

Amyloid- β Induces Chemokine Secretion and Monocyte Migration across a Human Blood-Brain Barrier Model

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Abstract

Background: Aside from numerous parenchymal and vascular deposits of amyloid β ($A\beta$) peptide, neurofibrillary tangles, and neuronal and synaptic loss, the neuropathology of Alzheimer's disease is accompanied by a subtle and chronic inflammatory reaction that manifests itself as microglial activation. However, in Alzheimer's disease, alterations in the permeability of the blood-brain barrier and chemotaxis, in part mediated by chemokines and cytokines, may permit the recruitment and transendothelial passage of peripheral cells into the brain parenchyma.

Materials and Methods: Human monocytes from different donors were tested for their capacity to differentiate into macrophages and their ability to secrete cytokines and chemokines in the presence of $A\beta$ 1-42. A paradigm of the blood-brain barrier was constructed utilizing human brain endothelial and astroglial cells with the anatomical and physiological characteristics observed in vivo. This model was used to test the ability of monocytes/macrophages to

transmigrate when challenged by $A\beta$ 1-42 on the brain side of the blood-brain barrier model.

Results: In cultures of peripheral monocytes, $A\beta$ 1-42 induced the secretion of proinflammatory cytokines TNF- α , IL-6, IL-1 β , and IL-12, as well as CC chemokines MCP-1, MIP-1 α , and MIP-1 β , and CXC chemokine IL-8 in a dose-related fashion. In the blood-brain barrier model, $A\beta$ 1-42 and monocytes on the brain side potentiated monocyte transmigration from the blood side to the brain side. $A\beta$ 1-42 stimulated differentiation of monocytes into adherent macrophages in a dose-related fashion. The magnitude of these proinflammatory effects of $A\beta$ 1-42 varied dramatically with monocytes from different donors.

Conclusion: In some individuals, circulating monocytes/macrophages, when recruited by chemokines produced by activated microglia and macrophages, could add to the inflammatory destruction of the brain in Alzheimer's disease.

Introduction

The neuropathology of Alzheimer's disease (AD) is characterized by the presence of numerous neuritic plaques containing cores and wisps of fibrillar amyloid- β ($A\beta$) peptide; these plaques are surrounded by activated microglia, reactive astrocytes, and dystrophic neurites (1). Fibrillar

$A\beta$ is also deposited in the vascular walls of intracerebral and leptomeningeal vessels (2). In addition, numerous neurons in the cerebral cortex and subcortical nuclei accumulate neurofibrillary tangles made of paired helical filaments (PHF) derived from the cytoskeletal protein tau (3). These neurons endure apoptotic death that may result from complex interactions between Alzheimer's presenilin mutation, trophic factor withdrawal, and $A\beta$ (4). The extracellular and intracellular presence of these insol-

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uble, nondegradable, and possibly irritating fibrillar structures appears to provoke a sustained inflammatory-like reaction, which is considered contributory to neurodegeneration (5). A large number of inflammatory components have been identified in AD lesions, among them the activated complement proteins (from C1q to C5b-C9 membrane attack complex), acute-phase reactants, cytokines, and chemokines (6). The principal cell involved in this response appears to be activated microglia (7–9). The potential contribution of circulating monocytes/macrophages to the inflammatory process of AD, however, remains to be elucidated.

Under normal circumstances, the brain parenchyma is separated from the circulation by a selective and efficient blood-brain barrier (BBB). Nevertheless, circulating monocytes could traverse the BBB if attracted by powerful chemotactic stimuli, as occurs in some experimental inflammatory states of the brain (10,11) or following intraparenchymal injections of chemokines (12). A breached BBB may also occur as a complication of hypoxic and ischemic episodes resulting from infarctions commonly observed in AD brains (13). A disturbed BBB in AD is also supported by gross anatomical and functional alterations of the cerebral microvessels (14,15) and by the occasional absence or attenuation of endothelial cells (16). In AD, the large number of amyloid deposits often associated with microvessels (17) [in relation to damaged smooth muscle and endothelial cells (16)], and the presence of multiple inflammatory molecules lend support to the possibility of monocytes transmigration into the vicinity of A β deposits. In the present investigation, we used a model of the human BBB that displays physiological regulation of molecular permeability and permits monocyte transmigration (18,19). We used this paradigm to explore the possibility of transmigration of peripheral monocytes that is facilitated by inflammatory cytokines and chemokines induced by A β in macrophages, endothelial cells, and astrocytes in the model.

