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Amyloid-β peptide–Receptor for Advanced Glycation Endproduct interaction elicits neuronal expression of macrophage-colony stimulating factor: A proinflammatory pathway in Alzheimer disease

(microglia/neurons/cerebrospinal fluid)

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ABSTRACT **In Alzheimer disease (AD), neurons are thought to be subjected to the deleterious cytotoxic effects of activated microglia. We demonstrate that binding of amyloid-beta peptide (Aβ) to neuronal Receptor for Advanced Glycation Endproduct (RAGE), a cell surface receptor for Aβ, induces macrophage-colony stimulating factor (M-CSF) by an oxidant sensitive, nuclear factor κB-dependent pathway. AD brain shows increased neuronal expression of M-CSF in proximity to Aβ deposits, and in cerebrospinal fluid from AD patients there was ≈5-fold increased M-CSF antigen ($P < 0.01$), compared with age-matched controls. M-CSF released by Aβ-stimulated neurons interacts with its cognate receptor, c-fms, on microglia, thereby triggering chemotaxis, cell proliferation, increased expression of the macrophage scavenger receptor and apolipoprotein E, and enhanced survival of microglia exposed to Aβ, consistent with pathologic findings in AD. These data delineate an inflammatory pathway triggered by engagement of Aβ on neuronal RAGE. We suggest that M-CSF, thus generated, contributes to the pathogenesis of AD, and that M-CSF in cerebrospinal fluid might provide a means for monitoring neuronal perturbation at an early stage in AD.**

The extracellular deposits of beta-amyloid (Aβ), which accumulate in the brain in Alzheimer disease (AD) as a component of the neuritic plaque, bring about neuronal damage and dysfunction (1–9), and microglial activation through specific interactions with these cells (10–14). Aβ perturbs cellular properties by multiple mechanisms, including induction of oxidant stress, challenging cellular antioxidant defenses and redirecting protein synthesis. Oxidant-related activation of the transcription factor nuclear factor-κB (NF-κB) (15), for example, induces expression of cytokines and cell adherence molecules, thereby modulating cell–cell interactions. Monitoring the formation of gene product(s) whose expression is triggered by oxidant stress resulting from Aβ-neuronal interactions could provide an index of neuronal dysfunction at a stage prior to irreversible cellular damage.

In this study, we demonstrate that binding of Aβ to the neuronal Receptor for Advanced Glycation Endproducts (RAGE) (16) generates oxidant stress, activating NF-κB and inducing expression of macrophage-colony stimulating factor (M-CSF). M-CSF released by neurons stimulates its cognate receptor, c-fms, on microglia, inducing activation with increased expression of the macrophage scavenger receptor (MSR) and apolipoprotein E (apoE). In addition, M-CSF-mediated microglial activation enhances cell proliferation and migration, as well as cell survival in the presence of toxic levels of Aβ. Increased expression of M-CSF in neurons near deposits of Aβ, together with elevated levels of M-CSF in cerebrospinal fluid of patients with AD, suggest that this factor is relevant to the pathophysiology of neuronal degeneration and microglial activation in AD.

METHODS

Cell Culture, RAGE-Related Reagents and Assays for Vascular Cell Adhesion Molecule 1 (VCAM-1) and M-CSF. Primary cortical neurons, microglia, BV-2 cells, transformed murine microglia (17) and human neuroblastoma cells (SK-N-SH, American Type Culture Collection) were grown as described (16). Monospecific rabbit anti-human or murine RAGE IgG (16, 18–19) and anti-VCAM-1 IgG (19) were employed, as well as purified recombinant human soluble RAGE and VCAM-1 (16, 19–20). ELISA for human VCAM-1 utilized a kit from British Biotechnology Products (Oxford, U.K.) and for human M-CSF the kit was obtained from R & D Systems. Northern blot analysis for VCAM-1 and M-CSF transcripts employed full-length cDNA probes (19, 21). Proliferation of BV-2 cells exposed to supernatant from Aβ-treated neuroblastoma cells was studied by counting change in cell number and incorporation of ^{3}H-thymidine. Integrity of BV-2 cells exposed to Aβ was studied by measuring suppression of the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (8). Cerebrospinal fluid samples, obtained within 3–20 hr of expiration of the patients and immediately frozen at −80°C, were from the National Neurological Research Specimen Bank. Synthetic Aβ (1–40, 1–42, or 25–35) was purchased from Quality Control Biochemicals (Hopkington, MA), and Aβ derived from AD brain was purified as described (22). Where indicated, Aβ (1–40) or albumin was linked covalently to Amino Link Coupling gel (Pierce) according to the manufacturer's recommendations. For Aβ and albumin, in each case ≈15 nmol peptide/protein were bound per ml gel. These reagents were not found to contain lipopolysaccharide based on

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Abbreviations: Aβ, amyloid-beta peptide; AD, Alzheimer disease; RAGE, Receptor for Advanced Glycation Endproduct; M-CSF, macrophage-colony stimulating factor; MSR, macrophage scavenger receptor; apoE, apolipoprotein E; CAT, chloramphenicol acetyltransferase; VCAM-1, vascular cell adhesion molecule 1; NF-κB, nuclear factor κB; NAC, *N*-acetylcysteine.

the Endospecy chromogenic assay (limit of detection <10 pg/ml; Seigaku Kogyo, Tokyo).

