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Rapid Communication

Tissue Factor Antigen in Senile Plaques of Alzheimer's Disease

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Tissue factor (tissue thromboplastin) is the primary initiator of the extrinsic coagulation pathway, triggering a proteolytic cascade when exposed to circulating coagulation factors. In this study, the distribution of tissue factor was examined immunohistochemically in Alzheimer's disease (AD) and control brains. Tissue factor was expressed diffusely in the neocortex, but in AD there was enhanced immunoreactivity in senile plaques. Although tissue factor might potentially contribute to the formation of senile plaques, it could also accumulate in the plaques as a secondary response to other biochemical perturbations. (Am J Pathol 1991, 139:491–494)

Alzheimer's disease (AD) is characterized by the accumulation of amyloid β protein (AβP) in senile plaques and blood vessels. AβP is derived from an amyloid β protein precursor (APP), which exists as an integral membrane protein in the normal brain and other tissues. Recent data indicate that production of an intact AβP involves altered proteolytic processing of APP.[1,2]

Several proteases and protease inhibitors have been identified that may be involved in senile plaque pathogenesis. The serine protease inhibitor α_1-antichymotrypsin (α_1-ACT),[3,4] multiple complement proteins,[5–7] including several with serine protease activity, and amyloid P component,[8,9] an elastase inhibitor,[10] have all been detected within senile plaques. APP, which contains a Kunitz-type serine protease inhibitor domain, is also present in a subset of plaques.[11–15] The secreted form of APP is identical to protease nexin-II.[16,17]

Much like the complement system, coagulation proteins comprise a catalytic cascade system that involves the controlled generation of several serine proteases. Tissue factor (coagulation factor III, tissue thromboplastin) is the primary initiator of the extrinsic coagulation pathway. In contrast to other coagulation factors, it is an integral membrane glycoprotein that does not circulate as a plasma protein.[18] It functions as a cofactor for coagulation factor VII(a), resulting in the formation of a bimolecular complex with serine protease activity, which can initiate the extrinsic pathway by activating factors IX and X.

Tissue factor (TF) is especially abundant in the brain. Using TF purified from human brain, both monoclonal and polyclonal antibodies have been developed and used in immunohistochemical studies to examine the distribution of TF in normal tissues.[19–22] In this study, TF was immunolocalized in brain tissue from patients with AD. There was diffuse expression of TF throughout the gray matter with focal accentuation of immunoreactivity in senile plaques.

Materials and Methods

Brain tissue was obtained postmortem from eight patients with Alzheimer's disease, ages 66 to 87 years, and four nondemented subjects, ages 35, 37, 61, and 80 years. Postmortem intervals ranged from 6 to 15 hours. The brains were fixed by perfusion with 10% neutral buffered formalin followed by immersion in the same fixative. Diagnoses were confirmed with the modified Bielschowsky and thioflavin S stains on paraffin-embedded tissue.[23]

Samples of neocortex (middle-frontal gyrus, superior-temporal gyrus, or both) for immunohistochemistry were taken before fixation of the brain and either frozen in isopentane cooled by liquid nitrogen or immersed in cold acetone and processed according to the AMeX proce-

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dure.[24] Immunohistochemical assay for TF was carried out using monoclonal antibody HTF1-7B8 (8 μg/mL) and the avidin–biotin–peroxidase complex procedure.[25] The specificity of this antibody, which was raised against TF purified from human brain, has been shown previously.[19,20] In control sections, the primary antibody was replaced with an antibody of the same IgG subclass (γ_1, k). Some slides were counterstained with thioflavin S after the immunohistochemical reaction.

In a separate series of controls, portions of fresh kidney and placenta were processed by rapid freezing or the AMeX method and the patterns of TF localization using these two methods were compared.

Results

The frozen and AMeX immunohistochemical methods yielded corresponding patterns of TF localization in brain, kidney, and placenta. However, the AMeX method provided better preservation of structural detail and better visualization of senile plaques.

In normal brain, TF was expressed diffusely throughout the cortex. Nonreactive neuronal perikarya stood out sharply against the strongly stained neuropil, which showed a delicate, uniform network of reactivity (Figure 1). The white matter had a fine, lacey pattern with accentuation in the glial tissue surrounding blood vessels. The adventitia of larger blood vessels was strongly stained.

