may offer a specific therapy to block plaque-induced microgliosis and, in this way, slow the neuronal loss and dementia of Alzheimer’s disease.

Alzheimer’s disease (AD) is a neurodegenerative dementia associated with loss of neurons and the appearance of reactive glia (1, 2). The neuropathological hallmarks of this disorder include neuritic and core senile plaques, which are complex aggregations of proteins composed largely of a 42-residue peptide, β-amyloid (Aβ1–42) (3). A preponderance of evidence indicates that a chronic imbalance in the production and clearance of Aβ initiates pathological responses, which include neuritic and synaptic abnormalities, neurofibrillary tangles, and loss of neurotransmitters (4). Although Aβ peptides found in senile plaques are widely believed to damage the nervous system (4), the mechanisms by which these molecules actually drive AD pathology remain uncertain. One pathway for Aβ-induced neuron damage may involve inflammatory cells, for it has long been recognized that reactive microglia are closely associated with neuritic and core plaques (5–8). Since reactive microglia release such bioactive agents as proteolytic enzymes, cytokines, free radicals, and nitric oxide (9–11), the immunopathology of AD is likely to involve microglial release of cellular poisons. Recent studies also show that exposure to neuritic and core plaques stimulated cultured human microglia to secrete a potent neurotoxic amine that was found in the AD brain (7). Moreover, Aβ1–42, the most abundant component of neuritic and core plaques (12) served as the major plaque-derived signal to evoke neuron-killing by microglia (13). As described here, Aβ13–16 (HHQK) is necessary for an initial microglial interaction with plaque through a cell-surface binding site involving heparan sulfate. Small peptides containing HHQK block plaque induction of neurotoxic microglia in vitro and reduce inflammation in vivo. Strategies exploiting HHQK-like agents to suppress plaque-microglia interactions offer a selective means to prevent AD immunopathology.

EXPERIMENTAL PROCEDURES

Cell Culture—Rat microglia were isolated from newborn animals using the method of Giulian and Baker (14), with recovery of a >98% homogenous population monitored by binding of the fluorescent probe, acetylated low density lipoprotein labeled with 1,1-diiododecyl-3,3,3’,3’-tetramethylindocarbocyanine (DiI-Ac-LDL). Human microglia (>98% homogeneity) were obtained from adult frontal cortical tissue isolated within a 6-h postmortem interval as described earlier (7) and grown in chemically defined medium. Cultured neurons prepared from embryonic rat hippocampus (13) consisted of process-bearing neurons (10–20% of total cell population) atop a bed of astrocytes (>70% of the cells) containing microglia (5–10% of the cells). In order to eliminate microglia, cultures were exposed to saporin (a ribosome-inactivating protein) coupled to acetylated LDL (Ac-LDL) (13). Saporin-Ac-LDL selectively bound to scavenger receptors and at 10 μg/ml, after 18 h, reduced microglial numbers to <0.1% of the total population, with no effect on viability of either neurons or astrocytes. After 14 days in vitro, cultures (with a final concentration of 0.6% fetal bovine serum) were exposed to test substances in the presence or absence of exogenous microglia for 72 h. Human microglia (250,000/chamber) were placed in Millicell chambers (0.4-μm filter insert with a 12-mm diameter; Millipore, Bedford, MA) atop cultures of rat hippocampal cultures depleted of microglia. All neuronal cultures were fixed in 3% paraformaldehyde at room temperature for 6 h and immunostained by overnight incubation with a mixture of anti-neurofilament antibodies (SMI-311, 1:150; RT-97, 1:150; Sternberger Monoclonals, Inc.) plus anti-MAP-2 (Boehringer Mannheim, 184959; 1:200) at 4 °C in the presence of 2% horse serum and 0.3% Triton X-100. Data were expressed as percent mean survival in relation to parallel untreated control cultures, after scoring eight randomly selected fields for each of three coverslips from at least three independent experiments.

