# Comparative histopathological studies on senile plaques and cerebrovascular amyloid of aged animals, especially of aged cynomolgus monkeys

# 老齢動物の老人斑および血管アミロイド症の比較病理組織学的研究：特にカニクイザルを中心に

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# General Introduction

In the developed countries including Japan, senile dementia and Alzheimer's disease (AD) have become a serious social problem in due to elongation of life span. Since the patients suffering from these diseases result in progressive neural deteriorations such as reduction of memories or destitution of intelligence, it is difficult for the patients to live in normal social interactions. In neuropathological examination, severe neuronal loss, neurofibrillary tangles, senile plaques and cerebrovascular amyloid are observed in the patients' brain. Although some genetic risk factors in AD have recently been identified, the role of the genes in the pathogenesis of AD has not been clarified completely. Therefore, development of animal models of AD is needed at present.

Senile plaques and cerebrovascular amyloid are found not only in humans but also in animals such as non-human primates, dogs, and bears. Since these lesions show characteristic features among each species, it is important to perform comparative histopathology on senile plaques and cerebrovascular amyloid. On the other hand, the occurrence of these lesions might be tightly associated with some genetical risk factors so that animal models are required to have obvious breeding histories and pedigree. In the present studies, cynomolgus monkeys belonging to Tsukuba Primate Center for Medical Science (TPC), National Institute of Health, Japan were mainly used and they have complete information of breeding and clinical histories and known pedigree. Their brains were examined on senile plaques and cerebrovascular amyloid by histopathological and immunohistochemical methods.

In the Chapter 1, comparative histopathological studies on

senile plaques and cerebrovascular amyloid were performed in aged cynomolgus monkeys and dogs, in addition to aged cats and a two-humped camel which have not be reported thus far. In the Chapter 2, incidence of the lesions with aging and distribution of the lesions throughout the brain were histopathologically examined by using cynomolgus monkeys with obvious pedigree. In the Chapter 3, immunohistochemical examinations were carried out on the constituents of the lesions including apo E known as a genetic risk factor of late-onset AD. Moreover, subtypes of amyloid β protein (Aβ) molecules with different carboxyl terminus in senile plaques and cerebrovascular amyloid were examined.

As a result, it has been found that 1) comparative histopathological analysis is a very important tool to clarify the mechanism of senile plaque formation, and 2) cynomolgus monkeys are very useful model for pathological analysis of Aβ deposition in AD.

# Chapter 1.

## Comparative Histopathological Studies on Senile Plaques and Cerebrovascular Amyloid in Aged Cynomolgus Monkeys, Dogs, Cats, and a Two-Humped Camel

## Introduction

Senile plaques, cerebrovascular amyloid, and neurofibrillary tangles are the most characteristic histopathological features observed in the brains of senescent humans with or without Alzheimer's dementia [38]. Senile plaques and cerebrovascular amyloid are also observed in other aged mammals, including non-human primates; apes such as orangutans [39], chimpanzees [9], and monkeys such as rhesus [33, 39, 48, 65, 69], cynomolgus [31], squirrel [66], and lemurian monkeys [4]; in addition, carnivores such as dogs [10, 39, 61-63, 68], a coyote [41], and an American black bear [64]. Moreover, only a lesion of senile plaques was found in a polar bear [6, 39]. Recently, we reported firstly senile plaques and cerebrovascular amyloid in aged cats and a two-humped camel; so that it was predicted senile plaque formation occurring in a wide variety of mammalian species [15].

Senile plaques are classified into two subtypes according to the category of humans, monkeys, and dogs [38, 48, 52, 61]. One type was mature or neuritic plaque with swollen neurites and amyloid deposits, which is further divided into classical plaque with central amyloid core and primitive plaque without one. The other type was diffuse plaque which showed amorphous form without amyloid deposition. Amyloid β protein (Aβ) is the most important component of two kinds of cerebral amyloidosis such as senile plaques and cerebrovascular amyloid [11, 25, 26].

To establish an animal model for cerebral amyloidosis, histopathological characteristics and incidence of senile changes in the brain in various animal species must be investigated. In

this chapter, senile plaques and cerebrovascular amyloid in aged animals of four species, i.e. cynomolgus monkeys, dogs, cats and a two-humped camel, were examined histopathologically. Furthermore, comparative pathological significance on cerebral amyloidosis was discussed.

## Materials and methods

*Animals*

Cynomolgus monkeys (*Macaca fascicularis*)

I examined the cerebrum of 6 aged cynomolgus monkeys (20 to 29 years old), which derived from Tsukuba Primate Center for Medical Science (TPC), National Institute of Health of Japan. Since the TPC originated monkeys were registered on their profiles such as age, sex, and detailed breeding and clinical histories, these records were listed in Table 1. In this study, a part of the brain was sampled from each specimen. The area examined was schematically illustrated in Fig. 1.

Dogs

I examined 12 aged dogs, 10 to 18 years old, which were necropsy cases in Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo (VPUT). All 12 animals did not show any severe neurological signs and abnormal behaviors. Gross lesions found at necropsy were summarized in Table 2.

Cats

Thirteen aged cats, 12 to 20 years old, were examined in this study, which were necropsy cases in VPUT. Neither

neurological signs nor abnormal behaviors had been shown except for one case, No. 95059. The animal showed convulsions and loss of reflexes by adenocarcinoma metastating to the brain. Gross lesions found at necropsy were listed in Table 3.

Two-humped (Bactrian) camel (*Camelus bactrianus*)

A female two-humped camel, which had been imported from the desert area of Central Asia 20 years ago and kept in a zoological farm in Kanagawa prefecture, Japan, became less active and was unable to stand due to swelling in the right axillary region. The prognosis was assessed as unfavorable, and the animal was euthanized and autopsied immediately after death. At autopsy, septicemia due to phlegmonous inflammation was considered to be the cause of death. On histopathological examination, however, systemic metastases of an ovarian adenocarcinoma, of which mass could not be seen macroscopically, were found.