Expression of apoE and macrophage scavenger receptor (MSR) was studied in BV-2 cells exposed to recombinant murine M-CSF (R & D Systems). Northern blot analysis for the murine type I MSR was performed using the full-length cDNA (obtained from American Type Culture Collection). Western analysis for MSR (23) utilized rabbit anti-murine MSR IgG (Serotec), and the major immunoreactive band on reduced SDS/PAGE (10%) was ≈60 kDa. Immunoblotting for apoE utilized anti-apoE IgG (Biodesign International, Kennebunkport, ME).

Electrophoretic Mobility-Shift Assay and Transient Transfection Assays. Nuclear extract was prepared from cultured neuroblastoma cells (SK-N-SH) exposed to Aβ alone or in the presence of other indicated agents and incubated with double-stranded ^{32}P-labeled oligonucleotide (35 fmol) comprising NF-κB sites (20 bp) from M-CSF (21) or VCAM-1 (19). Samples (5–10 μg/ml protein in each lane) were subjected to nondenaturing polyacrylamide/bisacrylamide (8%) gel electrophoresis followed by autoradiography. Transient transfection employed liposomes and human neuroblastoma cells (SK-N-SH; 5×10^6 cells) with a wild-type M-CSF promoter-reporter gene construct containing the NF-κB sites and chloramphenicol acetyltransferase (CAT) (21) either alone or in the presence of other agents, such as Aβ, NAC, anti-RAGE IgG, or nonimmune IgG. Simultaneous transfection with pSV-β-galactosidase was performed to control for efficiency of transfection, and data are reported as normalized CAT activity. Forty-eight hours after transfection, cell extracts were prepared for CAT and β-galactosidase assays.

Immunohistology and Cerebrospinal Fluid Analysis. Paraffin sections of formalin-fixed temporal lobe from AD and age-matched control brain were first stained with either rabbit anti-heme oxygenase type 1 (1:100; StressGen Biotechnology, Victoria, Canada), rabbit anti-human p50 IgG (5 μg/ml; Santa Cruz Biotechnology), rabbit anti-(α) human-VCAM-1 IgG (20 μg/ml) (19), rabbit α-human M-CSF IgG (5 μg/ml; Santa Cruz Biotechnology), rabbit α-human c-fms IgG (5 μg/ml; Santa Cruz Biotechnology), murine monoclonal α-Aβ IgG (5 μg/ml) (16), mouse anti-human CD68 (Dako) or rabbit/mouse nonimmune IgG (at the same concentration as immune IgG; and isotype-matched nonimmune murine IgG as a control for the murine mAbs) (16). Sites of primary antibody binding were visualized using the avidin/biotin alkaline phosphatase method (Sigma); double staining using antibody to Aβ or CD68 was performed as indicated using peroxidase-conjugated goat α-mouse IgG as the secondary antibody (Sigma). Samples of cerebrospinal fluid were obtained from the National Neurological Research Specimen Bank, and were analyzed using the ELISA for M-CSF.

RESULTS

Expression of M-CSF and VCAM-1 in AD Brain. Evidence of oxidant stress, associated with neuronal injury in AD, is manifested by expression of heme oxygenase type 1, malondialdehyde-lysine epitopes, and nuclear localization of the p50 subunit of NF-κB (16, 24–29). Cellular oxidant stress is observed both in neurons bearing neurofibrillary tangles, and in those without tangles close to Aβ plaques, indicating a relationship between the generation of free radicals and neuropathologic events. Neurons near senile plaques showed increased levels of heme oxygenase type 1 and p50 (Fig. 1 *a* and *c*, respectively); p50 was prominent in many nuclei (Fig. 1*c Inset*), suggesting NF-κB activation had occurred. The brains of age-matched controls, as well as those areas of AD brain without neuritic plaques, only showed minimal amounts of either heme oxygenase type 1 or p50 (Fig. 1 *b* and *d*, respectively). These results suggested that expression of NF-κB-regulated genes that participate in the inflammatory host response (30–32) might be activated in neurons in AD, leading us to focus on M-CSF (21, 33–34) and VCAM-1 (35–37), because of their potential to attract, stimulate, and retain microglia at sites of cellular perturbation. Immunostaining of AD brain showed