Preparation of Plaques, Proteins, and Peptides—Native amyloid protein aggregates were isolated from AD neocortical gray matter laden with neuritic and core plaques using discontinuous sucrose gradients, with recovery of neuritic/core plaque fragments from the 1.4/1.7 M sucrose interface (3). Native Aβ monomers (a mixture of Aβ1–42 and Aβ1–40) were extracted from these plaque fragments and separated by gel electrophoresis. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

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Aβ Peptides Block Induction of Neurotoxic Microglia

**Fig. 1.** Microglial activation requires a specific Aβ domain. Panel A, testing of various Aβ peptides in a neurotoxicity assay using hippocampal cultures supplemented with microglia showed a 70–80% killing of neurons after exposure for 72 h to human Aβ1–40, or Aβ1–42. Elimination of microglia from the cultures prevented neuron death. The pattern of neuron killing by synthetic peptides was similar to that elicited by native Aβ monomers purified from plaques. Interestingly, rodent Aβ1–40 (Gly3, Phe10, and Arg13) did not activate microglia. The Aβ peptides containing either the N terminus of the peptide (Aβ1–11, Aβ1–16, Aβ13–16, and Aβ1–28) or C terminus (Aβ17–42, Aβ25–35) alone were also inactive. Panel B, the capacity of Aβ1–42 (1 μmol/liter) to activate microglia was examined after modification of the N-terminal region by chemical or enzymatic methods. Altering residues in the 13 to 16 domain blocked the Aβ1–42 induction of neurotoxic microglia. Cyclohexanedione (CHD) modification of Arg6; tetranitromethane (TNM) modification of Tyr10; DEPC modification of His6, His13, His26 with hydroxylamine used to reverse the DEPC effect; transglutaminase (TNG) modification of Gln13; ethyl acetimidate (EAM) modification of Lys20 and Lys23; Aβ1–26Gln13Gln14 represents a synthetic peptide with replacements of His13 and His14.

**A**

- native Aβ monomer
- Aβ13-16 human
- Aβ11 human
- Aβ16 human
- Aβ1-1  human
- Aβ1-28 human
- Aβ17-42 human
- Aβ1-40 human
- Aβ1-40 rodent
- Aβ25-35 human

**B**

- Aβ1-42 No Modification
- CHD (Arg5)
- TNM (Tyr10)
- DEPC (His6,13,14)
- DEPC hydroxylamine
- TNG (Gln15)
- EAM (Lys16,28)
- Aβ1-42Gln13,Gln14

**Binding Assays**—Fluoresbrite carboxylate YG microspheres (1.0-μm diameters; 0.5 ml of 2.5% suspension; Polyscience Inc., Warrington, PA) were activated with 1% carbodiimide for 4 h at room temperature. Washed spheres were resuspended in 0.2 ml borate buffer (pH 8.5) in the presence of 300 μg of Aβ1–42 (SpheresAβ1–42) or 400 μg of bovine serum albumin (SpheresBSA) in 6% MeSO. After overnight mixing at room temperature, the spheres were washed extensively and blocked by 1 M glycine (pH 8.0) for 30 min. Isolated microglia (1,250,000/mm3) adherent to 13-mm glass coverslips in 24-well culture plates were mixed with 250,000 SpheresAβ1–42 in the presence or absence of Aβ peptides or glycosylaminoglycan (chondroitin sulfate and hepanan sulfate; Sigma) at 37°C. Mild trypsinization of microglia (5000 units/ml; Sigma) was carried out at 37°C for 60 min. Prior to binding, trypsin was inactivated by the addition of soybean trypsin inhibitor (500 μg/ml; T9003 Sigma) at a ratio of 1 mg of inhibitor to 10,000 units of enzymatic activity, and followed by multiple washings of cell cultures with medium containing 10% fetal bovine serum. Enzyme-treated control cultures included microglia that were incubated for 60 min with trypsin immediately inactivated by trypsin inhibitor prior to addition to cultures. Microglia were also exposed to heparin sulfate (heparin lyase, EC 4.3.3.4, from ICN; two consecutive treatments each of 0.01 unit/ml for 60 min) and AChD (chondroitin ABC lyase, EC 4.3.3.4, from Sigma; two consecutive treatments each of 0.01 unit/ml for 60 min) and chondroitinase (chondroitin ABC lyase, EC 4.3.3.4, from Sigma; two consecutive treatments each of 0.01 unit/ml for 60 min) and AChD (chondroitin ABC lyase, EC 4.3.3.4, from Sigma; two consecutive treatments each of 0.01 unit/ml for 60 min) and chondroitinase (chondroitin ABC lyase, EC 4.3.3.4, from Sigma; two consecutive treatments each of 0.01 unit/ml for 60 min) and chondroitinase (chondroitin ABC lyase, EC 4.3.3.4, from Sigma; two consecutive treatments each of 0.01 unit/ml for 60 min) and chondroitinase (chondroitin ABC lyase, EC 4.3.3.4, from Sigma; two consecutive treatments each of 0.01 unit/ml for 60 min). The glycosaminoglycan synthesis inhibitor, 4-methylumbelliferyl-β-D-xyloside (β-D-xyloside) was acquired from Sigma. Binding assays were carried out at 37°C for 4 h in chemically defined culture medium after which coverslips were dipped 10 times in phosphate buffer and fixed with 4% buffered paraformaldehyde. Microsphere adherence to cells was scored at x 200 magnification with phase/fluorescence microscopy. Data, expressed as mean percent inhibition of sphere binding, was calculated as (1 – (total number of spheres per field of treated group – background binding) / total number of spheres per field of untreated control cultures – background binding)) × 100%. Background binding was defined as nonspecific adherence of unmodified Fluoresbrite microspheres exposed to sister cultures under identical conditions. All values were based upon five coverslips from at least three independent experiments.

