Immunohistochemical Study on the Deposition of Apolipoprotein E in Cerebral and Islet Amyloidoses in Cynomolgus Monkeys (Macaca fascicularis)

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Abstract: To investigate the role of apolipoprotein E (apo E) in amyloidoses of cynomolgus monkeys, the localization of apo E in cerebral amyloid, including senile plaques and cerebrovascular amyloid, and in islet amyloid was examined immunohistochemically. Mature types of senile plaques with amyloid deposits and cerebrovascular amyloid showed intense immunoreactivity to both antisera to apo E and amyloid β protein (A β). In contrast, diffuse plaques without obvious Congoophilic amyloid showed weak immunoreactivity to antiserum to apo E, but intense reactivity to antiserum to A β . In addition, the number of these apo E-positive diffuse plaques was small compared with that of A β -positive plaques. On the other hand, diabetic islet amyloid that was negative with A β , reacted intensely with antiserum to apo E. These findings suggest that apo E plays an important role in amyloid fibril formation in several types of amyloidoses.

Key words: amyloidosis, apolipoprotein E, cynomolgus monkey

All types of human senile plaques and cerebrovascular amyloid consisting of amyloid β protein (A β) are immunoreactive to apolipoprotein E (apo E) [7, 10, 12, 14, 16]. Furthermore, ϵ 4 gene of apo E has been considered a genetic risk factor in late-onset familial and sporadic Alzheimer's disease [1, 10, 12] and *in vitro* studies have indicated that apo E accelerates the formation of amyloid fibril from A β [4, 14]. It has recently

been reported that inclusion body myositis consisting of A β was also immunoreactive to apo E [11]. On the other hand, systemic amyloid consisting of AA amyloid, but not of A β amyloid, is immunoreactive to apo E [3, 7]. These studies support the prediction that there is a close correlation between certain types of amyloid and apo E deposition.

Apo E deposition in senile plaques and cerebrovas-

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cular amyloid was also reported in non-human primates, such as chimpanzees and rhesus monkeys [2, 9]. Except for cerebral amyloid, however, no other apo E deposition in amyloid lesions has been reported in any species of monkey.

In the present study, we examined the localization of apo E in two different types of amyloidoses; cerebral amyloidosis and islet amyloidosis, in cynomolgus monkeys (*Macaca fascicularis*), and discuss the role of apo E in amyloidosis.

We examined a total nine aged cynomolgus monkeys (from 13 to 29 years old), whose histories are listed in Table 1. We used brain tissue from five of the monkeys with cerebral amyloidosis, and pancreases from four with islet amyloidosis.

Formalin-fixed and paraffin-embedded sections from these brains and pancreases were stained with hematoxylin and eosin, periodic acid methenamine silver (PAM), and alkaline Congo red. To identify the characteristic of amyloid with Congo red staining, the sections were also pretreated with potassium permanganate and formic acid. Immunohistochemical examination was performed using an avidin-biotin-peroxidase complex (ABC) method with Vectastain ABC

kit (Vector Laboratories, Burlingame, CA). For the primary antibodies, we used goat antiserum against human recombinant apo E (anti-apo E; Chemicon, Temecula, CA, U.S.A.; 1:800) and rabbit antiserum against synthetic peptide A β 1-40 (anti-A β ₁₋₄₀; Boehringer Mannheim Biochemica, Germany; 1:5). For the secondary antibodies, we used biotinylated rabbit serum against goat Ig G and goat serum against rabbit Ig G (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD; 1:100, respectively).

Cerebral amyloidosis was confirmed as senile plaques and amyloid angiopathy by both Congo red and PAM staining (Table 1). In the 5 brain specimens, senile plaques were then classified into either mature plaques characterized by Congophilic amyloid deposits and swollen neurites or diffuse plaques without those features. Congophilic cerebrovascular amyloid was frequently localized in the cortical capillaries (Case 1, 2, 3, and 5). Islet amyloid was found in all 4 of the diabetic cynomolgus monkeys (Table 1). In these diabetic cases, we frequently found that the most of hyalinized islets were replaced by severe amyloid deposition, with remaining islet cells being severely degenerated and decreased in number. These amyloid