*Histopathological examination*

Tissue samples from the brain were fixed in 10% neutral buffered formalin. Paraffin sections (4- or 6-$\mu$m-thick) were stained with hematoxylin and eosin (HE) or with periodic acid methenamine silver (PAM) for senile plaques and with alkaline Congo red for amyloid.

*Immunohistochemical examination*

Paraffin sections (4-$\mu$m-thick) were immunostained following 5 min treatment with 90% formic acid, as described previously [17]. The antibodies used were a rabbit antiserum against synthetic A$\beta$, consisting of 1-40 peptide (anti-A$\beta_{1\text{-}40}$; Boehringer Mannheim

Biochemica, Germany), as a primary antibody and a biotinylated goat anti-rabbit Ig G (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as a secondary antibody. The sections were then treated with avidin-biotin-peroxidase complex (ABC; Vectastain ABC kit, Vector Laboratories, Burlingame, CA) and finally visualized in 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) plus hydrogen peroxide solution.

## Results

### *Senile plaques and cerebrovascular amyloid in cynomolgus monkeys*

Five out of 6 cases had senile plaques which were mostly mature plaques including classical and primitive ones (Figs. 2-a,b and Table 1). The number of immature diffuse plaques was less than that of mature plaques (Fig. 2-c and Table 1). The senile plaques were often seen in the cortex of temporal lobe, although limited parts of the brains were examined in this study. Cerebrovascular amyloid was mostly observed in the parenchymal capillaries and arterioles of the cerebral cortex in 3 of 6 aged monkeys (Fig. 3-a, b and Table 1). Most of amyloid deposits on cortical capillaries localized in the area where senile plaques distributed densely. In these area, it was often seen that amyloid deposition to blood vessels was very adjacent to mature plaques. No cerebrovascular amyloid was observed on meningeal arterioles in all 6 cases (Table 1).

All the lesions of senile plaques and cerebrovascular amyloid detected by PAM and Congo red stainings intensely reacted to anti-$A\beta_{1-40}$ (Fig 4). Diffuse plaques could be detected more sensitively by anti-$A\beta_{1-40}$ than by PAM staining. Moreover, anti-$A\beta_{1-40}$ could detect more number of amyloid depositing blood vessels than by Congo red staining.

*Senile plaques and cerebrovascular amyloid in dogs*

Senile plaques were observed in the cerebral cortex in 10 out of 12 dogs, more than 12 years old (Table 2). The plaques were frequently localized in the frontal lobes and spread more wide area in the brain in older animals. Morphologically, canine senile plaques were classified into classical, primitive, and diffuse ones as well as humans and monkeys (Fig. 5-a~c). Diffuse plaques were most frequently observed in all 10 cases with the plaques, whereas a small number of primitive plaques was observed in the cortices of temporal lobes in 4 cases from 12 to 14 years old. The oldest dog (18 years old; case No. 95071) frequently had classical and primitive plaques, although the number of these plaques was less than that of diffuse plaques. In the case of cerebrovascular amyloid, amyloid deposits on meningeal arterioles were often seen in comparison with other examined animals such as cynomolgus monkeys, cats, and a camel (Fig. 6). A few number of amyloid deposition on capillaries was found in 4 cases (Table 2), whereas numerous capillary amyloid depositions were observed in the oldest case No. 95071 .

All the lesions of senile plaques and cerebrovascular amyloid

detected by PAM and Congo red stainings intensely reacted to anti-$A\beta_{1-40}$ (Fig. 7). The number of diffuse plaques detected by anti-$A\beta_{1-40}$ was more than that by PAM staining. Immunostaining by anti-$A\beta_{1-40}$ could detect more number of amyloid depositing blood vessels than that by Congo red staining.

*Senile plaques and cerebrovascular amyloid in cats*

Senile plaques were found in the cerebral cortices in 4 cats older than 18 years old (Table 3). The plaques, which located mainly in the temporal lobe in the 4 animals and additionally in the occipital lobe in one animal, No. 94123, were morphologically uniform. It was consisted of a coarse assembly of short silver staining-positive materials with an irregular margin (Fig. 8). No amyloid deposition was detected in the plaque by Congo red staining. Amyloid deposition was detected along a few cortical arterioles in one animal, No. 94123, by Congo red staining (Fig. 9 and Table 3). Congophilic vessels in the brain could not be seen in the other animals examined.

The feline senile plaque was positive for anti-$A\beta_{1-40}$, showing a short-filamentous or granular pattern with an irregular margin (Fig. 10-a). Filamentous Aβ deposition, which did not form senile plaque, was detected in the cortical neuropil and were sparsely distributed. The Aβ depositions were often located very adjoining neurons or capillaries (Figs. 10-b). In case No. 94123, the cortical capillaries and arterioles were also positive for anti-$A\beta_{1-40}$ (Fig. 10-c).

Neurofibrillary tangles were not observed in any of the

animals.

*Senile plaques and cerebrovascular amyloid in a camel*

On gross examination, the brain showed slight atrophy characterized by well-defined cerebral sulci. On histopathological examination of the brain with PAM staining, many senile plaques were detected. With HE and PAS stainings, glial cell proliferation and ceroid-lipofuscin deposition were observed in neuronal cells throughout the cerebral cortex and in macrophages around capillaries, as seen in aged individuals of various species. Eosinophilic intracytoplasmic inclusion bodies and extracellular polyglucosan bodies were occasionally observed in the both neocortex and medulla.