**In Vivo Studies**—Adult albino rats (250 g; Holtzman, Madison, WI) were anesthetized by intraperitoneal injection with 0.4 ml/100 g of body weight of a mixture containing chloral hydrate, 42.5 mg, and pentobarbital, 8.9 mg, in 1 ml of distilled water. Neuritic/core plaque fragments suspended in artificial cerebral spinal fluid (62.5 mM NaCl, 125 mM KCl, 0.62 mM NaH2PO4, 12.5 mM NaHCO3, 1.32 mM CaCl2, 1.07 mM MgCl2, 12.5 mM MgCl2, 12.5 mM MgCl2) were mixed with equal volumes of Aβ1–5 (1...
mmol/liter) peptide, of Aβ13–16 (1 mmol/liter) peptide, or of artificial cerebral spinal fluid alone. Solutions of synthetic human Aβ1–42 (1 mmol/liter) peptide were mixed with 2 volumes of Aβ1–5 (1 mmol/liter), 2 volumes of Aβ13–16 (1 mmol/liter), or 2 volumes of cerebral spinal fluid alone. Plaque suspensions (4 μl, total volume) using a 22-gauge catheter or Aβ solutions (1.5 μl, total volume) using a 33-gauge syringe were deposited bilaterally into the neocortex at 4.0 mm posterior to the bregma, 3.5 mm lateral from the midline, and 1.3 mm below the surface of the brain at a rate of 0.25 μl/min. Animals were sacrificed 7 days after injection with 0.8 ml/100 g of body weight of the anesthetic mixture, followed by cardiac perfusion with 50 ml of phosphate-buffered saline (pH 7.4) containing 25 units/ml heparin. Brains were then cut by razor blades into 2-mm thick sections using a coronal rat brain matrix (Ted Pella Inc., Redding, CA) and incubated in cell culture medium containing 250 ng/ml Dil-AC-LDL for 12 h at 37 °C (21). Slices were then fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, placed in 30% sucrose for 24 h, frozen, and cut into 32-μm thick sections. Each brain provided 50–60 serial sections. Labeled cells were viewed by fluorescence microscopy (523–557-nm excitation/595-nm emission).

