Effect of endothelial cell polarity on β-amyloid-induced migration of monocytes across normal and AD endothelium

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Departments of 1Biochemistry and Molecular Biology and 2Pathology, Keck School of Medicine, University of Southern California, Los Angeles, California 90033; 3Center for Aging, University of Rochester, Rochester, Rochester, New York 14642; and 4Department of Physiology, Columbia University, New York, New York 10032
Received 29 June 2001; accepted in final form 6 May 2002

Giri, Ranjit, Suresh Selvaraj, Carol A. Miller, Florence Hofman, S. D. Yan, David Stern, Berislav V. Zlokovic, and Vijay K. Kalra. Effect of endothelial cell polarity on β-amyloid-induced migration of monocytes across normal and AD endothelium. Am J Physiol Cell Physiol 283: C895–C904, 2002; 10.1152/ajpcell.00293.2001.—During normal aging and amyloid β-peptide (Aβ) disorders such as Alzheimer’s disease (AD), one finds increased deposition of Aβ and activated monocytes/microglial cells in the brain. Our previous studies show that Aβ interaction with a monolayer of normal human brain microvascular endothelial cells results in increased adherence and transmigration of monocytes. Relatively little is known of the role of Aβ accumulated in the AD brain in mediating trafficking of peripheral blood monocytes (PBM) across the blood-brain barrier (BBB) and concomitant accumulation of monocytes/microglia in the AD brain. In this study, we showed that interaction of Aβ1–40 with apical surface of monolayer of brain endothelial cells (BEC), derived either from normal or AD individuals, resulted in increased transendothelial migration of monocytes (HL-60 and THP-1) and PBM. However, transmigration of monocytes across the BEC monolayer cultivated in a Transwell chamber was increased 2.5-fold when Aβ was added to the basolateral side of AD compared with normal individual BEC. The Aβ-induced transmigration of monocytes was inhibited in both normal and AD-BEC by antibodies to the putative Aβ receptor, receptor for advanced glycation end products (RAGE), and to the endothelial cell junction molecule, platelet-endothelial cell adhesion molecule-1 (PECAM-1). We conclude that interaction of Aβ with the basolateral surface of AD-BEC induces cellular signaling, promoting transmigration of monocytes from the apical to basolateral direction. We suggest that Aβ in the AD brain parenchyma or cerebrovasculature initiates cellular signaling that induces PBM to transmigrate across the BBB and accumulate in the brain.

amyloid β-peptide; brain endothelial cells; monocytes; platelet-endothelial cell adhesion molecule; receptor for advanced glycation end products

ALZHEIMER’S DISEASE (AD) is a progressive neurodegenerative disease and the most frequent cause of dementia, affecting >5% of the population over the age of 65 yr. The neuropathology of AD is characterized by neuronal loss, numerous intraneuronal deposits of neurofibrillary tangles (NFT), senile plaques composed of extracellular proteinaceous deposits around reactive microglia, neuritic plaques, and cerebrovascular amyloid deposits (27). The major component of senile plaques and cerebrovascular deposits is amyloid β (Aβ), consisting of 39–43 amino acid residues, formed by the proteolytic processing of amyloid precursor protein (APP) (23). The accumulation of Aβ is thought to be an early feature of AD (15). The predominant forms of Aβ are the 1–40 and 1–42 fragments. Soluble Aβ1–40 is the major form of circulating Aβ, whereas amyloidogenic Aβ1–42, the major constituent of senile plaques, is present in minor amounts in the circulation (2, 13, 20, 22).

In Aβ-related cerebral vascular disorders (cerebral amyloid angiopathy (CAA) and the genetic amyloidogenic disease hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D)) and AD, one finds not only increased deposition of Aβ in the brain but also increased numbers of activated microglial cells in the parenchyma and monocytes/macrophages in the vessel wall (11, 25, 28, 31). However, relatively less is understood as to how Aβ in the circulation and the brain causes accumulation of microglia (macrophage-like cells) in the AD brain. Microglia are derived from hematopoietic cells (6, 7). Moreover, recent studies show that bone marrow-derived cells (monocytes) can migrate across the blood-brain barrier (BBB) and differentiate into microglia in the brain parenchyma (3, 16), adding further support to the view that microglial cells in the brain may be derived from peripheral hematopoietic cells.

Recent studies (4, 5) show that Aβ induces migration of monocytes across a monolayer of normal human brain endothelial cells that serves as a model of the BBB. Additionally, we show that the interaction of Aβ with its putative receptor, receptor for advanced glyca-
tion end products (RAGE), causes cellular signaling in normal human brain-derived microvascular endothelial cells (N-HBMVEC), which culminates in the expression of cell adhesion molecules and concomitant adhesion of monocytes. This was followed by the transmigration of monocytes across the human brain endothelial cell (HBEC) monolayer. We also observed that transmigration of monocytes was inhibited by antibody to platelet-endothelial cell adhesion molecule-1 (PECAM-1) (5), a molecule concentrated at endothelial cell junctions (1, 9). However, it is not clear how monocytes/microglial cells accumulate to a greater extent in the brain of AD patients compared with aged-matched controls. We hypothesize that migration of monocytes across the cerebrovascular endothelium in AD is augmented as a result of a differential response to Aβ of the basolateral surface of the endothelium in AD, compared with normal. Previous studies show that high-affinity binding sites for soluble (s) Aβ are preferentially localized to the apical surface of N-HBEC, similar to that observed for insulin (12). Thus interaction of Aβ with its receptor on the basolateral surface in the AD brain may favor migration of monocytes from the blood into the brain, but may be diminished in normal brain vascular endothelium because of reduced or absent Aβ binding to its putative receptor on the basolateral side.