The senile plaques were mostly diffuse one (Fig. 11) with a more clearly demarcated border than those seen in aged dogs. They were distributed throughout the cerebral cortex. A few primitive plaques were also seen in the frontal and temporal cortex. No plaques were seen in the hippocampus or the cerebellar cortices. None of the plaques were stained with Congo red, and congophilic amyloid was rarely detected along cortical capillaries (Fig. 12). The diffuse and primitive plaques and the cortical capillaries were strongly positive for anti-$A\beta_{1-40}$ (Fig. 13). Meningeal arterioles were neither Congophilic nor immunoreactive. Neurofibrillary tangles were not detected by any of the methods used in the present study.

## Discussion

In the present experiment, morphological differences of senile plaques were found among 4 species of animals, whereas the plaques were mainly consisted of the same component such as Aβ. Although the cause of species' difference was unknown, the possibilities were indicated as follows; differences of co-factor throughout Aβ deposits, of time course until senile plaque formation, and of precise constituents in senile plaques, among 4 species of animals.

Morphologically, senile plaques in cynomolgus monkeys were agreed with those in humans [38, 52, 70], and both senile plaques and cerebrovascular amyloid were closely related. Further, cynomolgus monkeys used in this study had their own profiles including breeding and clinical histories and known pedigree. Therefore, cynomolgus monkeys will be a useful model to investigate the correlation between senile plaques and cerebrovascular amyloid, and genetic analysis. Senile plaques in dogs were frequently found as diffuse ones as described by Uchida *et al*. [61]. In humans, appearance of numerous diffuse plaques was limited in the cases of middle aged patients in Down's syndrome [28]. Hence, dogs will be utilized for studying pathogenesis of diffuse plaques. Since senile plaques in cats appeared in the terminal age of their life span, it was very useful to search what is associated with the factor of time lag on Aβ deposits in cats contrary to cynomolgus monkeys and dogs. It was considered that Aβ deposition without formation of senile plaques in cats was a very important feature to investigate the starting stage of Aβ deposits which is not diffuse plaque generally known as an initial stage in

various species [52, 73]. Moreover, discovery of senile plaques in cats and a camel suggests the possibility of senile plaque formation in wide variety of mammalian species. This suggestion is also supported genetically, because amino acid sequence of Aβ in many mammalian species except for rodents have a complete homology [15].

On the other hand, cerebrovascular amyloid also showed different features among 4 species of animals. Cerebrovascular amyloid in dogs was frequently observed in meningeal arterioles, whereas that in cynomolgus monkeys was observed in capillaries. Few lesion of cerebrovascular amyloid was recognized in cats or a camel. In general, it has been known that cerebrovascular amyloid was consisted of Aβ40, a kind of Aβ subtypes [49]. Since I found recently not only Aβ40 in meningeal arterioles but also Aβ42(43) in capillaries in aged cynomolgus monkeys, the two sites of cerebrovascular amyloid might be produced by different proteolytic process [27, 35, 36]. Thus, the role of Aβ in the pathogenesis of cerebrovascular amyloid may have wide diversity among animal species. Therefore, to clarify the role of Aβ in the pathogenesis of cerebral amyloidosis, 4 species of animals we examined will be very useful models.

From these results, it is suggested that elucidation of Aβ deposits will be possible by comparative studies on senile plaques and cerebrovascular amyloid in each animal species.

## Abstract

Senile plaques and cerebrovascular amyloid are well known as histopathological hallmarks in the patients of Alzheimer's disease and these lesions are also found in some species of animals.

To investigate on senile plaques and cerebrovascular amyloid by comparative histopathology, I examined four species of aged animals such as cynomolgus monkeys, dogs, cats, and a two-humped camel. Mature plaques including classical and primitive ones and diffuse plaques were observed in cynomolgus monkeys and dogs. Mature plaques were frequently found in cynomolgus monkeys, whereas diffuse plaques were frequently observed in dogs. Furthermore, I found firstly senile plaques in cats and a camel. Senile plaques in a camel seemed to be diffuse plaques, but those in cats were not classified into any types of senile plaques described previously. The lesion of cerebrovascular amyloid in cynomolgus monkeys was often recognized in cortical capillary adjoining mature plaques while that in dogs often observed in cortical parenchymal and meningeal arterioles. A small number of cerebrovascular amyloid was found in cats and a camel.

These results suggest the possibility of senile plaque formation in wide variety of mammalian species. Moreover, it is suggested that elucidation of Aβ deposits will be possible by comparative studies on senile plaques and cerebrovascular amyloid in each animal species.

**Table 1 Clinical course of six aged cynomolgus monkeys, and occurence of senile plaques and cerebrovascular amyloid**

| Animal No. (Origin) | Sex | Age | Breeding history | Clinical history | Pathological findings at necropsy | Senile plaques | | | Cerebrovascular amyloid | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | CP | PP | DP | CAP | PA | MA |
| 1 (Cambodia) | Male | 29y8m | 41/147* Final breeding: 24y9m | Weight loss after obesity Hyperglycemia, Diabetes mellitus Vesicular stomatitis, Rash on hypogastrium Respiratory symptom: nasal discharge, cough and conjunctivitis | Diabetes mellitus Atrophy of testis, spleen and thyroid gland | - | + | - | - | - | - |
| 2 (Cambodia) | Female | 22y3m | 1/5** (stillbirth) Final delivery age: 6y11m Final menses: 3 month before deceased | Obesity, hyperglycemia Endometritis (operated biopsy) Anorexia, vomit and obstipation | Fatty degeneration of liver pulmonary edema Atrophy of glomerulus | + | + | + | + | + | - |
| 3 (Philippines) | Female | 23y1m | 0/10** Final menses: at decease | Weight loss after obesity Diarrhea, anorexia Abscess on forearm | Diabetes mellitus Septicemia | + | + | + | + | + | - |
| 4 (Cambodia x Vietnam) | Female | 20y10m | 6/13** (2 times partus caesarius) Final delivery age: 11y7m Final menses: 20y6m (menoxemia for the last 1 year) | ſeight loss after obesity yperglycemia, Diabetes mellitus ontinuous hemorrhage from gingiva nemia, facioedema | Diabetes mellitus Abscess of subcutaneous scalp Necropneumonia | - | - | - | - | - | - |
| 5 (Cambodia x Vietnam) | Female | 22y8m | 6/16** (1 time partus caesarius) Final delivery age: 13y2m Final menses: 3 month before decease (menoxenia | Obesity Sporadic diarrhea Cataract and swelling of right eye Abdominal mass Anorexia and obstipation | Rupture of endometritis | + | + | - | + | + | - |
| 6 (Vietnam) | Male | 23y4m | 21/48* Final breeding age: 16y4m | Weight loss after obesity Inguinal hernia Continuous hemorrhage from gingiva after exodontia, Fistulation from gingiva and infiltration of the abscess | Focal infection of gingival abscess Septicemia | + | + | - | + | + | - |