Materials and Methods

Chemicals

Synthetic A β 1-42 peptide was obtained from California Peptide Research Inc., (Napa, CA). The A β was dissolved as 1.0 mM stock in concentrated dimethylsulfoxide (DMSO, Fluka Chemie AG, Buchs, Switzerland) and kept at -75°C . The solution was negative for endotoxin (<10 pg/ml) by the Limulus Lysate Test (Sigma, St. Louis, MO). The stock solution was diluted in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA)

with 10% fetal calf serum (Hyclone, Logan, UT) as indicated. As a control medium, the tissue culture medium was used alone or with 1% DMSO.

Differentiation of Monocytes into Macrophages

Human blood monocytes were obtained from peripheral blood of healthy donors by a modification of the Recalde procedure (20). In 1 ml of RPMI medium with 10% autologous serum, 1×10^6 monocytes were incubated for 8 days alone or in the presence of A β 1-42 at indicated concentrations or control medium. The adherent cell density was estimated using the 3-(4-5-dimethylthiazol-2-yl)-2,5, diphenyl tetrazolium bromide (MTT, Sigma) assay. In this assay, the medium was removed from the cultures and the remaining nonadherent cells removed by two washes with RPMI medium. To each well, 400 μl of RPMI and 20 μl of MTT solution were added and incubated at 37°C for 4 hr. After removing the medium, 200 μl of acid isopropanol was added to each well and the purple solution was transferred to an ELISA microtiter plate and read at 540 nm. The optical density of medium and cells without MTT was subtracted from the optical density of MTT containing solutions.

Cultures of Peripheral Monocytes

Monocytes (1×10^6) were cultured in 24-well plates using 1 ml of RPMI medium with 10% autologous serum and various concentrations of A β 1-42. After 48 hr incubation, the medium was harvested for cytokine assays. RPMI media containing 10% autologous serum with or without DMSO at 1% or 0.1% were used as controls.

ELISA Assays of Cytokines and Chemokines

Tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-10, and IL-12 levels in the culture media were determined by a sandwich ELISA as previously described (21). Interleukin-1 β , human monocyte chemoattractant protein-1 (MCP-1), and IL-8 levels were determined using Quantikine ELISA kits (R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

Human macrophage inflammatory protein-1 α (MIP-1 α) and MIP-1 β levels were determined by specific sandwich ELISA protocols as described previously (22). Standards were prepared as a 2-fold serial dilution series of recombinant human MIP-1 α and MIP-1 β (R&D Sys-

tems). These chemokine sandwich ELISAs consistently detect concentrations of human MIP-1 α or human MIP-1 β >50 pg/ml.

Blood-Brain Barrier Model

The endothelial-astrocytic BBB model was constructed in 24-well tissue culture plate inserts with a CycloPoreR polyethylene terephthalate membrane bottom with 2×10^6 pores/cm² measuring 3 μ m in diameter (Collaborative Biomedical Products, Bedford, MA). The surfaces of the membrane were coated with rat tail collagen type I and with human fibronectin (Collaborative Biomedical Products) as previously described (19). Brain microvascular endothelial cells were isolated from nonepileptogenic lateral temporal cortex obtained during surgical ablation of epileptogenic foci in the medial temporal lobe. Human astrocytes were isolated as described previously (23). To prepare the model, 3×10^4 human astrocytes (HFA) in 300 μ l of DME-S medium containing Dulbecco's modified Eagle's medium/F12 (DME/F-12, Sigma), 10% fetal bovine serum, 25 mM Hepes, pH 7.4, 0.14% bicarbonate, 50 mg/ml endothelial cell growth supplement (H-Neurex, Upstate Biotechnology), 100 μ g/ml penicillin, 100 μ g/ml streptomycin, and 250 μ g/ml fungizone were plated on the lower surface of the membrane (representing the brain side) with the insert oriented in an upside-down position and the cell suspension contained on the insert by a cut-off bulb from a transfer pipette. After a 4-hr incubation, the insert was placed in the correct position in a 24-well tray and a 310- μ l aliquot of 0.4×10^4 brain endothelial cells in DME-S medium was plated in the upper chamber of the insert (representing the blood side of the model). Then 1 ml of DME-S medium was added to the lower chamber. The BBB model has been shown to have a low permeability coefficient to insulin (0.001 ± 0.0002 cm/min) and a restricted transmigration of monocytes (19).