We report here that interaction of Aβ with either apical or basolateral surfaces of an HBEC monolayer, when derived from either normal or AD individuals, differentially affects the transmigration of monocytes. Furthermore, we show here that interaction of Aβ with its putative receptor RAGE expressed on the basolateral surface of AD-HBEC initiates cellular signaling to allow monocytes to transmigrate from an apical to basolateral direction. Additionally, the transmigration of monocytes is blocked by antibody to PECAM-1, indicating the role of PECAM-1 junction molecule in mediating the transmigration of monocytes.

METHODS

Isolation and characterization of HBEC. Human brain capillaries were isolated from small fragments of cerebral cortex obtained from post mortem surgical resections from adults with seizure disorder and AD (66–92 yr) through the Neuropathology Core of the University of Southern California (USC) Alzheimer Disease Research Center (ADRC) as previously described (5). Brain specimens were cut into small pieces and homogenized in DMEM containing 2% fetal bovine serum (DMEM-S) with the use of a Dounce homogenizer with a loose fitting. The homogenate was centrifuged in 15% dextran in DMEM-S for 10 min at 10,000 g. The pellet containing crude microvessels was further digested in a solution containing 1 mg/ml collagenase-dispase in DMEM-S for 1 h at 37°C. Brain endothelial cells (BEC) were fluorescence-activated cell sorter (FACS) sorted using acetylated low-density lipoprotein (LDL) labeled with 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (di-Acetyl LDL) as previously described (12).

Human brain microvessels were plated on gelatin-coated tissue culture dishes or glass coverslips and cultured in RPMI 1640-based medium with 10% fetal bovine serum (FBS), 10% NuSerum, endothelial cell growth supplement (30 μg/ml) (Collaborative Biomedical Products; B&D, Bedford, MA), heparin (100 μg/ml), l-glutamine (2 mM), sodium pyruvate (1 mM), MEM nonessential amino acids, MEM vitamins, penicillin and streptomycin (100 U/ml; Irvine Scientific, Irvine, CA). Cultures were incubated at 37°C in a humid atmosphere of 5% CO2 and characterized as described previously (12). The BEC exhibit a cobblestone morphology and express factor VIII antigen and CD105 (12). These HBEC express γ-glutamyl transpeptidase indicative of BEC characteristics. Moreover, these HBEC populations contained <1% pericytes or glial cells, as determined by labeling with anti-smooth muscle actin and anti-glial fibrillary acidic protein, respectively (12). No microglial presence was detected as assessed by staining with anti-CD11b. HBEC were used between passages 2 and 5.

Cell culture of monocytes. The promyelocytic cell line HL-60 and human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 containing 10% heat-inactivated fetal calf serum (FCS). HL-60 cells differentiate into monocytic-like monocytes after treatment with 1 × 10⁻⁷ M 1a,25-dihydroxyvitamin D₃ (Bi- omol Research Laboratories, Plymouth Meeting, PA) for 3–4 days under culture conditions as described previously (19). We used these cells in the differentiated state for transmigration studies.

Isolation of peripheral blood monocytes. Peripheral blood human monocytes (PBM) were isolated from blood collected in EDTA as the anticoagulant as previously described (5). Briefly, 10 vol of blood sample (30 ml) were mixed with 1 vol (3 ml) of a solution composed of 6% dextran-500 in 0.9% NaCl in a 50-ml conical tube. The tube was allowed to stand at room temperature for 45 min, which resulted in the sedimentation of erythrocytes. The leukocyte-rich plasma was harvested, layered over Nyco-prep media, density 1.068 g/ml in EDTA as the anticoagulant as previously described (5). To either the upper chamber (top or apical side) or to the lower chamber (bottom or basolateral side) was then added Aβ or to the lower chamber (bottom or basolateral side) was then added Aβ₁₋₄₀ synthetic peptide (125 nM), and the contents were incubated at 37°C for time periods ranging from 30 min to 4 h. At the indicated time points, 50–μl aliquots were removed from the bottom compartment of the well. Cells were stained with 0.2% trypan blue, and trypan blue-excluded transmigrated monocytic cells were counted microscopically with the use of a hemacytometer grid. To keep the volume constant in the bottom compartment, an equal amount (50 μl) of medium was added after each removal of monocytes. For inhibition experiments, the pharmacological inhibitors were added 45 min before addition of Aβ₁₋₄₀, to
either the top compartment or the bottom compartment of the Transwell chamber. 