*: Number of females impregnated/matings
**: Number of pregnancies/matings

CP: Classical plaques PP: Primitive plaques DP: Diffuse plaques
CAP: Capillaries PA: Parenchymal arterioles MA: Meningeal arterioles

**Table 2 Aged dogs examined, gross pathological diagnosis, and occurence of senile plaques and cerebrovascular amyloid**

| Case No. | Age | Sex | Breed | Pathological diagnosis | Senile plaques | | | Cerebrovascular Amyloid | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | DP | PP | CP | CAP | PA | MA |
| 94054 | 14y6m | Male | Beagle | Necrotic enteritis, Leiomyoma of stomach | + | + | - | - | - | + |
| 95057 | 12y | C. male | Mongrel | Malignant thymoma | + | + | - | + | + | + |
| 95058 | 16y | Male | Whippet | Chronic nephritis | + | - | - | + | - | - |
| 95060 | 13y | Male | Mongrel | Mastocytoma | + | - | - | - | - | - |
| 95071 | 18y6m | S. female | Yorkshire Terrier | Chronic nephritis | + | + | + | + | + | + |
| 95072 | 12y6m | Male | Pomeranian | Systemic thrombosis | + | - | - | - | - | - |
| 95074 | 13y | C. male | Yorkshire Terrier | Adenocarcinoma of prostate gland | + | + | - | + | + | + |
| 95075 | 14y | C. male | Shih tzu | Chronic nephritis, purulent cystitis | + | + | - | - | + | + |
| 95077 | 12y | Male | Pomeranian | Atrophy of the kidney, hemorrhage of the brain | + | + | - | + | + | + |
| 95079 | 12y | Female | Saroukie | Rhabdomyosarcoma and its systemic metastases | - | - | - | - | - | - |
| 95080 | 10y2m | Female | West highland white terrier | Chronic pneumonia | - | - | - | - | - | - |
| 95093 | 12y | Male | Shiba | Hepatocellular carcinoma | + | - | - | - | - | - |

CP: Classical plaques PP: Primitive plaques DP: Diffuse plaques
CAP: Capillaries PA: Parenchymal arterioles MA: Menigeal arterioles

**Table 3 Aged cats examined, and occcurence of senile plaques and cerebrovascular amyloid**

| Case No. | Age | Sex | Breed | Pathological diagnosis | Senile | Cerebrovascular amyloid |
|---|---|---|---|---|---|---|
| 92140 | 12y | Female | Himalaya | Chronic nephritis, Lymphadenitis | - | - |
| 95037 | 12y | C. male | Tintil | Mesothelioma | - | - |
| 95019 | 13y | S. female | J.D.C. | Large granular lymphoma | - | - |
| 93018 | 14y | Mal | J.D.C. | Nephritis | - | - |
| 95088 | 14y | C. male | J.D.C. | Lymphosarcoma | - | - |
| 95059 | 15y | Female | J.D.C. | Adenocarcinoma and its systemic metastases | - | - |
| 92070 | 16y | Female | Persian | Mammary tumor and its metasitasis to lung | - | - |
| 93126 | 16y | Female | Tintil | Nasal carcinoma | - | - |
| 94079 | 16y | Female | J.D.C. | Chronic pancreatitis, pneumonia, Nephritis, Gastric ulcer, Degeneration of liver | - | - |
| 92076 | 18y | Female | J.D.C. | Adenocarcinoma and its systemic metastases | + | - |
| 93011 | 18y | S. female | J.D.C. | Chronic nephritis | + | - |
| 95085 | 18y | S. female | J.D.C. | Chronic pancreatitis, Gastric ulcer, Meningiom | + | - |
| 94123 | 20y | C. male | J.D.C. | Renal atrophy with perirenal cyst, Pulumonary edema | + | + |

JDC: Japanese Domestic Cat

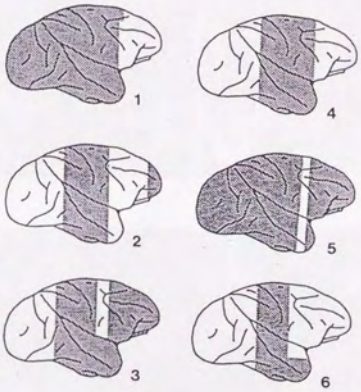



Fig. 1 Lateral view of sampling parts of the cerebrum in aged cynomolgus monkeys.