Table 1. Clinical course and histopathological features of 9 cynomolgus monkeys

Case No.	Age	Sex	Source	Anamnesis	Sampled tissue	DP ^{a)}	MP ^{b)}	CAA ^{c)}	IA ^{d)}
1	22y3m	Female	TPC ¹⁾	Fatty degeneration of the liver Endometriosis Pulmonary edema Atrophy of glomerulus	brain	+	+	+	NT
2	23y1m	Female	TPC	Diabetes mellitus Diarrhea, anorexia	brain	+	+	+	NT
3	23y4m	Male	TPC	Inguinal hernia Focal infection of gingival abscess Septicemia	brain	—	+	+	NT
4	26y	Female	SCL ²⁾	Diabetes mellitus	brain	+	+	+	NT
5	23y	Female	SCL	Diabetes mellitus	brain	—	+	+	NT
6	29y8m	Male	TPC	Diabetes mellitus Vascular stomatitis, rash on hypogastrium Conjunctivitis Atrophy of testis, spleen, and thyroid gland	pancreas	NT	NT	NT	+
7	23y5m	Female	TPC	Diabetes mellitus, lung abscess, pyonephritis	pancreas	NT	NT	NT	+
8	about 27y	Male	TPC	Diabetes mellitus, lung abscess, endocarditis	pancreas	NT	NT	NT	+
9	13y1m	Female	TPC	Diabetes mellitus, lung abscess	pancreas	NT	NT	NT	+

¹⁾ Tsukuba Primate Center for Medical Science, National Institute of Health, Japan, ²⁾ Laboratory Animal Science and Toxicology Laboratories of Sankyo Co., Ltd. ^{a)} Diffuse plaques, ^{b)} Mature plaques, ^{c)} Cerebral amyloid angiopathy, ^{d)} Islet amyloid. +: positive, —: negative, NT: not tested.

deposits both in the brains and islets were resistant to the potassium permanganate treatment, but were sensitive to the formic acid treatment.

Using immunohistochemistry, we found that mature plaques and cerebrovascular amyloid with Congophilic amyloid reacted strongly to anti-apo E and anti- $A\beta_{1-40}$ (Figs. 1-a and b, and Table 2). However, swollen neurites in mature plaques did not react to either antibody. Thus, when mature plaques examined the immunoreactivity for these two antibodies was similar. In contrast, diffuse plaques were stained strongly by anti- $A\beta_{1-40}$, whereas the immunoreactivity for anti-apo E was weak (Figs. 2-a, b, and Table 2). Furthermore, the cortical blood vessels positively stained by anti- $A\beta_{1-40}$ were also immunostained by anti-apo E (Figs. 1-a, b and Table 2). The number of diffuse plaques reacted to anti- $A\beta_{1-40}$ was about twice more than that to anti-apo E. In the cerebrum, anti-apo E detected a small number of astroglia and mononuclear cells around cortical blood vessels, whereas anti- $A\beta_{1-40}$ detected only senile plaques and cerebrovascular amyloid.

Islet amyloid immunoreacted to anti-apo E but not to anti- $A\beta_{1-40}$ (Fig. 3 and Table 2). The results of immunostaining with anti-apo E agreed with those of Congo red staining. The remaining islet cells, acinus and blood vessels in the pancreas, did not react to either anti-apo E nor anti- $A\beta_{1-40}$.

In the mature plaques with Congophilic amyloid in cynomolgus monkeys, extracellular portions, but not

the neurites, reacted to both anti-apo E and anti- $A\beta_{1-40}$. Cerebrovascular amyloid consisting of $A\beta$ also reacted to anti-apo E. Therefore, from these morphological features, we conclude that apo E is needed for the formation of Congophilic amyloid with $A\beta$.

Because a diffuse plaque ultrastructurally consists of a small number of amyloid fibrils, it contains fewer polymerized fibrils compared with mature plaques [15, 17]. Consequently, we considered that the diffuse plaques remained in a non-Congophilic form until amyloid fibrils well polymerized. In this study, diffuse plaques in cynomolgus monkeys reacted weakly to anti-apo E. Furthermore, the number of anti-apo E positive plaques was less than that of anti- $A\beta_{1-40}$ positive plaques. This suggests that apo E in diffuse plaques play a role in the polymerization of amyloid fibrils in early stage of the senile plaque formation. This finding supports previous *in vitro* results showing that apo E accelerates $A\beta$ polymerization [4, 14].

The amino acid sequence of apo E in cynomolgus monkey has about a 93% homology with that of human [5], and the $A\beta$ sequence has 100% homology [8]. In cynomolgus monkeys, all diffuse plaques react to anti- $A\beta_{1-40}$ [6], and in human, all diffuse plaques react to anti-apo E [10]. In our study, however, we found that only about half number of the diffuse plaques were detected by anti-apo E. For the anti-apo E, we used an antibody prepared against recombinant human apo E; as a result, the specificity of this anti-apo E in cyno-

Fig. 1. The immunoreactivity of mature plaques and capillaries with cerebrovascular amyloid (arrowheads) (a) to anti-apo E and (b) to anti- $A\beta_{1-40}$. Immunostain. a; $\times 480$, b; $\times 320$.