**32P labeling of cells and immunoprecipitation.** HBEC grown to confluence in 100 × 15-mm tissue culture dishes were washed with prewarmed phosphate-free RPMI 1640 (GIBCO/BRL) and radiolabeled with 0.20 μCi 32P (carrier free; ICN Biomedical, Irvine, CA) in 3 ml of the same medium for 4 h at 37°C as previously described (10). Briefly, the 32P-labeled monolayer of HBEC was washed with phosphate-free medium and incubated in 3 ml of the same medium in the presence or absence of Aβ1–40 for the indicated time periods. At the end of incubation, cells were washed, and cell lysate was prepared using 1 ml of lysis buffer containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40 (NP-40), 0.1 mM dithiothreitol (DTT), 0.5% sodium deoxycholate, 2 mM sodium orthovanadate, 50 mM sodium fluoride, 10 mM EDTA, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin A, and 1 μg/ml leupeptin. The lysate was centrifuged at 3,000 g for 10 min. PECAM-1 was immunoprecipitated from the supernatant by a polyclonal antibody to human PECAM-1. The immunocomplex was collected by centrifugation, washed three times with 300 μl of lysis buffer, and then solubilized in electrophoresis buffer [50 mM Tris-HCl, pH 6.8, 2% SDS, 5% (vol/vol) β-mercaptoethanol, 10% (vol/vol) glycerol, and 0.1% bromphenol blue]. The solubilized sample was subjected to electrophoresis on a 10% SDS-polyacrylamide gel followed by autoradiography. Radioactivity in the band corresponding to PECAM-1 was quantitated by scanning with an Ambis Radiographic Imaging Systems scanner (model 101; San Diego, CA).

**Immunohistochemical analysis of the human central nervous system.** AD patients and neurologically normal age-matched controls from the ADRC of USC were evaluated clinically and followed to autopsy. Included were males and females, ranging in age from 66 to 92 yr. Tissue blocks (1 cm³) were obtained postmortem (range 4–7 h; mean 5 h), fixed in 10% neutral buffered Formalin (pH 7.3; Sigma Chemical), and embedded in paraflin or snap-frozen in liquid nitrogen-chilled isopentane. Tissues were sampled from the superior and middle frontal gyrus (Brodman’s area 10) and cerebellum. Sections were stained with hematoxylin and eosin, thioflavin S, or the Gallyas modification of the Bielschowsky silver impregnation method. Thioflavin S-stained sections were viewed through a Zeiss fluorescence microscope with a narrow-band blue/violet filter at 400–455 nm. Diagnosis of AD was according to a modified protocol from the Consortium to Establish a Registry for Alzheimer’s Disease (18).

**Materials.** 1a,25-Dihydroxyvitamin D3 and calycin A were obtained from Biomol Research Laboratories; GF-109203X [protein kinase C (PKC) inhibitor] was obtained from Calbiochem-Novabiochem (San Diego, CA). dL-α-acetyl LDL was purchased from Biomedical Technologies (Stoughton, MA). An antibody to bovine PECAM-1 (XVD2 as ascites fluid) was developed in our laboratory (8), monoclonal antibody to human PECAM-1 (SEW 16) was kindly provided by Dr. Peter Newman (Blood Research Institute, Milwaukee, WI). Polyclonal antibody to RAGE in rabbits was developed in our laboratory (21). All other chemicals, unless otherwise mentioned, were obtained from Sigma (St. Louis, MO). X-ray film (Kodak X-Omat AR) was obtained from Eastman-Kodak (Rochester, NY). Amyloid peptide (Aβ1–40) was custom synthesized at the W. M. Keck facility at Yale University, purified, and characterized by analytical reverse phase HPLC, amino acid analysis, and laser desorption spectrophotometry as described earlier (12). It was dissolved in PBS before use.

**Statistical analysis.** Statistical analysis of the responses obtained from control and Aβ-treated HBEC was carried out by one-way analysis of variance (ANOVA), using Instat 2 (Graphpad, San Diego, CA) software program. The effects of inhibitors on Aβ-induced responses were analyzed by comparing the response of endothelial cells in the presence and absence of inhibitor. Student’s t-test was used for multiple comparisons. P values < 0.05 were considered significant.

**RESULTS**

**Effect of interaction of Aβ with the apical surface of N- and AD-BEC monolayer on the transmigration of monocytic cells.** With increased monocytes/microglial cells in cerebrovasculature and brain parenchyma of Aβ-related cerebral vascular disorders, including AD, we compared migration characteristics of monocytes across the monolayer of endothelial cells derived from either age-matched controls or AD patients. Our previous studies (5) show that Aβ1–40 augmented the adhesion and migration of either monocytic cells (THP-1 or differentiated HL-60 cells) or PBM across the monolayer of N-HBEC. The optimal concentration of Aβ1–40 was 125 nM, which did not affect morphology or cause toxicity to HBEC. As shown in Fig. 1A, addition of Aβ1–40 (125 nM) to the bottom compartment of the Transwell chamber, containing N-HBEC monolayer (apical side), resulted in a time-dependent increase in the migration of THP-1 monocytic cells. There was an ∼2.5-fold increase above the basal level in the migration of THP-1 monocytic cells by the 2-h time point in response to treatment with Aβ1–40 (125 nM). In AD-HBEC, Aβ1–40 (125 nM) also caused a time-dependent increase in the migration of THP-1 cells and showed an approximately twofold increase in transmigration above the basal level by the 2-h time point (Fig. 1A). There was <10% difference in the extent of transmigration of monocytes in response to Aβ1–40 when the same AD patient-derived BEC were used at either passage 4 or 5. These studies demonstrate that Aβ interaction with the apical surface of N-HBEC and AD-HBEC can mediate the migration of monocytes from the apical to the basolateral side. Moreover, Aβ1–40-induced transmigration of monocytes across the monolayer of AD-HBEC was relatively higher compared with N-HBEC at time points from 2 to 4 h (P value < 0.001).