Transmigration of Monocytes

To assess the monocyte/macrophage migration through the BBB model, 160 μ g of A β 1-42 in 35 μ l of medium was placed in the bottom of the lower chamber of six wells and air dried. Another six wells without A β were used as null controls. To three of the A β containing wells and three of the null control wells, 2×10^4 monocytes in 1 ml DME-S medium were added. After 16 hr of incubation, 5×10^5 monocytes in

DME-S medium were added to each of the 12 upper chambers. After a period of 24 hr incubation, the number of monocytes present in each of the chambers was determined using a hemocytometer chamber.

Results

A β 1-42 Elicits the Differentiation of Monocytes into Macrophages

Human peripheral blood monocytes were cultured in the presence of A β 1-42 and evaluated by the MTT method with respect to differentiation into macrophages. The macrophages were stained positively with CD68 antibody (clone KP-1) using the avidin-biotin-peroxidase technique (DAKO, Carpinteria, CA) (data not shown). Monocytes exposed to various doses of A β ranging from 1.5 to 12.5 μ g/ml differentiated into adherent macrophages with maximum effect at 3–6 μ g/ml, whereas higher doses were increasingly toxic (Fig. 1A). This response was also time dependent (Fig. 1B).

A β 1-42 Induces Secretion of Cytokines by Cultured Monocytes

Human monocytes were cultured in the presence of increasing concentrations of A β to induce secretion of cytokines. At 0.25 μ g/ml, A β had no effect on cytokine secretion. However, significant secretion of cytokines was noticed at 2.5 μ g/ml, with the largest response at 25 μ g/ml (Fig. 2). At this concentration, the secretion of TNF- α by 1×10^6 monocytes amounted to 1400 pg/ml, three times more than that stimulated by the control media containing DMSO (Fig. 2A). At the same concentration of A β (25 μ g/ml), the secretions of IL-6 and IL-12 were significantly increased, representing 140 pg/ml and 10.5 pg/ml, which is equivalent to about 7 and 20 times more than those observed in control values, respectively (Fig. 2B, C). A β 1-42 had no significant effect on the secretion of IL-10 up to 25 μ g/ml (Fig. 2D). A β 1-42 had only small effect on monocyte secretion of IL-1 β : 9.7 pg/ml in control medium, 8.1 pg/ml in medium with 8 μ g/ml A β 1-42, and 25 pg/ml in medium with 25 μ g/ml A β 1-42. On the other hand, bacterial lipopolysaccharide (LPS) (1 μ M) stimulated monocytes to release 293 pg/ml of IL-1 β , whereas LPS with A β 1-42 (25 μ g/ml) stimulated the secretion of 211 pg/ml of IL-1 β by monocytes.

During the course of this investigation, we noticed individual donor fluctuations in the