**RESULTS**

**Specific Regions of Aβ Are Required for Inducing Microglial Toxicity—**As reported earlier (13), native Aβ monomers isolated from senile plaques induced microglia to release neuron killing factors (Fig. 1A). Although the synthetic forms of human Aβ1–40 and Aβ1–42 were also potent stimuli for neurotoxic microglia, rodent Aβ1–40 was not (Fig. 1A). Since the only differences between the human and rodent peptides are found at residues 5, 10, and 13 (Arg5 → Gly; Tyr10 → Phe; His13 → Arg), it appeared that the N-terminal portion of human Aβ held some primary structure necessary to stimulate microglia. Accordingly, we altered full length human Aβ1–42 to mimic the rodent form. Cysteine cross-linking of the residue Arg5 or tetranitromethane (TNM) modification of Tyr10 had no effect upon Aβ activation of microglia (Fig. 1B), suggesting that neither residues 5 nor 10 are essential for cell stimulation. In contrast, diethylpyrocarbonate (DEPC) modifications of His6, His13, and His14, eliminated neuron killing. Reversal of the DEPC modifications by hydroxylamine restored Aβ as a stimulus, pointing to a need for the His residues. The inability of synthetic peptide Aβ1–42Gln13Gln14 to elicit neurotoxic responses (Fig. 1B) focused attention upon His13 and His14. Exploring residues neighboring His13 and His14, we found that both transglutaminase (TNG) cross-linking of Gln15 to ethylamine or acetylimidation of Lys16 and Lys28 by ethyl acetylimidate also blocked Aβ1–42 induction of neurotoxic microglia (Fig. 1B). Although residues His13, His14, Gln15, and Lys16 (the HHQK domain within Aβ) were required for induction of neurotoxic microglia, the HHQK peptide by itself did not trigger neuron killing (Fig. 1A). It was possible, therefore, that the HHQK domain served as a binding site allowing full length Aβ1–42 to interact with microglia. This hypothesis is supported by the fact that Aβ17–42 did not activate microglia while Aβ10–42 elicited neuron killing similar to that of Aβ1–42 (Fig. 2A).
TABLE I

Small peptide blockade of neurotoxic microglia

| Peptide     | Amino Acid Sequence | Blocked SpheresAβ1-42 binding | Blocked Aβ1-42 toxicity |
|-------------|---------------------|------------------------------|------------------------|
| β3-28       |                      | NA                           | NA                     |
| β1–10       |                      | +                            | NA                     |
| β1–20       |                      | +                            | +                      |
| β1–30       |                      | +                            | +                      |
| β1–40       |                      | +                            | +                      |
| β1–41       |                      | +                            | +                      |
| β1–42       |                      | +                            | +                      |
| β1–5        |                      | +                            | +                      |

HHQK Binding to Microglia Utilizes Membrane-associated Heparan Sulfate—To delineate further the role of the HHQK domain during Aβ binding to microglia, it was first necessary to produce a stable β-amyloid target for cell-based assays. Because Aβ peptides can rapidly aggregate when mixed with cell culture medium and precipitate from solution (12, 13, 22), we coupled peptides to 1-μm diameter fluorescent microspheres. These peptide-coupled spheres, or “artificial plaques,” were then added in concentrations of 250,000/well to homogeneous populations of cultured microglia (1,250 cells/mm²) at 37 °C; the binding of the spheres to cells was monitored by fluorescence microscopy. In general, spheres coupled to peptides containing the HHQK domain adhered to microglia far more rapidly than did spheres coated with agents lacking the domain. For example, after 4 h of incubation, 250 ± 11 SpheresAβ1–42 per mm² were bound to cells compared with 20 ± 8 SpheresAβ1–42 or 35 ± 10 SpheresBSA. The importance of the HHQK domain as a recognition site is evident in photomicrographs which show that far more SpheresAβ1–42 bound to microglia than did SpheresAβ1–42 (Fig. 3). Microglial avidity for HHQK-containing artificial plaques persisted throughout long term incubations of 72 h in microglia-neuron co-cultures. Moreover, binding of SpheresAβ1–42 to microglia was markedly reduced by mild trypsinization of intact cells (Fig. 4A), suggesting involvement of membrane surface proteins. Importantly, artificial plaques elicited patterns of microglial activity identical to those elicited by native plaques and by unbound peptides, since SpheresAβ1–42 and SpheresAβ1–42 brought about neuron killing while SpheresAβ1–42 did not (Fig. 2B).