**Effect of interaction of Aβ with basolateral side of N- and AD-BEC monolayer on the transmigration of monocytic cells.** With Aβ peptide in the AD brain increased toward the basolateral side of the cerebrovascular endothelium, we hypothesized that interaction of Aβ with the basolateral surface of the brain vascular endothelial cell monolayer would augment migration of monocytes. As shown in Fig. 1B, addition of Aβ1–40 (125 nM) to the bottom compartment of the N-HBEC monolayer, cultivated in a Transwell chamber, caused a 30% increase in the transmigration of THP-1 monocytic cells above the basal level. However, when Aβ1–40 was
(367 ± 142%; n = 10 for HBEC derived from 6 AD individuals) at 2 h. These results indicate that no significant differences in the migration of HL-60 cells between N- and AD-derived HBEC monolayers occur when Aβ1–40 is added to the top or apical side of the endothelial cell monolayer (P > 0.05). However, there was a substantial increase in the migration of monocytes across AD-HBEC (458 ± 152%; n = 10 derived from 6 AD individuals) compared with the N-HBEC (220 ± 76, n = 6 for HBEC derived from 5 normal individuals) monolayer in response to the interaction of Aβ with the basolateral surface. The data show a significant difference (<0.01) in the migration of HL-60 cells when Aβ1–40 is added to the basolateral side (bottom) between the AD- and N-HBEC monolayers (Fig. 2). These data indicate that Aβ induces a differential response in monocyte migration from the apical to basolateral direction, when Aβ interacts with the basolateral surface of endothelial cell monolayer derived from normal and AD individuals.

Involvement of PECAM-1 and RAGE in the transendothelial migration of HL-60 cells in response to interaction of Aβ1–40 with the apical or basolateral side of endothelial cells. Our recent studies (5) showed that migration of monocytes across the N-HBEC monolayer, mediated by the interaction of Aβ with the apical side, was abrogated by an antibody (Ab) to PECAM-1 (Ab-PECAM-1), indicating the role of PECAM-1 in regulating the flux of monocytes across N-HBEC. We examined whether PECAM-1 also affected the transmigration of monocytes across the AD-HBEC monolayer. As shown in Fig. 3A, addition of monoclonal Ab-PECAM-1 caused 80 ± 10% inhibition in the transmigration of HL-60 cells when Aβ1–40 (125 nM) was added to the apical side, data similar to those observed with N-HBEC (5). Similar results were obtained with polyclonal Ab-PECAM-1 (SEW 16; data not shown). Additionally, we previously observed that Ab-RAGE, a putative receptor for Aβ, inhibited Aβ-induced migration of monocytes across the N-HBEC monolayer. Thus we examined whether Ab-RAGE had any effect in AD-HBEC. Addition of Ab-RAGE (5 µg/ml) reduced by 75 ± 20% the transmigration of HL-60 cells in response to the addition of Aβ1–40 to the apical side (Fig. 3A). Doubling the amount of Ab-RAGE (10 µg/ml) did not further reduce the transmigration of monocytes (data not shown). Addition of an irrelevant Ab-HLA-ABC, used as a negative control, had no effect on the transmigration. These results indicate that in AD-HBEC, the transmigration of HL-60 monocytes mediated by the interaction of Aβ with the apical side involves both RAGE and PECAM-1.

We further examined whether PECAM-1 and RAGE were involved in the transmigration of HL-60 cells in response to interaction of Aβ with the basolateral side of AD-HBEC. As shown in Fig. 3B, Ab-RAGE and Ab-PECAM-1 inhibited 80 and 85%, respectively, the transmigration of HL-60 monocytes. Addition of both Ab-RAGE and Ab-PECAM-1 did not augment further inhibition, indicating that the effects of these antibodies were not additive (Fig. 3B). Studies (30) have dem-

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**Fig. 1.** Effect of interaction of amyloid β-peptide (Aβ)1–40 on the apical and basolateral surface of human brain endothelial cells (HBEC) on the transmigration of THP-1 monocytic cells. HBEC derived from normal (N) and Alzheimer's disease (AD) subjects were grown to confluence on a fibronectin-coated porous membrane of Transwell inserts. To the top compartment of the Transwell chamber, containing monolayer of either N-HBEC or AD-HBEC, was added THP-1 (0.5 × 10^5 cells/well) followed by the addition of Aβ (125 nM) to either the top compartment (A) or the bottom compartment (B). At the indicated time point, aliquots (50 µl) from the bottom compartment of the Transwell chamber were removed, and transmigrated THP-1 cells were counted in a hemocytometer. Data are means ± SD of individual experiments run in triplicate. Aβ1–40-treated AD-HBEC vs. Aβ1–40-treated N-HBEC: ***P < 0.001, **P < 0.01 (n = 3).