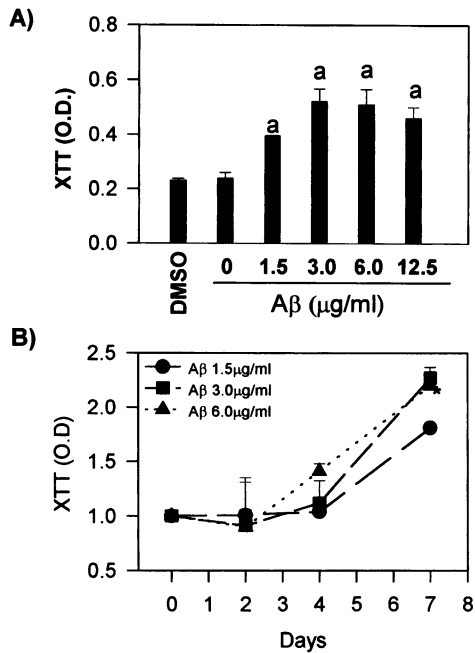


Fig. 1. A β induces differentiation of monocytes into macrophages. (A) Dose-dependent induction of macrophage differentiation by A β 1-42. Monocytes (5×10^5) were incubated for 7 days in the presence of different concentrations of A β 1-42, and the adherent macrophage population was estimated using the MTT assay. ^a, significantly different ($p < 0.001$) from DMSO control and 0 μ g/ml A β . (B) Time kinetics of monocyte differentiation into adherent macrophages induced by A β 1-42. *, 1.5 μ g/ml A β , significantly different from 3.0 and 6.0 μ g/ml A β , $p < 0.05$.

amount of cytokine secretion when the monocytes were stimulated with A β 1-42. Therefore, we investigated these variations in eight individual donors whose monocytes were treated by 2.5 μ M A β 1-42 (Table 1). With respect to TNF- α , monocyte cultures from five out of eight individuals showed a significant elevation of this cytokine, but cultures of two individuals showed an inhibition. Interleukin-6 and IL-12 demonstrated a substantial increase in seven and four donors, respectively. Interestingly, four individual cultures showed significant depression of IL-12 secretion. In the case of IL-10, only one out of eight individuals exhibited a profound rise in A β 1-42-induced secretion (Table 1).

A β 1-42 Induces Secretion of CC and CXC Chemokines by Cultured Monocytes

As with cytokine induction, in comparison to DMSO control, A β 1-42 induced secretion of CXC and CC chemokines in a dose-responsive

fashion, but with striking heterogeneity among donors (Table 2). The largest inductions of MIP-1 α and MIP-1 β were 2278-fold and 529-fold greater when compared with the smallest induction, respectively. The responses to A β 1-42 of monocyte MIP-1 α , MCP-1, and IL-8 secretion were dose related in the range of 0.5 to 50 μ g/ml (Fig. 3).

A β 1-42 with Monocytes Stimulate Monocyte Transmigration Across the BBB Model

The BBB model was utilized to test the potential transmigration of monocytes. As described in detail in Materials and Method, the BBB model consists of a monolayer of human endothelial cells derived from cerebral microvessels and human astrocytes separating the vascular side (upper chamber) from the brain parenchymal side (lower chamber). In a control experiment, 5×10^5 human monocytes were placed in the upper chamber and only medium in the lower chamber. After 24 hr an average of 700 monocytes was found in the lower chamber (Fig. 4). When 2×10^4 monocytes were introduced into the lower chamber prior to the experiment, the migration of monocytes from the upper to the lower chamber increased to an average of 3000. Addition of A β 1-42 (10 μ l, 25 μ M) at the bottom of the lower chamber, followed by medium, resulted in the transmigration of 20,000 monocytes in 24 hr. The transmigration of monocytes increased to 63,000 when the lower chamber contained both A β 1-42 and 20,000 monocytes (Fig. 4). The monocytes remaining in the upper chamber were correspondingly reduced. The increase in the proportion of transmigrated monocytes in the presence of A β and a small number of monocytes varied with different donors (data not shown). Examination of the model by transmission electron microscopy revealed monocyte activation and endothelial cell retraction. Phase contrast microscopy showed macrophages within the holes of the A β dot on the bottom (Fig. 5).