In Alzheimer's disease, there was diffuse neuropil reactivity similar to that seen in the control brains. In addition, senile plaques showed enhanced immunoreactivity for tissue factor and stood out against the background as darkly stained structures (Figure 1). They exhibited a linear and granular meshwork with a texture similar to the surrounding neuropil. Dilated, dystrophic neuritic processes were not distinctly visualized. In classical plaques, the amyloid core was nonreactive, although the surrounding corona stained strongly (Figure 2). Some plaques exhibited intense immunoreactivity at the edge of the amyloid core, but not within the core. Thioflavin S stained the amyloid core of classical plaques, but fluorescence of immunostained structures, such as the plaque corona, was blocked by the prior immunohistochemical reaction (Figure 2). When compared with controls, the Alzheimer's cases also showed greater expression of TF in the pial-glial network at the surface of the brain.

Discussion

Tissue factor is expressed uniformly in normal brain parenchyma, more intensely in cortex than white matter.

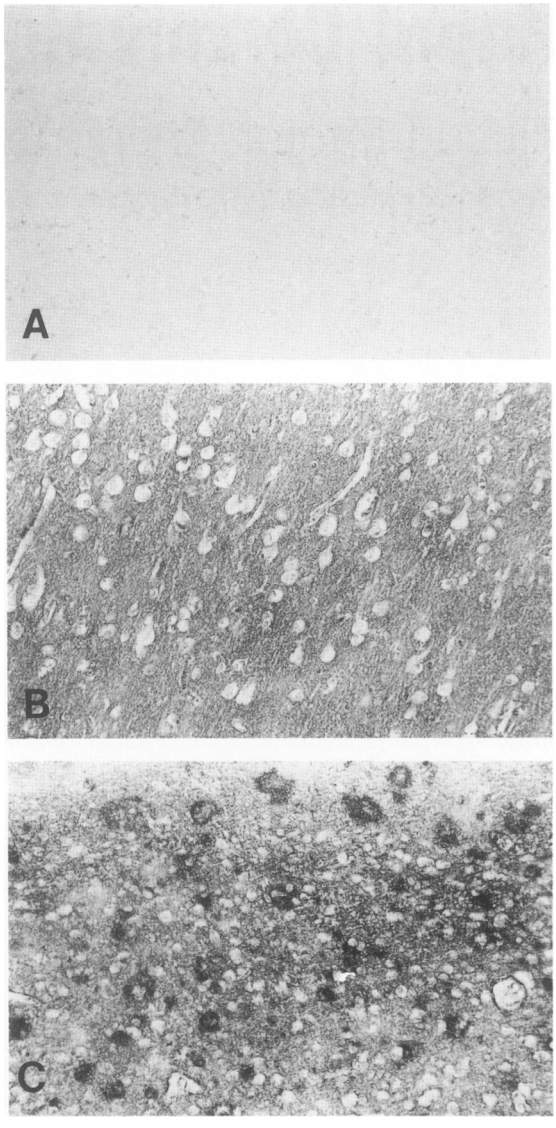

Figure 1. **A:** *There is no immunostaining of brain using IgG immunoglobulin as a negative control.* **B:** *Diffuse cortical reactivity for tissue factor antigen is shown in normal brain using MAb HTF1-7B8.* **C:** *In the AD brain, there is enhanced immunoreactivity for tissue factor antigen in senile plaques. Immunoperoxidase, all ×85.*

This has been described by others.[21,22] The pattern of localization suggests that TF is expressed by both neurons and astrocytes. Similar localization is seen in Alzheimer's disease, but in addition, there is enhanced immunoreactivity for TF in senile plaques.

At present, TF has one recognized function: it forms a tight complex with coagulation factor VII(a) and thereby functions as a cofactor in the enzymatic activation of coagulation factors IX and X. TF is an integral membrane protein that is normally sequestered from circulating factor VII and other coagulation factors.[20,21] The specific activity of TF is highest in brain, lung, and placenta, and much lower in liver, kidney, and spleen.[26] Tissue injury

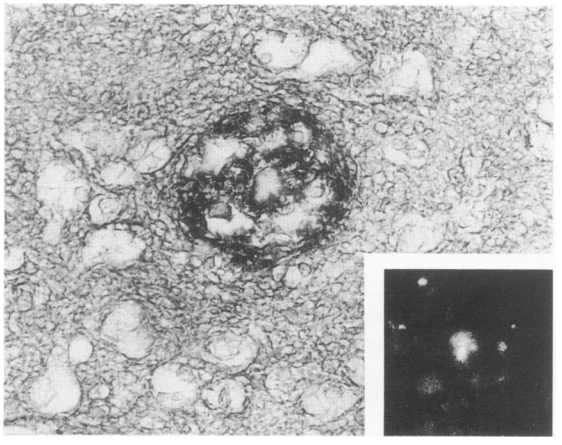

Figure 2. *Tissue-factor antigen is detected in the corona of a classical senile plaque, but the amyloid core is nonreactive. Counterstaining with thioflavin S shows the amyloid core in this same plaque (inset), but the immunostained corona does not fluoresce. Both, ×340.*

presumably exposes TF to circulating factor VII, resulting in complex formation and initiation of the clotting cascade via the extrinsic pathway. Alterations in the blood brain barrier allowing access of plasma proteins to the brain parenchyma could result in local coagulation factor activation.