To determine whether this effect was unique to BEC derived from the two AD patients or was common to other AD patients or specific to THP-1 monocytic cells, we studied the transmigration of vitamin D3-differentiated HL-60 monocytic cells in six AD-HBEC and five N-HBEC cultures. As shown in Fig. 2, there was an approximately threefold increase in transmigration of HL-60 monocytic cells in response to interaction of Aβ1–40 with N-HBEC (372 ± 139%; n = 11 for HBEC derived from 5 normal individuals) and AD-HBEC (618 ± 142%; n = 10 for HBEC derived from 6 AD individuals) at 2 h. These results indicate that no significant differences in the migration of HL-60 cells between N- and AD-derived HBEC monolayers occur when Aβ1–40 is added to the top or apical side of the endothelial cell monolayer (P > 0.05). However, there was a substantial increase in the migration of monocytes across AD-HBEC (458 ± 152%; n = 10 derived from 6 AD individuals) compared with the N-HBEC (220 ± 76, n = 6 for HBEC derived from 5 normal individuals) monolayer in response to the interaction of Aβ with the basolateral surface. The data show a significant difference (<0.01) in the migration of HL-60 cells when Aβ1–40 is added to the basolateral side (bottom) between the AD- and N-HBEC monolayers (Fig. 2). These data indicate that Aβ induces a differential response in monocyte migration from the apical to basolateral direction, when Aβ interacts with the basolateral surface of endothelial cell monolayer derived from normal and AD individuals.

Involvement of PECAM-1 and RAGE in the transendothelial migration of HL-60 cells in response to interaction of Aβ1–40 with the apical or basolateral side of endothelial cells. Our recent studies (5) showed that migration of monocytes across the N-HBEC monolayer, mediated by the interaction of Aβ with the apical side, was abrogated by an antibody (Ab) to PECAM-1 (Ab-PECAM-1), indicating the role of PECAM-1 in regulating the flux of monocytes across N-HBEC. We examined whether PECAM-1 also affected the transmigration of monocytes across the AD-HBEC monolayer. As shown in Fig. 3A, addition of monoclonal Ab-PECAM-1 caused 80 ± 10% inhibition in the transmigration of HL-60 cells when Aβ1–40 (125 nM) was added to the apical side, data similar to those observed with N-HBEC (5). Similar results were obtained with polyclonal Ab-PECAM-1 (SEW 16; data not shown). Additionally, we previously observed that Ab-RAGE, a putative receptor for Aβ, inhibited Aβ-induced migration of monocytes across the N-HBEC monolayer. Thus we examined whether Ab-RAGE had any effect in AD-HBEC. Addition of Ab-RAGE (5 µg/ml) reduced by 75 ± 20% the transmigration of HL-60 cells in response to the addition of Aβ1–40 to the apical side (Fig. 3A). Doubling the amount of Ab-RAGE (10 µg/ml) did not further reduce the transmigration of monocytes (data not shown). Addition of an irrelevant Ab-HLA-ABC, used as a negative control, had no effect on the transmigration. These results indicate that in AD-HBEC, the transmigration of HL-60 monocytes mediated by the interaction of Aβ with the apical side involves both RAGE and PECAM-1.

We further examined whether PECAM-1 and RAGE were involved in the transmigration of HL-60 cells in response to interaction of Aβ with the basolateral side of AD-HBEC. As shown in Fig. 3B, Ab-RAGE and Ab-PECAM-1 inhibited 80 and 85%, respectively, the transmigration of HL-60 monocytes. Addition of both Ab-RAGE and Ab-PECAM-1 did not augment further inhibition, indicating that the effects of these antibodies were not additive (Fig. 3B). Studies (30) have dem-
onstrated that migration of T cells across activated HBEC is blocked by antibody to intracellular adhesion molecule-1 (Ab-ICAM-1); thus we examined the effect of monoclonal Ab-ICAM-1 on transmigration of HL-60 cells. As shown in Fig. 3B, there was no significant effect by Ab-ICAM-1. Similar findings were obtained using an irrelevant Ab-HLA-ABC. Our results indicate that Aβ-mediated migration of monocytes across a HBEC monolayer from the apical to basolateral direction can occur as a result of the interaction of Aβ with RAGE, which is expressed on both the apical and the basolateral surfaces of AD-HBEC.