Discussion

A subtle and chronic local inflammatory reaction involving reactive glial cells is one of the major pathological events in the AD brain (5,6). Microglial activation and numerous proinflammatory molecules, including major histocompatibility complex type I and type II cell-surface glycoproteins, proinflammatory

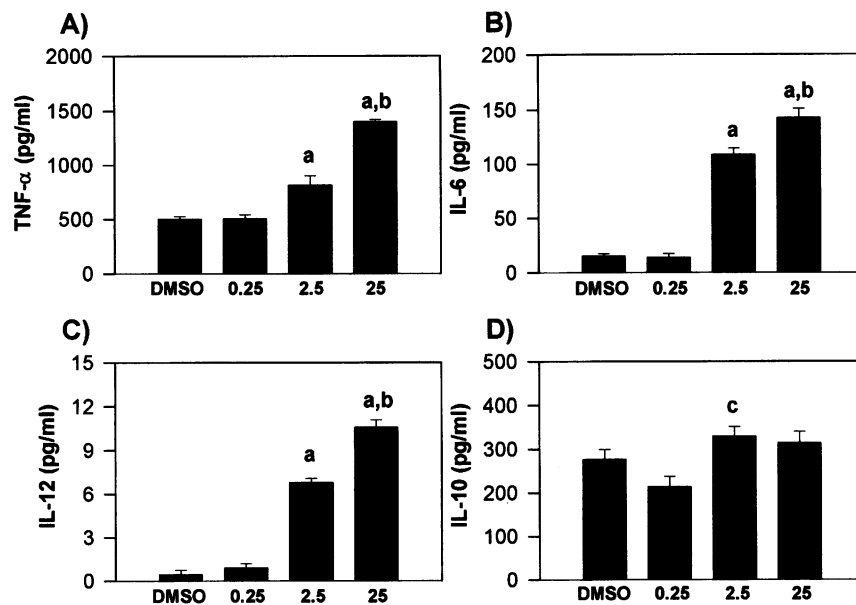


Fig. 2. A β induces monocyte secretion of cytokines. Monocytes (5×10^5) were cultivated in RPMI medium with 10% autologous serum in the presence of indicated concentrations of A β or DMSO. Media were harvested 48 hr later and the concentrations of (A) TNF- α , (B) IL-6, (C) IL-12, and (D) IL-10 were determined by the cytokine ELISA assay. ^a, significantly different from DMSO control and 0.25 μ g/ml of A β , $p < 0.05$; ^b, significantly different from 2.5 μ g/ml of A β , $p < 0.05$; ^c, significantly different from 0.25 μ g/ml of A β , $p < 0.05$.

cytokines, chemokine MCP-1, protease inhibitors, eicosanoids, coagulation factors, cell adhesion molecules, advanced glycation end products, and complement components, have been identified in association with the amyloid deposits and the neurofibrillary tangles (24–29). Recent evidence based on clinical-pathological correlation has suggested that inflammation plays a pivotal role in the pathogenesis of AD and the development of dementia. In a small number of individuals with the number and distribution of histopathological lesions necessary to fulfill the diagnosis of AD (30) but without symptoms of dementia, inflammatory markers and microgliosis were absent (31). In the transgenic mice overexpressing the human amyloid- β precursor protein, despite an im-

pressive number of cortical amyloid deposits, the inflammatory reaction was initially not recognized (32), but it has been recently identified. In another transgenic mouse model expressing the human APP with a familial AD gene mutation, the animals showed microglial activation related to A β deposits, and the microglial activation preceded the onset of neurodegeneration (33). In the AD brain, microglial activation has been shown to be associated with apolipoprotein E (APO E) ϵ -4 gene dosage (34), suggesting that the well-known association of APO E allele ϵ 4 with sporadic AD (35) may be related to the chronic inflammatory reaction in the AD brain.

In vitro cultures of human microglia obtained from rapid autopsies from individuals

Table 1. Effects of A β 1-42 on monocyte cytokine secretion

Donor	TNF- α		IL-6		IL-10		IL-12	
	pg/ml	A β /DMSO %	pg/ml	A β /DMSO %	pg/ml	A β /DMSO %	pg/ml	A β /DMSO %
1	541	62	69	149	343	114	3.4	567
2	249	151	48	138	160	73	0.8	31
3	818	166	109	690	329	133	6.8	850
4	644	90	59	148	433	122	0.8	21
5	7653	1667	504	545	196	86	10.7	369
6	90	766	34	61	20	500	1.0	143
7	2942	1681	6151	9181	1221	51	23.2	19
8	3737	102	860	130	100	88	151.0	34

Table 2. Effects of A β 1-42 on chemokine secretion by cultured monocytes from different donors