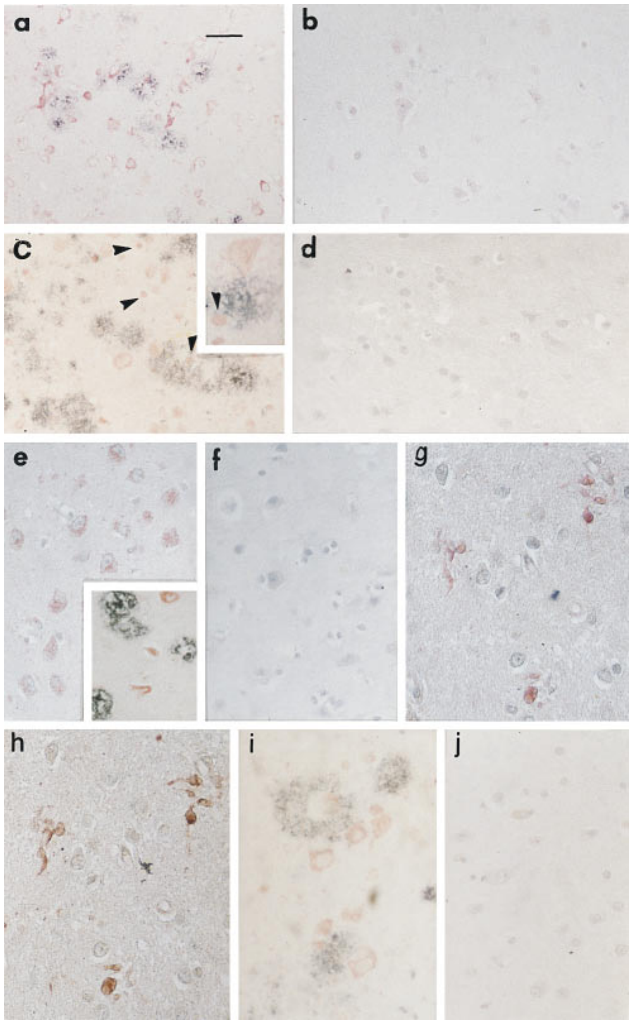

FIG. 1. Expression of markers of oxidant stress (H0–1, *a* and *b*; p50, *c* and *d*), M-CSF (*e* and *f*), and VCAM-1 (*i* and *j*) in neurons proximate to Aβ, and expression of c-fms (*g*) in microglial in AD brain. *a* and *c* demonstrate increased expression of H0–1 and p50, respectively, in neurons of temporal lobe from AD brain, and *b* and *d* show weak staining for the same antigens in age-matched controls. The arrows and inset in *c* show nuclear localization of p50 in certain neurons. *e* and *i* display increased neuronal staining for M-CSF and VCAM-1 in AD brain, respectively, versus low levels of these antigens in age-matched controls (*f* and *j*, respectively). The inset in *e* shows location of M-CSF and plaques in double-stained AD brain (red is M-CSF; black is Aβ). *g* and *h* shows double immunostaining of the same section and depicts increased expression of c-fms in microglia in AD brain (*g*), identified by staining for CD68 (*h*); there is no staining for this antigen in age-matched controls (data not shown). These results are representative of immunohistologic analyses of five AD and four age-matched control brains (postmortem time of 2–6 hr). [Bar = 56 μm (*a–f, j*) and 12 nm (*g–i*).]

M-CSF and VCAM-1 (Fig. 1 *e* and *i*, respectively) colocalized in neurons near accumulations of Aβ. The increased expression of M-CSF in neurons near Aβ deposits is illustrated in the inset in Fig. 1*e* in which double-staining of AD brain shows M-CSF in red and Aβ in black. In contrast, age-matched control brain tissue showed minimal staining for M-CSF and VCAM-1 antigens (Fig. 1 *f* and *j*, respectively). That M-CSF might contribute to the pathogenesis of AD was suggested by the presence of c-fms, the receptor for M-CSF (38) on microglia (39) (Fig. 1 *g* and *h*; show double immunostaining of microglia for c-fms and CD68, respectively), which was absent in age-matched controls (data not shown). However, VLA-4, the counterligand for VCAM-1(40), was poorly expressed on microglia and other cells in AD brain, based on studies with both mono- and poly-clonal antibodies (data not shown).

Expression of M-CSF and VCAM-1 by Neuroblastoma Cells Exposed to Aβ. In view of neuronal expression of M-CSF and the presence of c-fms on microglia, we further studied mechanisms underlying production of M-CSF and its potential effect on cellular targets. Exposure of neuroblastoma cells to synthetic Aβ 1–40 increased expression of M-CSF in a dose-dependent manner, a result of *de novo* protein synthesis inhibited by cycloheximide (Fig. 2 *A* and *B*; synthetic Aβ had no detectable lipopolysaccharide and addition of polymyxin B had no effect on Aβ-induced M-CSF production). Similar results were observed when either synthetic Aβ 25–35 or Aβ purified from AD brain, mainly Aβ 1–42 (22), was studied, and in a limited number of experiments with primary cultures of rat cortical neurons (data not shown). Two critical steps in the pathway through which Aβ induced M-CSF included interaction of amyloid-β peptide with RAGE on the neuronal cell surface and subsequent induction of cellular oxidant stress; M-CSF expression was blocked by anti-RAGE IgG, but not nonimmune IgG, and by the antioxidant *N*-acetylcysteine (NAC) (Fig. 2*B*). Increased production of M-CSF antigen was associated with enhanced levels of M-CSF transcripts (Fig. 2*C*) and activation of NF-κB, the latter shown by gel shift analysis with a probe spanning the NF-κB DNA binding motif in M-CSF (21) (Fig. 2*D*; compare lanes 1 and 2). Appearance of nuclear binding activity in extracts from Aβ-treated neuroblastoma cells was specific for the NF-κB probe, as shown by competition studies [Fig. 2*D*, lane 6; no competition was observed with excess unrelated Sp1 probe (data not shown)], and was prevented by anti-RAGE IgG or NAC (Fig. 2*D*, lanes 3 and 5, respectively). Transient transfection of neuroblastoma cells with a promoter-reporter (CAT) construct bearing the NF-κB site from the M-CSF gene showed increased expression following exposure of cells to Aβ; this was blocked by antibody to RAGE or NAC (Fig. 2*E*). These data indicate that expression of M-CSF in neuronal cells consequent to binding of Aβ to RAGE is driven by oxidant-mediated activation of NF-κB, followed by increased levels of M-CSF transcripts and the protein.