Any of the cellular elements of senile plaques, such as neurites, astrocytes, and microglial cells, might be involved in the local accumulation of TF. Microglia are of interest since they share many characteristics with mononuclear phagocytes in other tissues, including the production and response to numerous cytokines and the production and release of various proteases.[27,28] Tissue factor can be induced in peripheral blood monocytes by interleukin-1 (IL-1),[29,30] the C5a fragment of complement,[31] and activated T-lymphocytes.[32] Similar induction of TF in microglia could provide a local source of TF in senile plaques. Although brain IL-1 is increased in Alzheimer's disease,[33] it has yet to be determined whether microglia are capable of TF expression.

This study raises the question whether TF contributes to the pathogenesis of the senile plaque, or whether its excess in the senile plaque represents a secondary response to local biochemical alterations. TF shares some features with APP in that both are integral membrane glycoproteins with large extracellular portions, and both show diminished expression after phorbol ester-induced activation of protein kinase C.[34,35] Alterations in protein kinase C activity[36,37] and protein phosphorylation in Alzheimer's disease provide mechanisms that could influence TF metabolism. Future studies are needed to determine the activities of other coagulation proteins in the brain, mechanisms of TF processing and degradation, and the possibility of other functions for the TF protein.

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References

1. Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL: Evidence that β-amyloid protein in Alzheimer's disease is not derived by normal processing. Science 1990, 248:492–495
2. Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, Oltersdorf T, McClure D, Ward PJ: Cleavage of amyloid β peptide during constitutive processing of its precursor. Science 1990, 248:1122–1124
3. Abraham CR, Selkoe DJ, Potter H: Immunohistochemical identification of the serine protease inhibitor α_1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell 1988, 52:487–501
4. Picken MM, Larrondo-Lillo M, Coria F, Gallo GR, Shelanski ML, Frangione B: Distribution of the protease inhibitor α_1-antichymotrypsin in cerebral and systemic amyloid. J Neuropathol Exp Neurol 1990, 49:41–48
5. Eikelenboom P, Hack CE, Rozemuller JM, Stam FC: Complement activation in amyloid plaques in Alzheimer's dementia. Virchows Arch [B] 1989, 56:259–262
6. McGeer PL, Akiyama H, Itagaki S, McGeer EG: Activation of the classical complement pathway in brain tissue of Alzheimer patients. Neurosci Lett 1989, 107:341–346
7. McGeer PL, Akiyama H, Itagaki S, McGeer EG: Immune system response in Alzheimer's disease. Can J Neurol Sci 1989, 16:516–527
8. Coria F, Castano E, Prelli F, Larrondo-Lillo M, Van Duinen S, Shelanski ML, Frangione B: Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. Lab Invest 1988, 58:454–458
9. Duong T, Pommier EC, Scheibel AB: Immunodetection of the amyloid P component in Alzheimer's disease. Acta Neuropathol (Berl) 1989, 78:429–437
10. Li JJ, McAdam KPWJ: Human amyloid P component: An elastase inhibitor. Scand J Immunol 1984, 20:219–226
11. Perry G, Lipphardt S, Mulvihill P, Kancherla M, Mijares M, Gambetti P, Sharma S, Maggiora L, Cornette J, Lobl T, Greenberg B: Amyloid precursor protein in senile plaques of Alzheimer disease. Lancet 1988, 2:746
12. Palmert MR, Podlisny MB, Witker DS, Oltersdorf T, Younkin LH, Selkoe DJ, Younkin SG: Antisera to an amino-terminal peptide detect the amyloid protein precursor of Alzheimer's disease and recognize senile plaques. Biochem Biophys Res Commun 1988, 156:432–437
13. Ghiso J, Tagliavini F, Timmers WF, Frangione B: Alzheimer's disease amyloid precursor protein is present in senile plaques and cerebrospinal fluid: Immunohistochemical and biochemical characterization. Biochem Biophys Res Commun 1989, 163:430–437
14. Joachim C, Games D, Morris J, Ward P, Frenkel D, Selkoe D: Antibodies to non-beta regions of the beta-amyloid pre-