Donor	MIP-1 α		MIP-1 β	
	pg/ml	A β /DMSO %	pg/ml	A β /DMSO %
1	104,803	7800	40,270	1034
2	215,458	897,700	70,137	89,000
3	125,118	521,300	41,114	42,800
4	21,106 \pm 5175	42,200	N.D.	
5	112,425 \pm 2904	106,000	40,169 \pm 1,570	11,200
6	142 \pm 39	394	84 \pm 12	168
7	180 \pm 46	900	250 \pm 139	625

with AD secreted 100-fold or more C1q, IL-1 β , IL-6, and TNF- α than those cells from nondemented subjects (36). The secretion of these molecules showed a substantial dose-dependent elevation when the cells were exposed to nM

quantities of A β 1-42 (37). In a similar experiment, human microglia demonstrated the ability to secrete large quantities of C1q following active phagocytosis of A β 1-42 plated at the bottom of the culture well (37).

In this study we have demonstrated the proinflammatory effects of A β 1-42 on peripheral monocytes. In a dose- and time-dependent manner, A β induced the differentiation of monocytes into macrophages and the hypersecretion of inflammatory cytokines, including

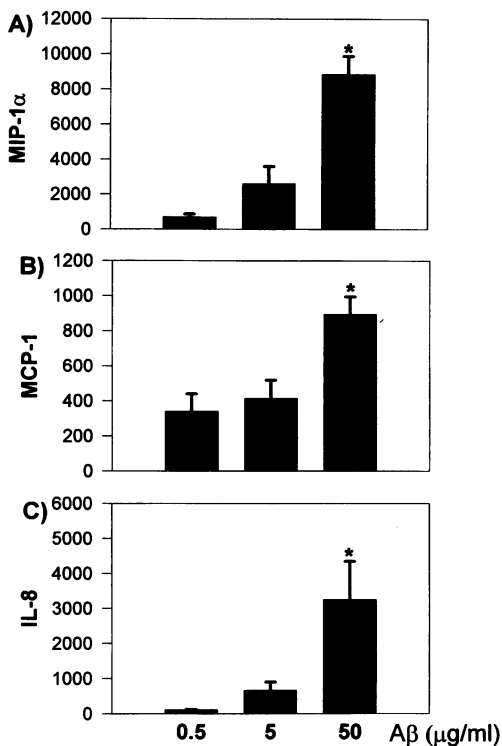


Fig. 3. A β induces monocyte secretion of chemokines. Monocytes (5×10^5) were cultivated in RPMI medium with 10% autologous serum in the presence of indicated concentrations of A β . Media were harvested 48 hr later and the concentrations of (A) MIP-1 α , (B) MCP-1, and (C) IL-8 were determined by the chemokine ELISA assay. *, post-hoc analysis (Fisher's LSD) significantly different from A β 0.5 and 5 μ g/ml, $p < 0.05$.

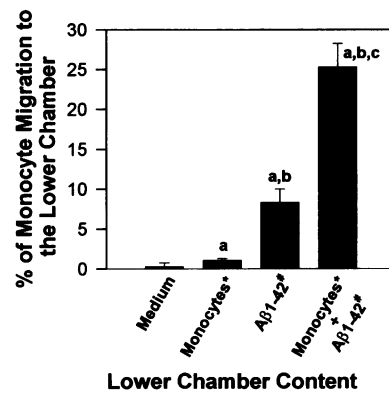


Fig. 4. Effect of A β 1-42 on monocyte migration across the blood-brain barrier model. The BBB models were prepared for migration by placing control medium, a dot of A β (160 μ g), monocytes (2×10^4), or both A β and monocytes in the lower chamber, and incubated overnight. The next day, 5×10^5 monocytes were added into the upper chamber and 24 hr later, cell counts in each chamber were determined using a hemocytometer chamber. *, 2×10^4 of monocytes in the lower chamber; #, 160 μ g of A β 1-42 in the lower chamber; a, significantly different from medium control ($p < 0.05$); b, significantly different from monocyte groups ($p < 0.05$); c, significantly different from A β alone ($p < 0.001$).