Exposure of microglia to M-CSF resulted in perturbation of a spectrum of cellular properties relevant to the pathologic picture in AD: chemotaxis, expression of MSR and apoE, proliferation and enhanced survival in the presence of toxic levels of Aβ. Stimulation of transformed murine microglia (BV-2 cells) (17) with purified, recombinant M-CSF induced cell migration; this was both dose-dependent and directional, as shown by checkerboard analysis, indicative of true chemotaxis (Fig. 3*A*). Based on these data, we tested the biologic activity of M-CSF in conditioned media of neuroblastoma cells exposed to Aβ 1–40 using the chemotaxis assay. For these experiments, the peptide was immobilized on beads so it could be removed from conditioned media. The observed migration of BV-2 cells in the presence of conditioned medium obtained from neuroblastoma cells exposed to Aβ was blocked by anti-M-CSF IgG, but not nonimmune IgG (Fig. 3*B*). Furthermore, neuroblastoma cells exposed to beads with immobilized albumin did not release chemotactic activity into culture supernatants (Fig. 3*B*). M-CSF also enhanced expression of the MSR in BV-2 cells; Northern analysis displayed increased expression of type I MSR transcripts and immunoblotting demonstrated increased antigen in BV-2 cells incubated with M-CSF (Fig. 3 *C* and *D*, respectively). As MSR has been proposed as a microglial receptor for Aβ (41) and is also expressed at increased levels in AD (42), this suggests a mechanism whereby neuronal stimulation of RAGE could enhance subsequent microglial-Aβ interaction. M-CSF also enhanced BV-2 cell expression of apoE, as shown by the increased level of antigen (Fig. 3*E*). Although multiple cells in AD brain, including microglia, have been shown to produce apoE, possibly underlying its increased expression in AD (43–47), our data suggest a means for increased expression by microglia at the site of Aβ deposits and perturbed neurons. In addition to enhancing cell growth, M-CSF also