The HHQK domain represents an unusual cluster of basic amino acids that is thought to exist on the hydrophilic surface of Aβ fibrils (22). The availability of this cluster to cell surfaces is of further interest since HHQK also functions as a charged site which binds to heparan sulfate or heparan sulfate-containing glycosaminoglycans (23–25). To investigate the role of heparan sulfate and HHQK binding to cells, we modified the surface membranes of microglia by enzymatic cleavage. Heparitinase eliminated SpheresAβ1–42 binding (Fig. 4A) to microglia while treatment with chondroitin sulfatase had no effect. The selective sensitivity to heparitinase suggested the need for microglia-associated heparan sulfate in the recognition of HHQK. Moreover, when microglia were incubated with excess amounts of soluble heparan sulfate, chondroitin sulfate, or dextran sulfate, only heparan sulfate blocked SpheresAβ1–42 binding to cells (Fig. 4B). To determine if heparan sulfate was needed for induction of neurotoxic behavior, we next reduced levels of glycosaminoglycans in long term co-cultures and tested for neuron killing induced by plaques. Heparitinase treatment alone caused a partial reduction in neurotoxicity (Fig. 4C). Because microglia synthesized glycosaminoglycans during the 72-h assay, we both cleaved cell-surface heparan sulfate and then blocked further glycosaminoglycan production by addition of 1 mM β-d-xyloside (26). Heparitinase plus β-d-xylose prevented neuronal killing (Fig. 4C), while chondroitinase, with or without β-d-xylose, showed only a small effect. These observations suggested that the binding of cell-associated heparan sulfate to the HHQK domain of Aβ was necessary for plaque activation of neuron-killing in microglia.

Since the interaction between Aβ1–42 and microglia involved cell-surface recognition of the HHQK domain, agents that compete with HHQK recognition might reduce Aβ1–42 avidity for microglia. Accordingly, synthetic peptides derived from Aβ1–42 were added to homogeneous cultures of microglia in the presence of SpheresAβ1–42. As shown in Fig. 5, peptides containing the HHQK domain (Aβ10–16, Aβ10–20, Aβ12–28, or human Aβ1–40) effectively suppressed SpheresAβ1–42 binding to microglia, while peptides lacking HHQK (Aβ1–5, Aβ17–42, rodent Aβ1–40, Aβ25–35, or human Aβ1–42Gln13Gln14) did not. Further testing of other derivatives from human Aβ1–42 confirmed that only those peptides containing HHQK influenced Aβ1–42 binding to cells (Table I). Thus, it appeared that competition by HHQK for a binding site found within full length human Aβ, as well as competition by soluble glycosaminoglycan for cell-surface heparan sulfate, inhibited microglial interactions with β-amyloid.

Small Peptides Containing The HHQK Domain Suppress Induction of Neurotoxic Microglia—Since peptides containing the HHQK domain blocked Aβ1–42 adherence to microglia, they might also impede Aβ1–42 induction of microglial neurotoxicity. To test this possibility, various peptides (each at 10
Aβ Peptides Block Induction of Neurotoxic Microglia

Peptides Block Induction of Neurotoxic Microglia

Aβ1–42, coupled to fluorescent microspheres and the SpheresAβ1–42, were incubated with microglia for 4 h following pretreatment of cells with trypsin (5000 units/ml at 37 °C for 60 min) followed by inactivation with soybean trypsin inhibitor, with heparitinase (heparin lyase, EC 4.2.2.8; two consecutive treatments each of 0.01 unit/ml for 60 min), or with chondroitinase (chondroitin ABC lyase, EC 4.3.3.4; two consecutive treatments each of 0.02 unit/ml for 60 min). Binding by SpheresAβ1–42 to microglia was reduced by trypsin and heparitinase, but not chondroitinase. Panel B, competition with ligands again suggested the involvement of a heparan sulfate-containing site on microglia with reduction of binding SpheresAβ1–42 in the presence of heparan sulfate (10 μmol/liter) but not by dextran sulfate (100 μmol/liter) or chondroitin sulfate (10 μmol/liter). Panel C, plaque induction of neurotoxicity in microglia involves a heparan sulfate-containing site. Microglia mixed with hippocampal neurons were treated with combinations of β-D-xyloside (1 mM), heparitinase (0.02 unit/ml), or chondroitinase (0.04 unit/ml), and then exposed to plaques. Enzyme treatments alone, particularly that of heparitinase, brought on some reduction in neurotoxic activity during the 72-h neurotoxicity assay; however, a combination of both enzymatic degradation of heparan sulfate plus competitive blockade of glycosylation by β-D-xyloside completely eliminated plaque activation of microglia.