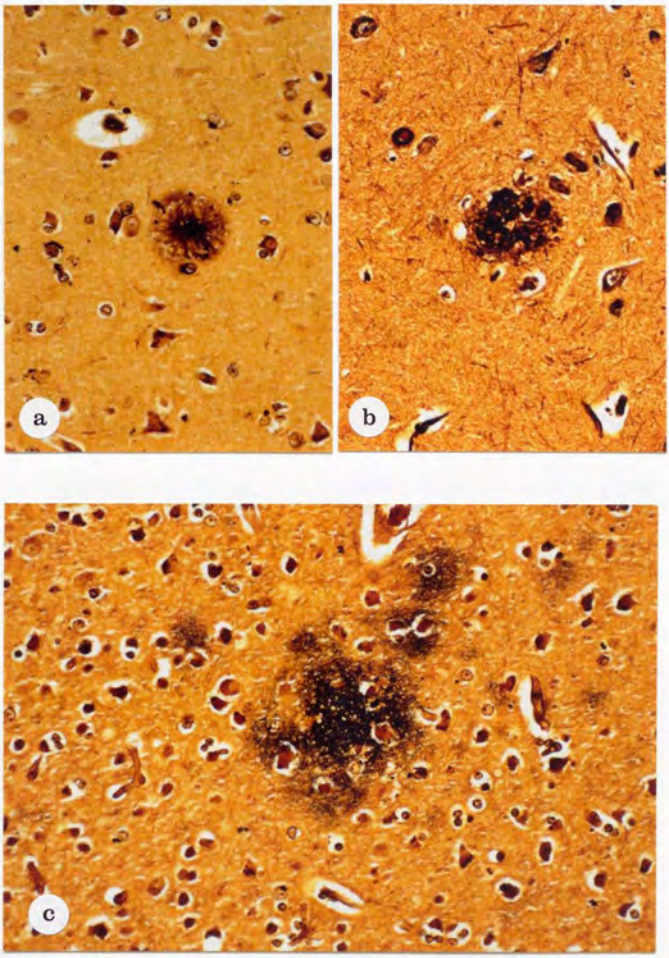



**Fig. 2 Senile plaques in the cerebral cortex of aged cynomolgus monkeys. (a) A classical plaque. PAM. x320. (b) A primitive plaque. PAM. x320. (c) Diffuse plaques. PAM. x320.**

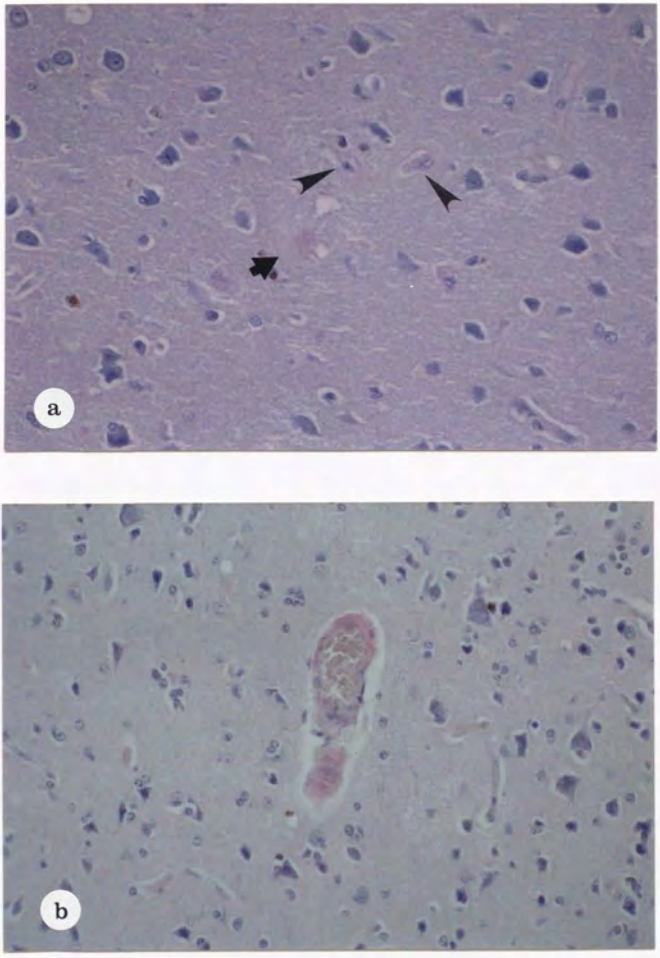



Fig. 3 Amyloid deposition to a classical plaque and cortical blood vessels in aged cynomolgus monkeys. (a) A classical plaque (arrow) and capillaries (arrowheads). Congo red. x320. (b) Cortical arterioles. Congo red. x320.

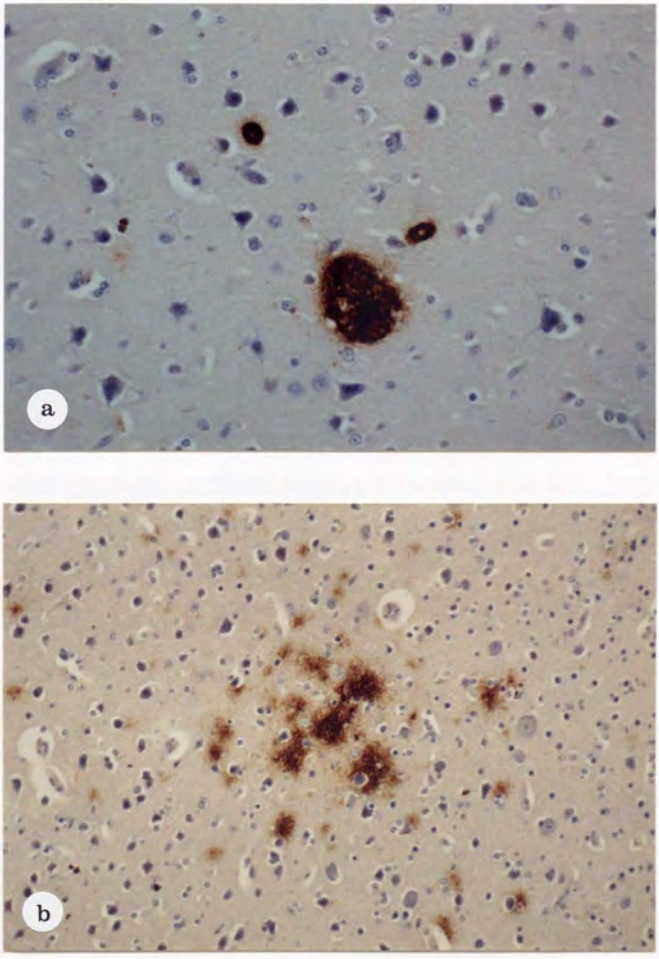



Fig. 4 Immunostainings of senile plaques and cerebrovascular amyloid to anti-$A\beta_{1-40}$ in aged cynomolgus monkeys. (a) A primitive plaque and capillaries. x320. (b) Diffuse plaques. x160.