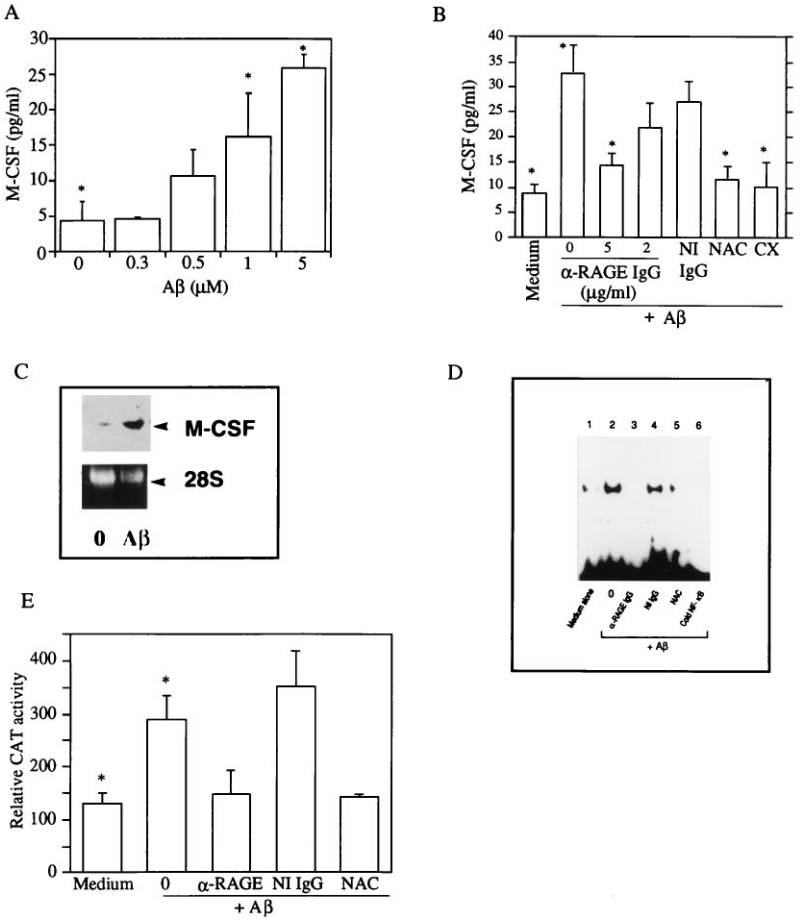

FIG. 2. Expression of M-CSF by human neuroblastoma cells exposed to Aβ. (*A*) Neuroblastoma cells (2×10^6 cells per well; SK-N-SH) in Opti-MEM (GIBCO) were incubated for 8 hr at 37°C with Aβ 1–40 at the indicated concentration, and supernatants were centrifuged and assayed for M-CSF antigen. (*B*) Neuroblastoma cells were incubated with Aβ (1–40; 1 μM) for 8 hr at 37°C alone or in the presence of α-RAGE IgG (5 μg/ml), nonimmune (NI) IgG (5 μg/ml), NAC (20 mM), or cycloheximide (CX; 50 μg/ml). Cultures were preincubated with antibodies or NAC for 1 hr. Results with cultures exposed to medium alone are designated "medium." ELISA was performed on cell-free supernatants as in *A*. (*C*) Northern blot analysis was performed on samples from neuroblastoma cells (5×10^6) incubated with medium alone (0) or medium with Aβ (1–40; 1 μM) for 6 hr at 37°C using ^{32}P-labeled cDNA for M-CSF (*Upper*). (*Lower*) Ethidium bromide staining of the same gel reveals approximately equal intensity of the 28S band. (*D*) Electrophoretic mobility-shift assay was performed on nuclear extracts harvested from neuroblastoma cells (2×10^7) incubated for 6 hr at 37°C in Opti-MEM (medium alone; lane 1) or medium supplemented with Aβ (1–40; 1 μM) alone (lane 2) or Aβ + α-RAGE IgG (10 μg/ml; lane 3), nonimmune IgG (10 μg/ml; NI; lane 4) or NAC (20 mM; lane 5). As indicated, an 100-fold molar excess of unlabeled NF-κB oligonucleotide was added (lane 6). (*E*) Liposome-mediated transient transfection of neuroblastoma cells (1.6×10^6) was performed using a promoter-reporter gene construct comprising the wild-type NF-κB sites from the M-CSF promoter. Where indicated, Aβ (1 μM), α-RAGE IgG (10 μg/ml), nonimmune IgG (10 μg/ml; NI), or NAC (20 mM) were also present (preincubation with these agents was performed as above). "Medium" indicates cultures subjected to transient transfection as above, but incubated in medium alone. Cotransfection was performed with pSV-β-galactosidase and results are reported as relative CAT activity. *, $P < 0.05$ compared with controls incubated with medium alone.