μmol/liter), were added to neuron cultures containing microglia in the presence of human Aβ1–42 (1 μmol/liter). Only Aβ1–28, Aβ1–16, Aβ10–20, Aβ10–16, or Aβ13–16 prevented neuron killing (Table I, Fig. 6A) elicited either by Aβ1–42 in solution or by SpheresAβ1–42. Increasing concentrations of blocking peptides, as shown for Aβ10–16 (Fig. 6B), reduced both the number of SpheresAβ1–42 binding to cells and the degree of neuron killing. The plateau of induced neurotoxicity suggested further that Aβ1–42 binding sites, which participated in microglial activation, had become saturated (Fig. 6B). Dose-response curves (Fig. 6C) showed two distinct patterns of inhibition, with Aβ1–16, Aβ10–16, and Aβ13–16 the more potent blocking agents (exhibiting ED50 values of about 30 nmol/liter) and Aβ13–20 and Aβ1–28 less potent (ED50 values of 250 nmol/liter). These differences in blocking efficacy might be due to the presence of hydrophobic residues 17–20 (Leu-Val-Phe-Phe) in the less potent blocking peptides. It should also be noted that none of the blocking peptides prevented induction of neurotoxic microglia (27) following exposure to zymosan (yeast-wall) particles or to lipopolysaccharide (Fig. 6D, LPS). Such data indicate that HHQK suppresses only microglial toxicity resulting from Aβ exposure and does not inhibit cell killing capacity in general.

Although HHQK-containing peptides inhibited Aβ1–42 activation of microglia, it remained uncertain whether such protective effects would occur when cells were exposed to native plaques, which consist of complex mixtures of brain proteins including Aβ peptides (13). To test this possibility, rat or human microglia were placed in cell chambers and mixed with neurite/core plaque fragments recovered at autopsy from AD cortical tissues. Peptides Aβ1–16, Aβ13–20, Aβ10–16, and Aβ13–16 each blocked neurotoxic activity of either rat (Fig. 7A) or human (Fig. 7B) cells stimulated by plaques, while peptides lacking the HHQK domain (Aβ1–5, Aβ1–11, Aβ17–42, Aβ25–35, or Aβ36–42) had no effect. Thus, the presence of small peptides containing HHQK not only impair Aβ1–42 recognition by microglia but also plaque induction of neurotoxic cells.

If Aβ1–41 activates rat microglia in culture, it follows that placement of Aβ1–42 into the rodent brain should elicit a similar response. Penetrating injury inflicted by a small needle to the neocortex of adult rat will induce a reactive microgliosis that dissipates within 4 days (21). Synthetic human Aβ1–42 or fragments of native plaques infused into the neocortex will produce a reactive microgliosis extending beyond the interval brought about by simple needle trauma (28). Accordingly, infusions of Aβ peptides or native plaque fragments were used to assess the immunosuppressive effects of HHQK upon β-amyloid-dependent inflammation in vivo. Seven days
after infusion of 0.5 nmol of Aβ1–42 into rat neocortex, injection sites contained a prominent number of reactive mononuclear phagocytes (Fig. 8A). This local inflammation was markedly reduced, however, if 1.0 nmol of Aβ10–16 is co-injected into the same site (Fig. 8B). Importantly, co-injection of 1.0 nmol of Aβ1–5, a control peptide lacking HHQK, did not suppress reactive cells (Fig. 8C). Moreover, neuritic/core plaque fragments placed within the neocortex of rats (Fig. 9A) elicited an inflammatory response that was also attenuated by 1.0 nmol of Aβ13–16 (Fig. 9B). In this way, both in vitro and in vivo experiments confirm that HHQK-containing peptides reduce microglial responses brought on by plaques or by Aβ1–42.