Table 2. Characterization of cerebral and islet amyloidoses in cynomolgus monkeys

	Cerebral amyloidosis			Islet amyloidosis
	DP ^{a)}	MP ^{b)}	CAA ^{c)}	
anti-apo E ¹⁾	+	++	++	++
anti-A β ₁₋₄₀ ²⁾	++	++	++	—

^{a)} Diffuse plaques, ^{b)} Mature plaques, ^{c)} Cerebral amyloid angiopathy. ¹⁾ goat anti-serum to human recombinant apo E, ²⁾ rabbit antiserum to synthetic peptide A β ₁₋₄₀. ++; Strongly positive, +; Positive, —; Negative.

Fig. 2. The immunoreactivity of diffuse plaques (a) to anti-apo E and (b) to anti-A β ₁₋₄₀. Diffuse plaque showed weak reaction to anti-apo E. Immunostain. a; $\times 480$, b; $\times 450$. **Fig. 3.** The immunoreactivity of hyalinized islets with amyloid deposits to anti-apo E. Immunostain. $\times 160$.

molgus monkey might be less than that in human.

A previous study reported that in humans, systemic amyloid and prion amyloid plaques without A β also reacted to anti-apo E [7]. Our study is the first to confirm that apo E deposits in diabetic islets in cynomolgus monkeys. Both of these results suggest that apo E is related with not only to cerebral amyloidosis but also to several types of amyloidoses.

The $\epsilon 4$ gene of apo E is considered a risk factor in late-onset familial and sporadic Alzheimer's disease, and a patient with $\epsilon 4/4$ genes is more susceptible to A β deposits [10, 12]. The apo E gene of a cynomolgus monkey belongs to the $\epsilon 4$ type at corresponding amino acid in human, the residue 112 from cysteine (an $\epsilon 3$ type) to arginine (an $\epsilon 4$ type) [5]. In cerebral amyloidosis, to investigate interaction of apo E and A β in cynomolgus monkey, genetic analysis may be criti-

cal. Therefore, the cynomolgus monkeys used in our study were very useful for investigating apo E for pathogenesis of amyloid deposits, because their profiles, including breeding, clinical histories and known pedigree, were well-documented in Tsukuba Primate Center.

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References

1. Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. 1993. *Science* 261: 921–923.
2. Gearing, M., Rebeck, G. W., Hyman, B. T., Tigges, J., and Mirra, S. S. 1994. *Proc. Natl. Acad. Sci. USA* 91: 9382–9386.
3. Linke, R. P. 1984. *J. Histochem. Cytochem.* 32: 322–328.
4. Ma, J., Yee, A., Brewer, H. B. Jr., Das, S., and Potter, H. 1994. *Nature* 372: 92–94.
5. Marotti, K. R., Whitted, B. E., Polites, H. G., and Melchoir, G. W. 1989. *Nucleic Acids Res.* 17: 1778.
6. Nakamura, S., Nakayama, H., Goto, N., Ono-Ochikubo, F., Sakakibara, I., and Yoshikawa, Y. (submitted to *J. Med. Primatol.*).
7. Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E., and Ikeda, K. 1991. *Brain Res.* 541: 163–166.
8. Podlisny, M. B., Tolan, D. R., and Selkoe, D. J. 1991. *Am. J. Pathol.* 138: 1423–1435.
9. Poduri, A., Gearing, M., Rebeck, G. W., Mirra, S. S., Tigges, J., and Hyman, B. T. 1994. *Am. J. Pathol.* 144: 1183–1187.
10. Rebeck, G. W., Reiter, J. S., Strickland, D. K., and Hyman, B. T. 1993. *Neuron* 11: 575–580.
11. Sarkozi, E., Askanas, V., and Engel, W. K. 1994. *Am. J. Pathol.* 145: 1280–1284.
12. Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B. J., Hulette, C. M., Joo, S. H., Pericak-Vance, M. A., Goldgaber, D., and Roses, A. D. 1993. *Proc. Natl. Acad. Sci. USA* 90: 9649–9653.
13. Selkoe, D. J. 1991. *Neuron* 6: 487–498.
14. Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., and Roses, A. D. 1993. *Proc. Natl. Acad. Sci. USA* 90: 1977–1981.
15. Tagliavini, F., Giaccone, G., Frangione, B., and Bugiani, O. 1988. *Neurosci. Lett.* 93: 191–196.
16. Wisniewski, T. and Frangione, B. 1992. *Neurosci. Lett.* 135: 235–238.
17. Yamaguchi, H., Nakazato, Y., Shoji, M., Takatama, M., and Hirai, S., 1991. *Acta. Neuropathol.* 82: 13–20.