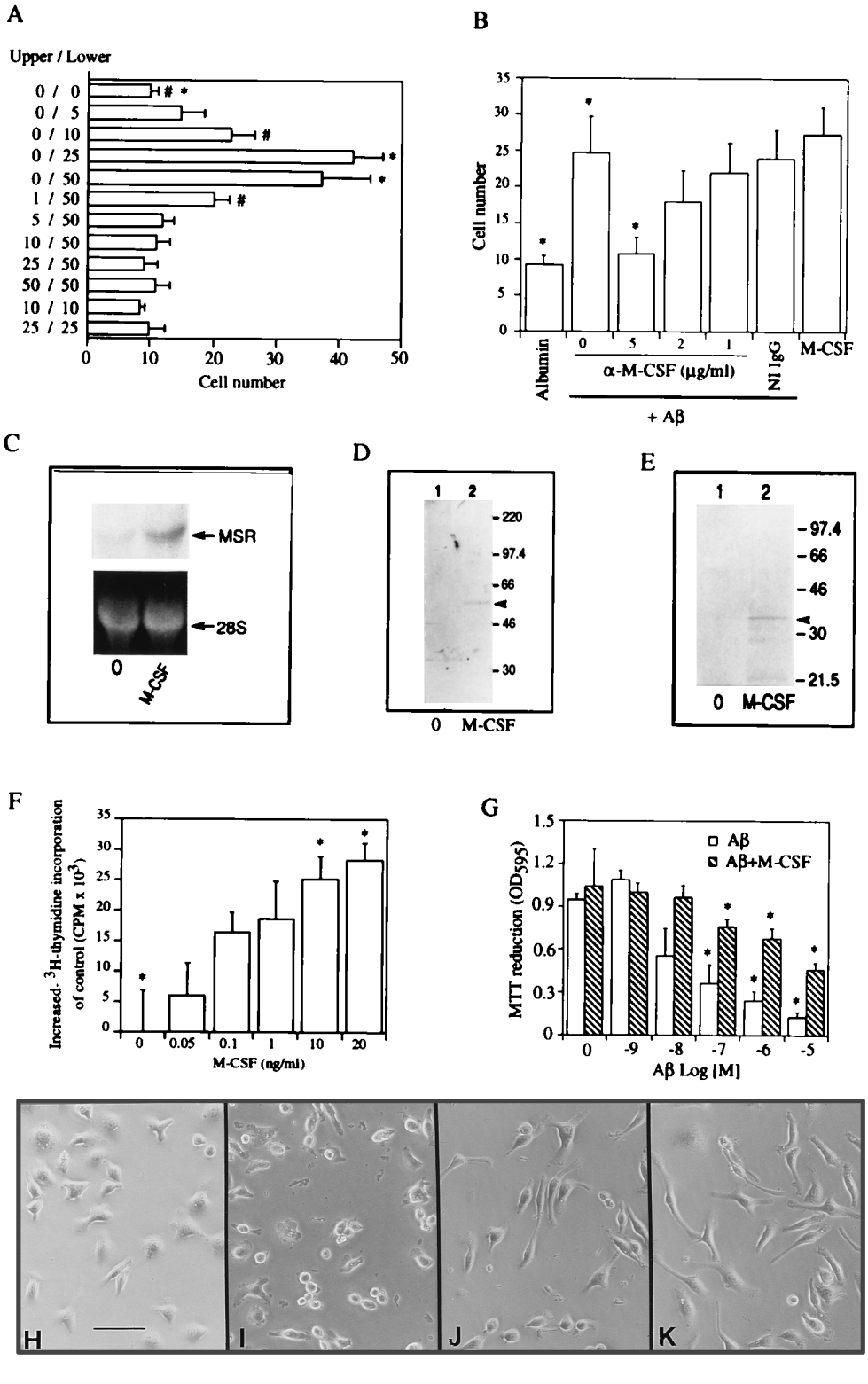

FIG. 3. M-CSF induces activation of BV-2 cells and enhances their survival in the presence of toxic levels of Aβ. (*A*) Chemotaxis of BV-2 cells was studied using microchemotaxis chambers: 1×10^4 cells were added to the upper compartment and M-CSF (ng/ml) was added to the upper and/or lower compartment as indicated. After 1.5 hr at 37°C, cells reaching the lower surface of the chemotaxis chamber membrane were visualized with Wright's stain and counted. Data are reported as cells per high-power field (HPF) based on counting nine fields per well. (*B*) Neuroblastoma cells (2×10^6) were incubated with Aβ (1–40; 1 μM) or albumin (1 μM) immobilized on Amino Link Coupling gel for 8 hr at 37°C. Beads were removed by centrifugation, and the supernatant was used to perform chemotaxis assays as indicated in *A* (above). Where indicated, anti-M-CSF IgG (α-M-CSF; at the indicated concentration) or nonimmune IgG (5 μg/ml) was added. (*C*) BV-2 cells (3×10^6) were exposed to medium alone (0) or medium containing M-CSF (50 ng/ml; M-CSF) for 72 hr at 37°C in Opti-MEM, and samples were harvested for Northern blot analysis using ^{32}P-labeled cDNA probe for murine MSR. The lower panel shows ethidium bromide staining of the same gel, and demonstrates approximately equal loading of RNA. (*D*) BV-2 cells (3×10^6 cells) were exposed to medium alone (0) or medium containing M-CSF (50 ng/ml) for 72 hr at 37°C, and samples were obtained for Western blot analysis (the latter utilized α-MSR IgG, as described in the text). Migration of simultaneously run molecular mass standards is shown on the side of the gel in kDa. (*E*) BV-2 cells were exposed to medium alone (0) or medium containing M-CSF (25 ng/ml), and cell lysates were prepared for reduced SDS/PAGE followed by immunoblotting using antibody to mouse apoE. The arrowhead indicates migration of the immunoreactive band in *D* and *E*). (*F*) BV-2 cells (5×10^4 per well) were incubated in medium alone (0) or medium supplemented with M-CSF (the indicated concentration), and [^{3}H]thymidine incorporation was determined. (*G*) BV-2 cells (as above) were incubated in medium alone (0) or medium supplemented with M-CSF (50 ng/ml) and were exposed to Aβ (25–35) at the indicated concentrations for 24 hr. 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction was determined 4 hr later. *, $P < 0.01$ or #, $P < 0.05$ compared with controls. (*H–I*) Photomicrographs of BV-2 cells incubated in medium alone (*H*), medium + Aβ (1–42; 2.5 μM) (I), medium + Aβ (1–42; 2.5 μM) + M-CSF (50 ng/ml) (*J*), or medium + M-CSF (50 ng/ml) (*K*) after 24 hr. (Bar = 200 μM.)

promoted BV-2 proliferation (Fig. 3*F*), and the survival of BV-2 cells exposed to toxic levels of Aβ (Fig. 3 *G–K*). BV-2 cells incubated with a range of Aβ 25–35 concentrations showed evidence of cellular perturbation based on suppression of MTT (Fig. 3*G*), a general indicator in change of cellular redox status (8), and morphologic changes (for the latter studies Aβ 1–42 was used), including withdrawal of cell processes and rounding up of cell bodies (compare BV-2 cells exposed to Aβ, Fig. 3*I*, with untreated BV-2 cells, Fig. 3*H*). Preincubation of BV-2 cells with M-CSF enhanced MTT reduction in the presence of Aβ by 2–4-fold, though not completely back to levels observed in untreated control cultures at higher concentrations of Aβ (Fig. 3*G*). In parallel, M-CSF treatment allowed BV-2 cells exposed to Aβ to maintain a spread phenotype with extended processes (Fig.