**DISCUSSION**

Gliosis is an important feature of AD pathology and is closely associated with senile plaques and neuronal injury. Histological study shows, for example, that nearly all neuritic and core plaques are surrounded by clusters of reactive microglia (7, 29). In vitro studies confirm that quiescent microglia found in normal brain will become reactive when placed in contact with isolated neuritic/core plaque fragments, as demonstrated by changes in morphology and release of neurotoxins (7). Such cell culture observations support the idea that immune responses contribute to the neuronal pathology of AD. Further study has shown that plaque-activated microglia produce a neurotoxic amine which can also be recovered from brain tissues heavily laden with clusters of reactive microglia (7). Moreover, this toxin was found to destroy hippocampal pyramidal cells. Since the hippocampus participates in memory and cognition (2), it is reasonable to implicate microglia-derived toxins as destroyers of cognitive function in AD patients (13).

To delineate early events of AD immunopathology, we examined plaque activation of cultured microglia. Fractionation studies of native neuritic and core plaques have shown that Aβ peptides, and not such plaque-associated components as α2-antichymotrypsin or apolipoprotein E, were the principal stimulants which drove microglial reactivity (13). Dose response curves revealed that full-length human Aβ1–42 and Aβ1–40 were the most effective stimuli for neurotoxic microglia while...
rodent Aβ1–40 (Arg⁵ → Gly; Tyr¹⁰ → Phe; His¹³ → Arg) was inactive (13). As described here, chemical modifications of synthetic peptides uncovered the significance of human Aβ primary structure by showing that Aβ13–16 (the HHQK domain) was necessary for induction of neurotoxic microglia (Table I). These observations led us to study the binding of Aβ peptides to microglia. Such experiments were hampered by the tendency of Aβ1–42 to rapidly form aggregates and fibrils as it was mixed with cell culture medium. In order to provide a stable reagent for quantitative assays, we coupled Aβ1–42 to microspheres to create artificial plaques suitable for binding studies. Such spheres elicited neurotoxicity, as did native plaques. Excess concentrations of small Aβ peptides containing the HHQK domain prevented microglial binding to full length Aβ1–42 and eliminated induction of microglial neurotoxicity. Moreover, mild trypsinization of microglia suggested that surface proteins

**FIG. 7.** The HHQK domain of Aβ suppresses neurotoxic microglia activated by plaques in culture. Panel A, suspensions of neuritic/core plaque fragments were incubated with rat microglia placed in cell chambers (each chamber containing 250,000 microglia) atop rat hippocampal cultures. Incubation with various Aβ peptides (all at 10 μmol/liter) revealed that only those agents containing the residues 13–16 blocked neuron killing. As shown in panel B, neurotoxic behavior of human microglia (250,000 cells/chamber) was also elicited by either Aβ1–42 (1 μmol/liter) or plaque suspensions placed in the chambers. Microglia did not damage neurons when co-incubated with the protecting peptides Aβ10–16 or Aβ13–16 (10 μmol/liter). The control peptide Aβ1–5 (10 μmol/liter), lacking the HHQK domain, had no protective effect.

**FIG. 8.** The HHQK peptide suppresses Aβ-induced inflammation in rat neocortex. Panel A, fluorescence photomicrograph shows that reactive mononuclear phagocytes (labeled with DiI-Ac-LDL) persisted for 7 days after infusion of 0.5 nmol of human Aβ1–42 into the neocortex of adult rat. This inflammatory response was suppressed by co-infusion of 1.0 nmol of Aβ13–16 (panel B) but not by 1.0 nmol of Aβ1–5 (panel C). The left hemisphere in each of eight animals was infused with Aβ1–42 with the contralateral side receiving mixtures of Aβ1–42 plus either Aβ1–5 or Aβ13–16. Photomicrographs are representative of the inflammatory responses seen in 32-μm thick serial sections recovered from the sites of plaque deposition. Bar = 20 μm.