Effect of tyrosine kinase and protein kinase inhibitors on the transmigration of HL-60 monocytes across the AD-HBEC monolayer. Our recent studies (5) reveal that inhibitors of PKC and tyrosine kinase blocked Aβ-mediated migration of monocytes across the N-HBEC monolayer. As shown in Fig. 4, Genistein, a tyrosine kinase inhibitor, blocked (~65%) the migration of HL-60 cells in response to the interaction of Aβ1-40 with the basolateral side of the AD-HBEC monolayer. Additionally, we observed that the PKC inhibitor GF-109203X blocked (~50%) the transmigration of HL-60 monocytes. However, the protein phosphatase inhibitor calyculin A did not significantly affect the Aβ-mediated migration of monocytes. Because Aβ-mediated signaling in N-HBEC (5) resulted in increased expression of cell adhesion molecules (CAMs;

Fig. 3. Effect of antibodies to platelet-endothelial cell adhesion molecule-1 (PECAM-1) and receptor for advanced glycation end products (RAGE) on transendothelial migration of HL-60 human monocytic cells across AD-HBEC monolayer. AD-HBEC grown to confluence on Transwell membranes were preincubated with monoclonal antibody (Ab) to PECAM-1 (Ab-PECAM-1; 5 μg/ml), monoclonal Ab-RAGE (5–10 μg/ml), monoclonal Ab-HLA-ABC (10 μg/ml), and monoclonal Ab to intracellular adhesion molecule-1 (Ab-ICAM-1; 5 μg/ml) for 30 min before the addition of HL-60 monocytic cells (0.5 × 10⁶ cells/well). Aβ1-40 (125 nM) was added to either top (A) or bottom compartment (B) of the Transwell chamber. Transmigrated monocytes were counted at 2 h. Data are means ± SD of 3 independent experiments. Aβ1-40 vs. in the presence of Ab: P < 0.001.
ICAM-1, vascular CAM-1 (VCAM-1), and E-selectin through the activation of the transcription factor nuclear factor (NF) NF-κB, determined whether pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB activation, would affect the transmigration of monocytes. As shown in Fig. 4, PDTC reduced by ~70% the transmigration of monocytes in response to the interaction of Aβ1-40 with the basolateral side of an AD-HBEC monolayer. These results indicate that cellular signaling emanating from the interaction of Aβ with RAGE expressed on the basolateral surface in AD-HBEC involves downstream activation of tyrosine kinase and PKC, culminating in amplified transmigration of monocytes.

Aβ1-40-induced phosphorylation of PECAM-1 in HBEC. Treatment of 32P-labeled HBMVEC with Aβ1-40 (125 nM) resulted in a time-dependent (0.5–4 h) increase in 32P incorporation into PECAM-1 (130-kDa band; data not shown). The phosphorylation of PECAM-1 increased to a maximal level at the 2-h time point after exposure to Aβ1-40 (125 nM) and then decreased by the 4-h point. The incorporation of 32P into PECAM-1 at 2 h was approximately fivefold higher relative to untreated HBMVEC at the zero time point (data not shown). As shown in Fig. 5, the addition of GF-109203X (20 nM), a selective PKC inhibitor, reduced by ~90% the Aβ1-40-induced incorporation of 32P into PECAM-1 (P < 0.001). As previously observed (10) in endothelial cells exposed to hypoxia, the addition of phosphatase inhibitor calyculin A (2 nM) with Aβ1-40 resulted in an approximately twofold increase in the incorporation of 32P into PECAM-1 relative to Aβ1-40 alone-treated HBMVEC (Fig. 5). Moreover, we observed that Aβ1-40-induced phosphorylation of PECAM-1 was blocked by Ab-RAGE but not with irrelevant Ab-HLA-ABC (Fig. 5), indicating that phosphorylation of PECAM-1 emanates as a result of cellular signaling on interaction of Aβ1-40 with receptor RAGE expressed on HBMVEC (12). The effect is specific for Aβ1-40, as amyloid peptide with reverse orientation Aβ3-40 had a minimal effect on the phosphorylation.

Effect of interaction of Aβ with the apical and basolateral surfaces of N- and AD-HBEC monolayers on the transmigration of PBM. Studies were undertaken to determine whether transmigration of PBM behaved in the same way as monocyctic cell lines (THP-1 and HL-60) in response to Aβ. As shown in Fig. 6A, addition of Aβ to the apical side of N-HBEC resulted in an approximately threefold increase in the transmigration of PBM at 2 h. However, addition of Aβ1-40 to the bottom compartment of the Transwell chamber, i.e., the basolateral side of the endothelial cell monolayer (N-HBEC), resulted in an ~1.6-fold increase in the transmigration of PBM (Fig. 6A). However, addition of Aβ to the apical or the basolateral side of the AD-BEC monolayer from two different AD patients yielded a significant time-dependent increase in the transmigration of PBM (Fig. 6B). There was an approximately five- and sixfold increase in the transmigration of PBM across the AD-HBEC monolayer at 2 h in response to Aβ added to the apical (top) and basolateral (bottom) side of the endothelial cell monolayers, respectively (Fig. 6B). To examine whether this effect was specific for monocytes, we utilized T cells from a normal human donor. As shown in Fig. 6C, Aβ added from either the apical or the basolateral side did not significantly increase the migration of resting T cells across the AD-HBEC monolayer.

Role of RAGE and PECAM-1 in the transmigration of PBM. Because we observed that transmigration of PBM across AD-HBEC was significantly augmented in response to the addition of Aβ to the basolateral side of the monolayer, we determined whether RAGE and PECAM-1 were involved in this process. As shown in Fig. 6D, the addition of Ab-RAGE and Ab-PECAM-1 reduced transmigration of PBM by ~60 and 80%, respectively. However, a control Ab-HLA-ABC had no significant effect on the transmigration of PBM. These
studies indicate that Aβ interaction with RAGE expressed on the basolateral side of the AD-HBEC may cause cellular signaling culminating in the transmigration of PBM. An Ab-PECAM-1, an adhesion molecule concentrated at endothelial cell junctions, blocks this effect.