3*J*), resembling the control cultures (Fig. 3*H*). BV-2 cells incubated with M-CSF alone displayed features consistent with differentiation, displaying increased size and extension of cellular processes (Fig. 3*K*). Similar results were obtained in a limited number of studies in which primary cultures of rat microglia replaced BV-2 cells.

Aβ, via RAGE, induces neurons to express a potent activator of microglia, M-CSF. Consistent with expression of genes regulated by NF-κB in neurons in AD, we also observed expression of the cell adherence molecule VCAM-1. This was further analyzed using neuroblastoma cells in which Aβ-induced cell surface VCAM-1 expression was observed to be dose-dependent (Fig. 4*A*). Expression of VCAM-1 by neuroblastoma cells exposed to Aβ was blocked by anti-RAGE IgG, but not by nonimmune IgG, indicating a central role for RAGE (Fig. 4*B*). In addition, NAC inhibited Aβ-induced appearance of enhanced amounts of VCAM-1 in neuroblastoma cells (Fig. 4*B*). Similar to Aβ-induced expression of M-CSF in neurons, oxidant-mediated induction of nuclear binding activity for the NF-κB DNA binding motif in the VCAM-1 promoter (Fig. 4*C*) was observed; a gel shift band of strikingly increased intensity was observed with nuclear extracts from Aβ-treated neuroblastoma cells (compare lanes 2 and 3 in Fig. 4*C*). In addition, elevated levels of VCAM-1 transcripts were seen in neuroblastoma cell cultures exposed to Aβ (Fig. 4*D*). These data delineate a mechanism, triggered by Aβ-RAGE interaction, whereby neurons in AD express VCAM-1.

M-CSF in the Cerebrospinal Fluid of AD Patients. Expression of M-CSF by neuroblastoma cells/neurons exposed to Aβ might act not only to stimulate microglia locally, but, in addition, M-CSF released by perturbed neurons and drained into cerebrospinal fluid might also serve as a marker of ongoing neuronal perturbation in AD patients. ELISA for M-CSF in cerebrospinal fluid obtained from patients with AD (n = 54) showed ≈5-fold increase, compared with age-matched controls (n = 16) ($P <$ 0.01; Fig. 5). There was no correlation between M-CSF antigen in peripheral blood and the presence of AD (data not shown). Although M-CSF levels were not elevated in cerebrospinal fluid from patients with multiple sclerosis (n = 15), the presence of this mediator in cerebrospinal fluid is certainly not diagnostic for AD. For example, there was a smaller increase in M-CSF in cerebrospinal fluid from patients with amyotrophic lateral sclerosis (n = 10), and increased levels of M-CSF from patients with Parkinson disease were comparable to what was observed in AD (n = 14) (Fig. 5). In addition, "control samples" from patients with various tumors, especially those likely to be metastatic to the brain, showed highly variable M-CSF levels in cerebrospinal fluid (data not shown). The cerebrospinal fluid samples used for these studies were obtained postmortem, with times varying from 3–20 hr after expiration of the patient. No correlation was observed in any patient/control group with respect to postmortem time and the level of M-CSF, only the underlying disorder (AD, Parkinson disease, etc.) was a significant determinant of M-CSF antigen in cerebrospinal fluid.

DISCUSSION

Previous studies have shown that Aβ itself directly induces microglial chemotaxis and products of cellular activation, such as cytokines, growth factors, and reactive oxygen and nitrogen intermediates (10–14). Much of the Aβ in AD brain is present in a relatively insoluble form close to neurons, and our studies suggest an indirect mechanism for amplifying Aβ-induced microglial perturbation: binding of Aβ to neuronal RAGE induces activation of NF-κB, which drives expression of M-CSF. M-CSF, a soluble mediator, at least in part, presumably reaches microglia at distant sites, drawing them toward loci of neuronal perturbation and inducing cell activation, including increased proliferation, enhanced expression of MSR and apoE, and increased survival in the presence of otherwise toxic levels of Aβ. The latter findings suggest that local expression of M-CSF in the vicinity of Aβ deposits could account for the presence of increased numbers