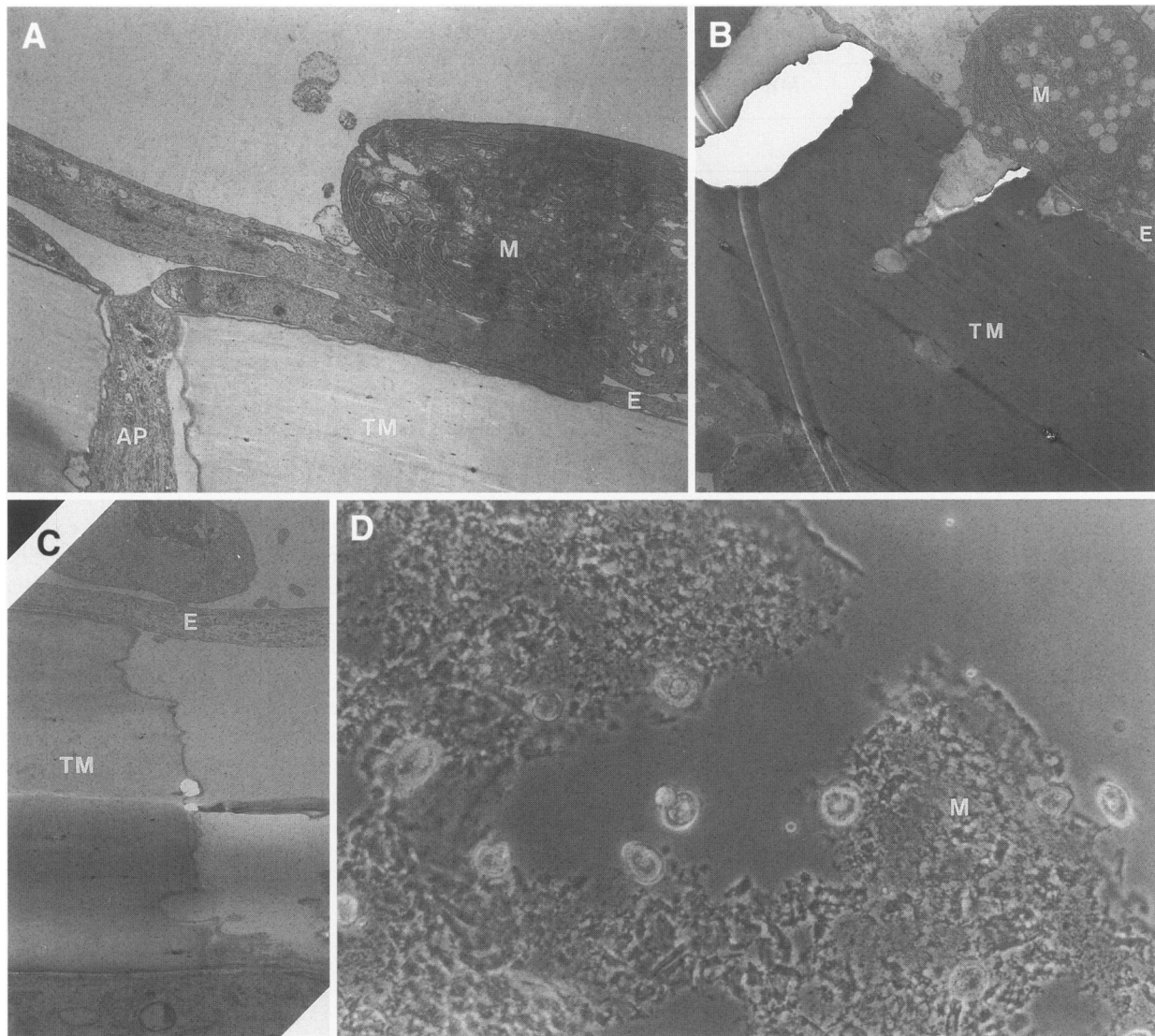


Fig. 5. Microscopic features of monocyte migration across the blood-brain barrier model. The models were constructed with human brain microvascular endothelial cells on the upper surface and human astrocytes on the lower surface of a porous terephthalate membrane. Monocytes (2.5×10^5) were placed in the upper chamber at the onset of migration. Either A β dot with 10,000 monocytes (M) (A and B; transmission electron micrographs, X 9,000) or no A β and no monocytes (C; transmission electron micrograph, X 9,000) was placed in the lower chamber. Note monocyte "activation" with abundant endoplasmic reticulum and adhesion to