**DISCUSSION**

In AD and Aβ-related cerebral vascular disorders (CAA and HCHWA-D), increased deposition of amyloid peptide (Aβ) in both the brain parenchyma and the cerebral vasculature occurs. These changes are accompanied by an accumulation of monocytes/macrophages in the vessel wall and activated microglial cells in the adjacent parenchyma (11, 25). Relatively little is known of the putative relationship of Aβ to the accumulation of these inflammatory cells in the brain. There are two major views regarding the developmental origin of microglial cells, one being that they are derived from neuroepithelial cells (17) and the other...
that they are derived from hematopoietic cells (7). Early studies of Hickey and Kimura (7) showed that peripheral hematopoietic cells (e.g., monocytes) cross the BBB and then differentiate to microglial cells in the brain parenchyma. These observations support the idea that microglial cells can arise from peripheral hematopoietic cells. Recent studies of Egliandis and Mezey (3) give further credence that microglial cells in the brain are most likely derived from hematopoietic cells. They showed that implantation of male bone marrow-derived cells into female mice resulted in the appearance of these male donor cells in the brain 3 days after the implant. After a few weeks, these cells differentiated into microglial cells in the brain as revealed by immunoreactivity toward glial antigenic marker. These studies thus provide compelling evidence that hematopoietic cells cross the BBB and act as microglial progenitor cells. In vivo studies confirm that Aβ induces the activation and migration of monocytes across a rat mesenteric vascular bed (24), indicating that a similar phenomenon occurs in the brain vasculature.

We previously demonstrated (5) that interaction of Aβ at nanomolar concentrations with a monolayer of N-HBEC initiates cellular signaling, leading to adhesion and transmigration of monocytes. Significantly, the intracellular mechanisms of BEC responses to Aβ culminate in the upregulation of CAMs (ICAM-1, VCAM-1, and E-selectin). These CAMs participate in the adhesion of monocytes. The expression of CAMs mediated by Aβ is blocked by Ab-RAGE (5). These results suggest that interaction of Aβ with its putative receptor RAGE, expressed on HBEC, results in cellular signaling that promotes monocytes to adhere to CAMs. These results are in accordance with previous findings (12) in which binding of 125I-labeled Aβ1–40 to a monolayer of cultured endothelial cells derived from normal brain was abrogated by Ab-RAGE. Moreover, these studies (12) also showed that binding of 125I-Aβ1–40 occurred on the apical side of the monolayer of HBEC. These observations imply that interaction of circulating sAβ with the vessel wall could induce signaling and allow PBM to adhere to the cerebrovasculature and subsequently induce their diapedesis into the brain.

The Aβ-induced migration of monocytes across the N-HBEC monolayer was blocked by Ab-PECAM-1 (platelet-endothelial cell junction molecule-1). These results indicate the involvement of PECAM-1 in mediating the transmigration. Our previous studies (19) show that oxidant stress-induced transmigration of monocytes across monolayers of human umbilical vein endothelial cells (HUVEC) was inhibited by Ab-PECAM-1. Thus PECAM-1 plays a role in the transmigration of monocytes. Vaporciyan et al. (26) report that PECAM-1 is concentrated at endothelial cell junctions and may play a role in the transmigration of both neutrophils and monocytes in vivo. The fact that the PECAM-1 molecule is also expressed in both normal and AD brain vasculature (data not shown) and in HBEC (29) suggests that circulating Aβ can induce the migration of PBM across the vasculature of the BBB via endothelial cell junction molecule PECAM-1.

The present work documents that interaction of Aβ1–40 with the apical surface of AD-derived BEC also mediates migration of both PBM and human mononuclear cells (THP-1 and vitamin D3-differentiated HL-60 cells), as has been previously observed with a monolayer of N-HBEC (5). In both N- and AD-derived HBEC, the extent of increase in transmigration of monocytes, in response to interaction of Aβ1–40 with the apical surface, was not significantly different. There was an approximately threefold increase (368 ± 141%) in the transmigration of monocytes from the apical to basolateral direction in response to interaction of Aβ1–40 with the apical surface of the AD-derived HBEC monolayer. Similarly, in the N-HBEC monolayer there was an approximately threefold increase (372 ± 139%) above the basal level in response to the interaction of Aβ1–40 with the apical surface of N-HBEC. Moreover, transmigration of monocytes across the AD-HBEC monolayer, in response to the interaction of Aβ1–40 with apical surface, was inhibited by both Ab-RAGE and Ab-PECAM-1, as we have previously (5) observed with N-HBEC. Our data suggest that in both normal and AD brain vasculature, circulating sAβ1–40 interaction with RAGE expressed on brain endothelium can induce cellular signaling that stimulates monocytes to transmigrate. The cellular signaling involves activation of redox-sensitive pathways, as Aβ1–40-mediated transmigration of monocytes in HBEC has been previously shown to be attenuated by the antioxidants probucol and vitamin E (5). The exact sequence of cellular signaling events downstream of Aβ-RAGE interaction remains to be elucidated.