cursor protein detect a subset of senile plaques. Am J Pathol 1991, 138:373–384
15. Cras P, Kawai M, Siedlak S, Mulvihill P, Gambetti P, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G: Neuronal and microglial involvement in β-amyloid protein deposition in Alzheimer's disease. Am J Pathol 1990, 137:241–246
16. Oltersdorf T, Fritz LC, Schenk DB, Lieberburg I, Johnson-Wood KL, Beattie EC, Ward PJ, Blacher RW, Dovey HF, Sinha S: The secreted form of the Alzheimer's amyloid precursor protein with the Kunitz domain is protease nexin-II. Nature 1989, 341:144–147
17. Van Nostrand WE, Wagner SL, Suzuki M, Choi BH, Farrow JS, Geddes JW, Cotman CW, Cunningham DD: Protease nexin-II, a potent anti-chymotrypsin, shows identity to amyloid β-protein precursor. Nature 1989, 341:546–549
18. Carson SD: Tissue factor-initiated blood coagulation. Prog Clin Pathol 1984, 9:1–14
19. Carson SD, Ross SE, Bach R, Guha A: An inhibitory monoclonal antibody against human tissue factor. Blood 1987, 70:490–493
20. Faulk WP, Labarrere CA, Carson SD: Tissue factor: Identification and characterization of cell types in human placentae. Blood 1990, 76:86–96
21. Drake TA, Morrissey JH, Edgington TS: Selective cellular expression of tissue factor in human tissues: Implications for disorders of hemostasis and thrombosis. Am J Pathol 1989, 134:1087–1097
22. Fleck RA, Rao LVM, Rapaport SI, Varki N: Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. Thromb Res 1990, 57:765–781
23. Khachaturian ZS: Diagnosis of Alzheimer's disease. Arch Neurol 1985, 42:1097–1105
24. Sato Y, Mukai K, Watanabe S, Goto M, Shimosato Y: The AMeX method: A simplified technique of tissue processing and paraffin embedding with improved preservation of antigens for immunostaining. Am J Pathol 1986, 125:431–435
25. McComb RD, Moul JM, Bigner DD: Distribtion of type VI collagen in human gliomas: Comparison with fibronectin and glioma-mesenchymal matrix glycoprotein. J Neuropathol Exp Neurol 1987, 46:623–633
26. Pitlick FA, Nemerson Y: Purification and characterization of tissue factor apoprotein. Methods Enzymol 1976, 45:37–48
27. Dickson DW, Mattiace LA, Kure K, Hutchins K, Lyman WD, Brosnan CF: Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. Lab Invest 1991, 64:135–156
28. Giulian D, Baker TJ, Shih LCN, Lachman LB: Interleukin 1 of the central nervous system is produced by ameboid microglia. J Exp Med 1986, 164:594–604
29. Lasser A: The mononuclear phagocytic system: A review. Hum Pathol 1983, 14:108–126
30. Carlsen E, Prydz H: Activation of monocytes—More than one process: Differential effect of cytokines on monocytes. Scand J Immunol 1988, 27:401–404
31. Muhlfelder TW, Niemetz J, Kreutzer D, Beebe D, Ward PA, Rosenfeld SI: C5 chemotactic fragment induces leukocyte production of tissue factor activity. J Clin Invest 1979, 63:147–150
32. Gregory SA, Edgington TS: Tissue factor induction in human monocytes: Two distinct mechanisms displayed by different alloantigen-responsive T cell clones. J Clin Invest 1985, 76:2440–2445
33. Griffin WST, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CL III, Araoz C: Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 1989, 86:7611–7615
34. Brozna JP, Carson SD: Monocyte-associated tissue factor is suppressed by phorbol myristate acetate. Blood 1988, 72:456–462
35. Buxbaum JD, Gandy SE, Cicchetti P, Ehrlich ME, Czernik AJ, Fracasso RP, Ramabhadran TV, Unterbeck AJ, Greengard P: Processing of Alzheimer β/A4 amyloid precursor protein: Modulation by agents that regulate protein phosphorylation. Proc Natl Acad Sci USA 1990, 87:6003–6006
36. Clark EA, Leach KL, Trojanowski JQ, Lee VMY: Characterization and differential distribution of the three major human protein kinase C isozymes (PKCα, PKCβ, and PKCγ) of the central nervous system in normal and Alzheimer's disease brains. Lab Invest 1991, 64:35–44
37. Masliah E, Cole G, Shimohama S, Hansen L, DeTeresa R, Terry RD, Saitoh T: Differential involvement of protein kinase C isozymes in Alzheimer's disease. J Neurosci 1990, 10:2113–2124