endothelial cells (E) and engagement for migration after retraction of endothelial cells (A and B). TM, terephthalate membrane; AP, astrocytic process. In the model without A β or monocytes in the lower chamber, the monocyte in the upper chamber appeared much smaller and without extensive endoplasmic reticulum (C). After transmigration into the lower chamber, the monocytes were seen as differentiated macrophages within the holes of the A β dot. Transmigrating monocyte/macrophages adhere to and lyse A β 1-42 dot on the bottom of the lower chamber (D; phase contrast, X 200).

TNF- α , IL-6, and IL-12, and chemokines IL-8, MCP-1, MIP-1 α , and MIP-1 β . Interestingly, among the different donors, there were wide fluctuations in the amount of cytokine and chemokine secretion elicited by A β , which may be a reflection of the individual's immunological responsiveness.

In the AD brain, a large number of amyloid cores are intimately associated with the endothelial basal lamina and in many instances they appear to totally obliterate the lumen of the vessel (17,38). These cores of fibrillar A β are surrounded by reactive microglia (5,24). MIP-1 α , MIP-1 β , MCP-1, and IL-8 are induced in mono-

cyte cultured in the medium with A β 1-42. The elevated concentration of chemokines in the brain could permit the transmigration of monocytes from the circulation, as illustrated in our BBB model. It is possible that some of the reactive microglia-like cells surrounding the amyloid plaque cores may be derived from peripheral monocytes/macrophages. Transformation of peripheral monocytes into microglia-like cells has been demonstrated in vitro on monolayers of astrocytes (39) and recently in vivo during autoimmune inflammation of the axotomized rat facial nucleus (11). Nonetheless, human microglia isolated postmortem from patients with amyotrophic lateral sclerosis appeared identical to spine-bearing microglia derived from normal adult brain (40).

Transmigration of hematogenous cells through the BBB has been documented in inflammatory processes of the brain, such as infections triggered by bacterial (41) or viral diseases (42,43), as well as by autoimmune diseases, such as multiple sclerosis (44). A breach of the BBB has been induced experimentally after injection of IL-8 and MIP-2 into rodent hippocampus, resulting in the transmigration of leukocytes (12). Gross disruptions in the BBB are also observed in head trauma, hemorrhagic and ischemic stroke (45), acute hypertensive (46) and hypotensive (47) crisis, neurodegenerative diseases (48), and in a variety of toxic conditions. It has been contended that the physical integrity of the cerebral microvasculature and the capacity to repair the BBB may be impaired as a result of aging (49). It has also been proposed that a breakdown of the BBB may be directly responsible for the pathogenesis of sporadic AD or at least synergistically participate with other pathogenetic mechanisms in the development of this dementia (15,50). Chronic monocyte transmigration could also result in subtle damage to the BBB. Interestingly, under normal circumstances a limited amount of A β , bound to a variety of transporter molecules such as the APO J and APO E, crosses the BBB (51,52). Perturbations in the BBB would also allow the passage of larger quantities of circulating A β linked to other already demonstrated carriers such as albumin (53), α 2-macroglobulin (54), transthyretin (55), and amyloid-P component.

In conclusion, numerous studies have shown that A β can invoke the secretion of proinflammatory factors by monocytic cells. In AD, increased chemokine and cytokine concentrations in brain parenchyma may attract circulat-

ing monocytes/macrophage to migrate across the BBB. The recruitment of peripheral monocytic cells into the cerebral cortex could further intensify the already existing inflammatory reaction, but this may be limited to individuals whose peripheral monocytes are hyperreactive to A β . Identification of such individuals among AD patients and the general population may shed light on the role played by the chronic inflammatory reaction in AD.

Acknowledgments

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