However, increased numbers of monocytes/macrophages and the clustering of microglia in perivascular space of the cerebrovasculature of patients with AD and Aβ-related disorders (CAA and HCHWA-D) remain intriguing as does the mechanism by which these inflammatory cells accumulate in these diseases (11, 25, 31). Because in AD and Aβ-related disorders (CAA and HCHWA-D) one finds (11) increased amounts of Aβ associated with the basolateral side of the vascular endothelium, we hypothesized that this may have a significant influence on the accumulation of inflammatory cells from the peripheral blood circulation in the vasculature and eventually into the brain parenchyma.

Endothelial cells in vivo are polarized with the apical side interacting with circulating blood cells and the basolateral side interacting with matrix components. In the brain microvasculature, the basolateral surface of endothelial cells can interact with both amyloid peptides associated with the basolateral surface of vascular endothelium and soluble/fibrillar forms of amyloid peptide accumulated in the brain. Here, we show that interaction of Aβ with the basolateral side of the monolayer of AD-HBEC results in an ~4.5-fold increase (458 ± 151%) in the transmigration of monocytes. In contrast, the interaction of Aβ with the basolateral side of the monolayer of N-HBEC resulted in an
approximately twofold increase (220 ± 76%) in the transmigration of monocytes. These results indicate that there are inherent differences in the transmigration of monocytes across an endothelial cell monolayer derived from normal and AD individuals, in response to interaction with amyloid peptide. It remains to be determined whether there are differences in the binding of Aβ and expression of Aβ putative receptors (RAGE, Scavenger Receptor, SR-A; Ref. 12) or an alternate signaling mechanism to cause increased migration of monocytes from the apical to basolateral side in response to the interaction of Aβ with the basolateral side of the AD-HBEC monolayer.

The involvement of both RAGE and PECAM-1 in the transmigration of PBM and monocyctic cells in response to the interaction of Aβ with the basolateral side of the AD-HBEC monolayer is supported by data showing that Ab-RAGE and Ab-PECAM-1 reduce transmigration by ~60 and 80%, respectively. Furthermore, our studies show that interaction of Aβ with the basolateral side of the monolayer of AD-HBEC causes cellular signaling by activation of both tyrosine kinases and PKC, as the pharmacological inhibitor of tyrosine kinase (Genistein) and PKC (GF-109203X) reduce ~65 and 50% of transmigration of monocyctic cells, respectively. Moreover, Aβ-mediated transmigration of monocytes was inhibited by PDTC, an inhibitor of NF-κB activation. These studies indicate that Aβ-mediated activation of PKC in BEC may lead to activation of NF-κB, as has been shown for tumor necrosis factor-α (TNF-α)-mediated activation of PKC in Jurkat cells, leading to activation of NF-κB (14). Our previous studies (19) have shown that oxidant stress (t-butyl hydroperoxide) causes an increase in the transmigration of monocytes across the monolayer of HUVEC and a concomitant increase in the phosphorylation of PECAM-1. Both transmigration of monocytes and phosphorylation of PECAM-1 were inhibited by PKC inhibitor, suggesting that there may be a causal or direct effect of PECAM-1 phosphorylation in the transmigration of monocytes. In the present study, we also observed that Aβ caused an increase in the phosphorylation of PECAM-1 in HBEC, suggesting that phosphorylation of PECAM-1 may have a direct or causal relationship in facilitating migration of monocytes. Overall, these results indicate that amyloid peptide present on the cerebrovasculature or in the brain parenchyma of AD may elicit cellular signaling to allow monocytes to migrate from the lumen side of the BBB, leading to accumulation in the cerebrovasculature and eventually the brain. The migration of monocytes involves PECAM-1 cell junctional molecule expressed on AD-BEC.

Our results show that interaction of Aβ with either the apical or basolateral surface of a monolayer of HBEC initiates cellular signaling leading to transmigration of monocytes from the apical to basolateral direction. We show that the increase in the transmigration of monocytes across BEC monolayers derived from normal and AD individuals is not significantly different in response to the interaction of Aβ with the apical surface. These results indicate that trafficking of PBM across the BBB can occur in response to amyloid peptide present in the peripheral circulation or because of accumulation of amyloid peptide in the brain. However, interaction of Aβ with the basolateral surface of the monolayer of N-HBEC shows a modest increase (2-fold) compared with AD-HBEC, which shows a substantial increase (4.5-fold) in the transmigration of monocytes. The transmigration of monocytes across AD-HBEC, in response to interaction of amyloid peptide with basolateral surface, occurs as a result of interaction of Aβ with the receptor RAGE. Furthermore, we show that PECAM-1 localized at endothelial cell-to-cell junctions regulates the trafficking of monocytes in response to the interaction of amyloid peptide with the apical and basolateral surface of BEC. We suggest that increased diapedesis of monocytes brought about by the interaction of Aβ with the basolateral surface of the vascular endothelium of BBB in AD individuals may contribute to the increased presence of inflammatory cells (monocytes/macrophages) and activated microglial cells seen in Aβ-related vascular disorders such as AD.

This work was supported by National Institute on Aging Grant PO1-AG-16233 (to B. V. Zlokovic).

REFERENCES