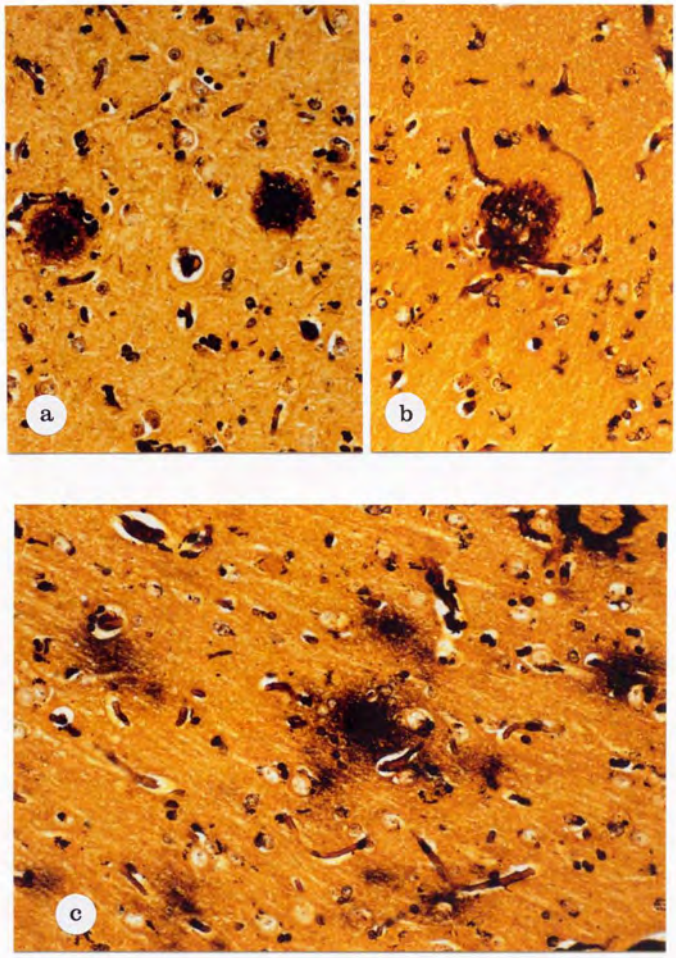



Fig. 5 Senile plaques in the cerebral cortex of aged dogs. (a) Two classical plaques. PAM. x320. (b) A primitive plaque. PAM. x320. (c) Diffuse plaques. PAM. x320.

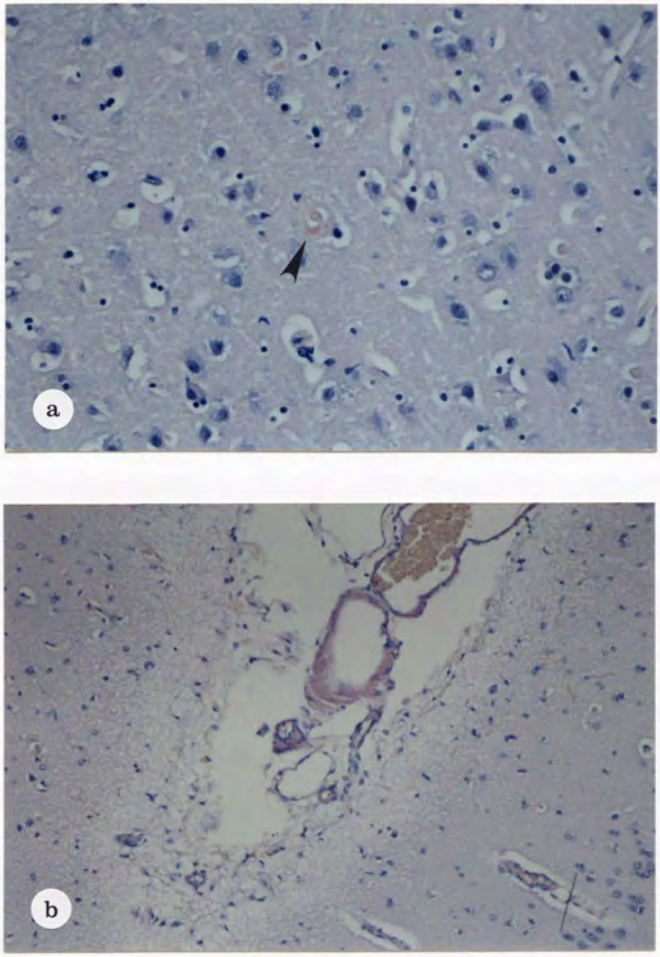



Fig. 6 Amyloid deposition to cortical blood vessels in aged dogs. (a) A capillary (arrowhead). Congo red. x320. (b) Meningeal arterioles. Congo red. x160.