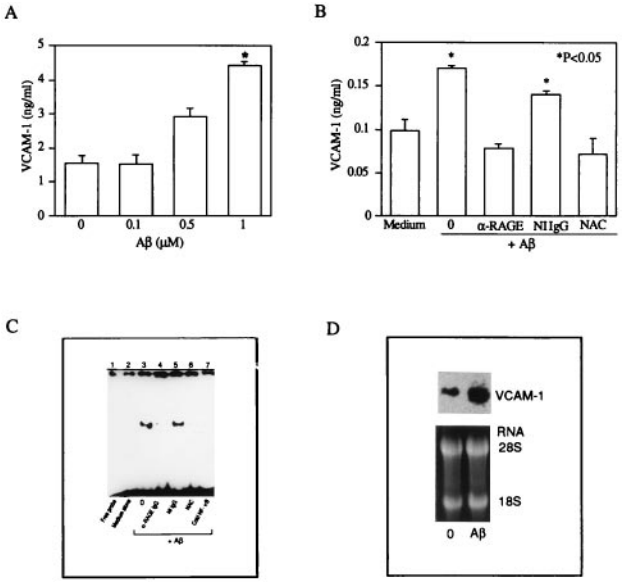

FIG. 4. Exposure of human neuroblastoma cells to Aβ induces expression of VCAM-1 (*A, B,* and *D*) and is associated with activation of NF-κB (*C*). (*A*) Neuroblastoma cells were incubated with Aβ, as described for Fig. 2 *A* and *B*, and expression of VCAM-1 antigen in cell lysates was evaluated by ELISA (55). (*B*) Neuroblastoma cells were incubated with Aβ (1–40) alone (0) or in the presence of anti-RAGE IgG (α-RAGE), nonimmune (NI) IgG or NAC as in Fig. 2, and then ELISA for VCAM-1 was performed. "Medium" denotes cultures exposed only to medium (i.e., no other additions). *, $P < 0.05$. (*C*) Neuroblastoma cells (2×10^7 per lane) were incubated with Aβ (1 μM) and nuclear extracts were prepared and incubated with ^{32}P-labeled oligonucleotide from the VCAM-1 promoter for gel shift assay. The same procedure as in Fig. 2*D* was followed except that lane 1 contains free probe in this case. (*D*) Neuroblastoma cells (10^7) were incubated with medium alone (0) or Aβ (1–40; 1 μM) for 6 hr at 37°C, RNA was harvested and subjected to Northern blot analysis using ^{32}P-labeled cDNA probe for VCAM-1. (*Lower*) Ethidium bromide staining of gels to visualize RNA loading.

of microglia expressing apoE and MSR, whereas neurons, which do not express c-fms, have died or withdrawn from such loci. A possible link between these findings and pathogenicity of Aβ, for example, is the recent report of chaperone-like properties of apoE, inducing Aβ into a pathological β-sheet secondary structure (48). Consistent with the hypothesis that M-CSF is a factor relevant to AD is our pilot data in which addition of Aβ to microglia caused expression of M-CSF (data not shown), providing, in addition, an autocrine mechanism in which Aβ-stimulated microglia produce M-CSF, which further stimulates microglial c-fms receptors. The extent to which activation of microglia by M-CSF contributes to neuronal dysfunction and pathologic lesions in AD remains to be determined. Use of osteopetrotic (49) mice, which lack M-CSF due to a structural gene mutation, in combination with already existing amyloid precursor protein transgenic paradigms (50–51), may shed light on the role of M-CSF in AD-type pathology. In contrast, although expression of VCAM-1 is also induced in Aβ-perturbed neurons, it may not be actively involved in AD pathogenesis, as few cells express the counterligand. The selective expression of cell adherence molecules and cytokines in AD brain is further supported by our observation that AD brain does not display increased levels of monocyte chemotactic protein 1, a chemokine often present when M-CSF is produced (data not shown). Taken together, our data provide insight into a RAGE-dependent mechanism through which neurons can trigger and promote microglial activation by expressing M-CSF, thus participating in the pathogenetic process, rather than being only passive targets of products from microglia clustered around deposits of Aβ.

Our findings also suggest that M-CSF levels in AD patients could be a marker of neuronal oxidant stress/inflammation.

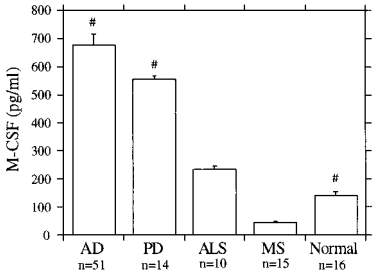

FIG. 5. M-CSF in the cerebrospinal fluid of patients with AD. Cerebrospinal fluid samples from patients with the indicated disorders or age-matched controls were obtained postmortem (3–20 hr after expiration) and studied by ELISA for M-CSF. Means ± SE and statistical significance by ANOVA. #, $P < 0.01$.

Although expression of M-CSF in AD is likely to result from both Aβ-RAGE-dependent and independent pathways, the presence of M-CSF in cerebrospinal fluid might serve as an indicator of the ongoing cellular damage. The ability of antioxidants to suppress Aβ-induced NF-κB activation is intriguing in view of *in vitro* experiments showing that probucol diminished Aβ-mediated cellular stress (52), albeit such antioxidants do not suppress all manifestations of Aβ-induced cellular toxicity (53). Furthermore, blockade of NF-κB activation may contribute to the potential benefit of anti-inflammatory agents in AD (28). Finally, there is the possibility that M-CSF levels in cerebrospinal fluid might be useful for monitoring an early phase of neuronal stress and, additionally, for evaluating the effectiveness of neuroprotective therapeutic agents.

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