**FIG. 9.** The HHQK peptide suppresses plaque-elicited inflammation in rat neocortex. Panel A, fluorescence photomicrographs of DiI-Ac-LDL (+) inflammatory cells in rat neocortex 7 days after implantation of neuritic/core plaque fragments. Panel B, contralateral neocortex received plaque fragments plus 2.0 nmol of Aβ13–16. HHQK suppression of plaque-induced inflammation in vivo suggests that a similar protective effect may be achieved in AD brain. The left hemisphere in each of three animals was implanted with plaques while the contralateral side received plaques plus Aβ13–16. Photomicrographs are representative of the inflammatory responses seen in 32-μm thick serial sections recovered from the sites of plaque deposition. Bar = 20 μm.
were involved in this binding interaction. Further work showed Aβ induction of neurotoxicity required heparan sulfate (sensitivity to heparinase and d-xyllose blockade) associated with microglial surfaces for an HHQK binding site. Since other investigators have previously identified residues 12–17 of Aβ (VHHQK) as a binding domain for heparan sulfate (23–25), we suggest that membrane-associated heparan sulfate plays an important role in the immunopathology of AD by promoting plaque accessibility to brain inflammatory cells.

Current thinking holds that Aβ, the major constituent of neuritic and core plaques, participates in the neuron destroying events that cause the dementia of AD. Some laboratories consider Aβ peptides as direct cell poisons (15), while others view these peptides as only one part of a more complex pathogenic process (30). Observations using different in vitro models support either viewpoint. For example, Aβ25–35, a synthetic peptide, will damage a variety of cells including tumor cells, fibroblasts, and astrocytes when applied in μmol/liter concentrations (13, 31, 32). This nonspecific cytotoxic action of Aβ25–35, however, is clearly different from the indirect neuron-killing mediated by Aβ activation of brain inflammatory cells described here. First, the immune-mediated process requires the presence of microglia, while the action of Aβ25–35 is direct and independent of glia (13). Second, drug inhibition studies show the involvement of the neuronal NMDA receptors in microglia-derived toxicity (7, 13), while free radical production appears necessary for the action Aβ25–35 (33). Third, Aβ1–42 will activate neurotoxic microglia at 10 nmol/liter or less (Fig. 2A), while Aβ25–35 kills cells at ≥30 μmol/liter (13). And fourth, the microglia-derived toxicity is selective to neurons, while Aβ25–35 acts indiscriminately upon a wide range of cell types. Since the validity of any disease model will be judged by its approximation of AD pathogenesis, it should be noted that Aβ25–35 has not been found to exist as a natural product either in AD brain or in cultured cells (34–36).

Immune suppression therapies have been suggested to limit neuronal damage brought about by microglia during stroke, trauma, and AD (7, 21, 37–39). We believe that immune-mediated neuron killing, as mimicked by the culture system used here, has many features common to cellular events occurring in AD brain, including a specificity of neuron destruction, selective activation by neuritic/core plaques (versus diffuse plaques), and a high sensitivity to naturally occurring human Aβ peptides (versus rodent peptides). Importantly, this microglia-dependent toxicity model allows further opportunity to search out key steps in the immunopathology of AD and to identify immune suppression strategies applicable to the treatment of dementia. Data presented here form the basis for a novel treatment strategy to suppress microgliosis in AD. Small peptides with the HHQK domain compete with plaques for binding to microglia and in this way block plaque induction of neuron-killing behavior. Importantly, HHQK blockade of neuron-killing has specificity for Aβ and did not prevent microglial activation by other immuno-stimulants such as zymosan and lipopolysaccharide. Such a selective targeting of plaque-microglia interactions is desirable, for it would spare systemic responses to other immune challenges. Agents that mimic the HHQK domain of β-amyloid plaques might block immune-mediated pathology unique to Alzheimer’s disease.

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