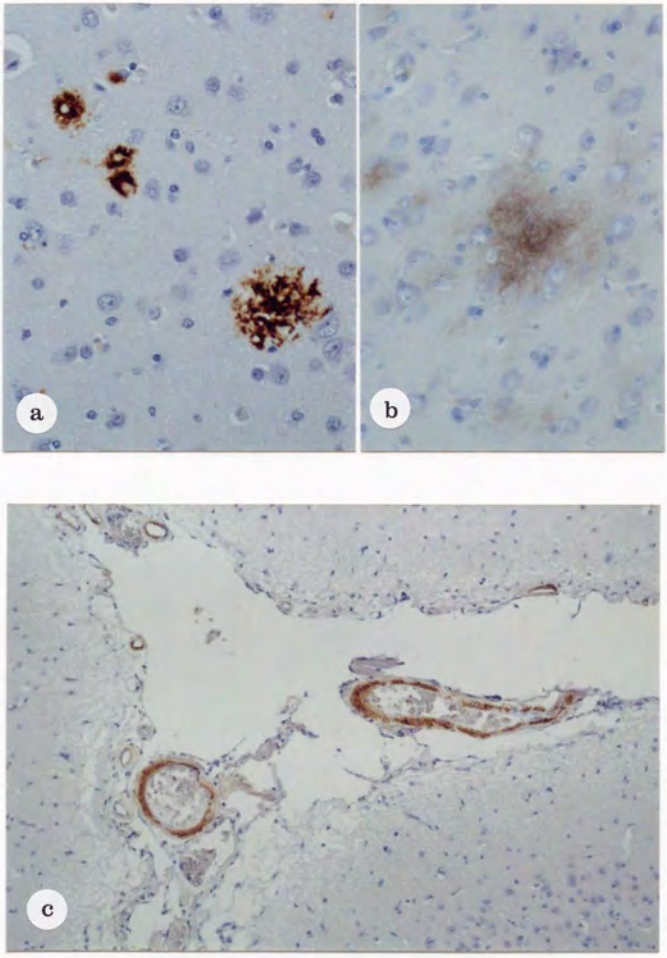



Fig. 7 Immunostainings of senile plaques and cerebrovascular amyloid to anti-$A\beta_{1-40}$ in aged dogs. (a) A primitive plaque and capillaries. x320. (b) Diffuse plaques. x320. (c) Meningeal arterioles. x130.

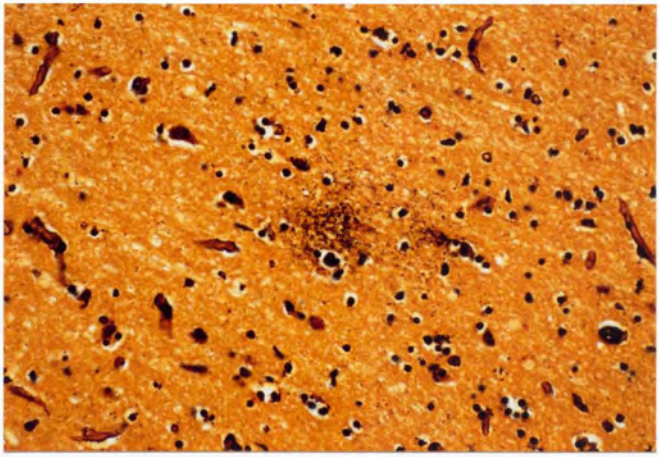


Fig. 8 Senile plaques in the cerebral cortex of very aged cats. PAM. x320.

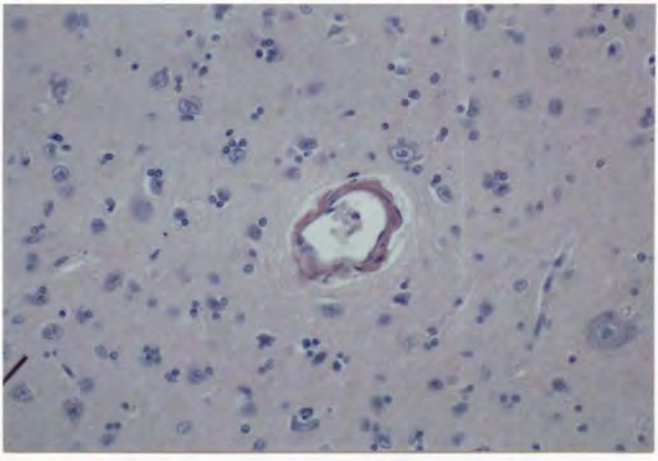


Fig. 9 Amyloid deposition to a cortical artery in a very aged cat. Congo red. x320.

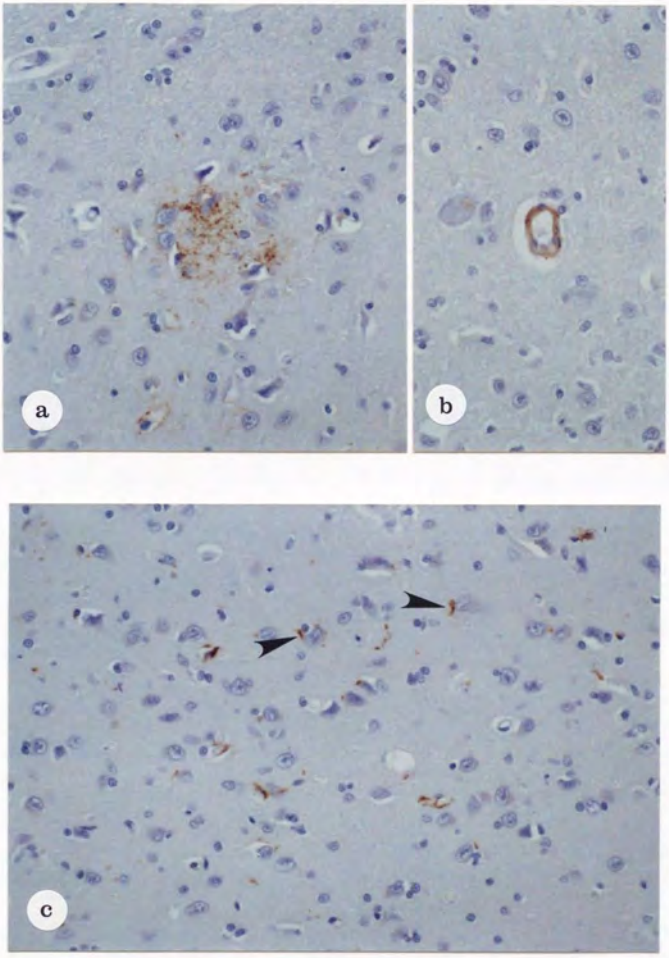



Fig. 10 Immunostainings of senile plaques, cerebrovascular amyloid, and Aβ deposition without these two lesions to anti-$A\beta_{1-40}$ in aged cats. (a) Senile plaques. x320. (b) A cortical artery. x320. (c) Aβ deposition in the cortical neuropil around neurons (arrowheads) where did not form senile plaque. x320.

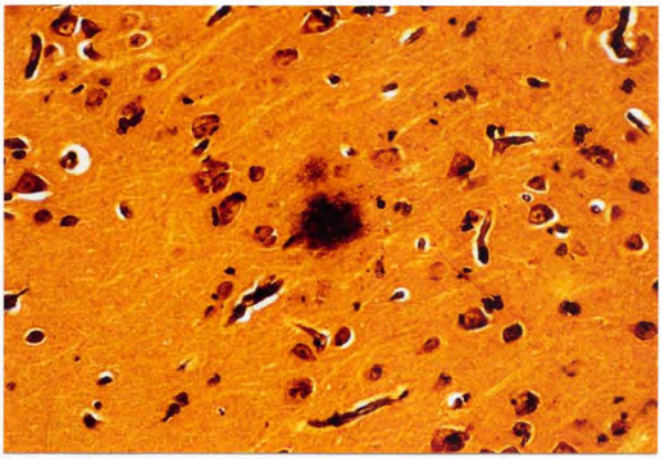


Fig. 11 Senile plaques in the cerebral cortex of an aged camel. PAM. x320.

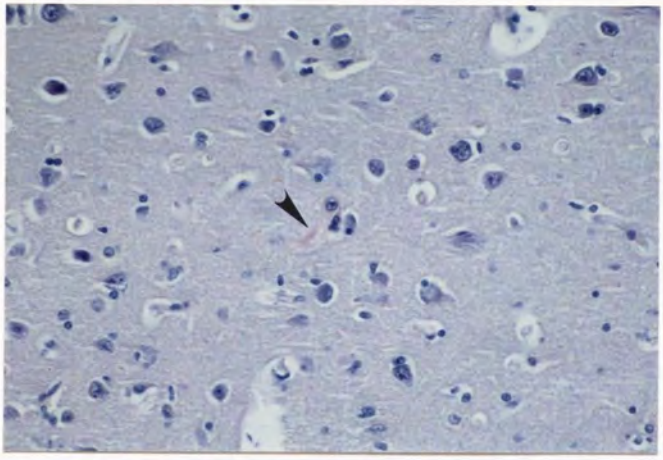


Fig. 12 Amyloid deposition to a cortical capillary of an aged camel. Congo red. x320.

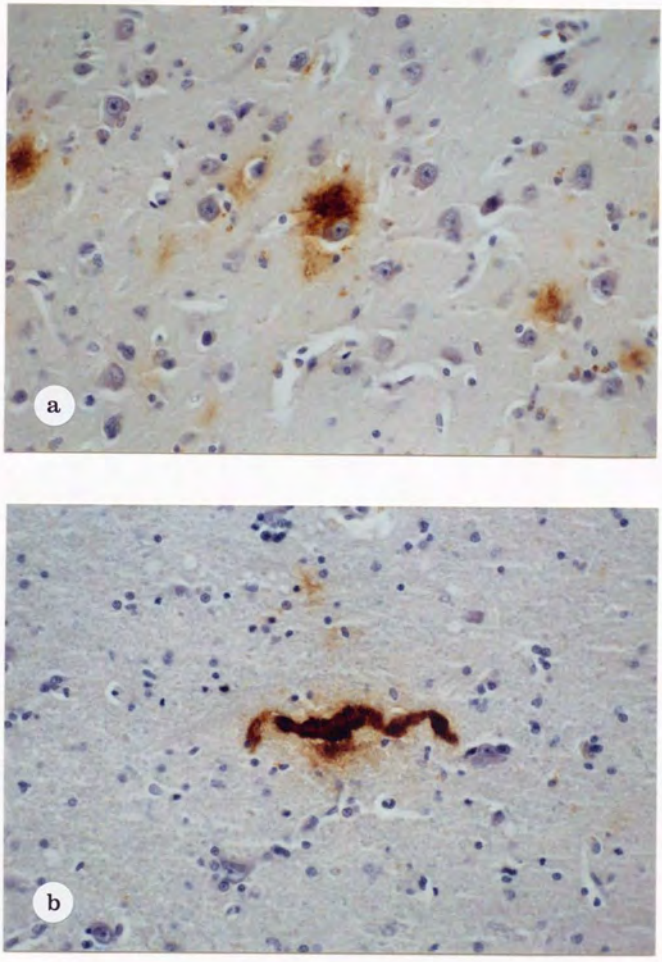



Fig. 13 Immunostainings of senile plaques and cerebrovascular amyloid in an aged camel. (a) Senile plaques. x320. (b) A capillary (arrowhead). x320.

# Chapter 2.

## Histopathological Characteristics and Incidence of Senile Plaques and Cerebrovascular Amyloid in Cynomolgus Monkeys

## Introduction

The patients with familial Alzheimer's disease (FAD) show severe amyloid β protein (Aβ) deposits in senile plaques and cerebrovascular amyloid in the brain compared with normal age-matched humans [38]. As for the cause of FAD, some genes have been known as genetic risk factors. Aβ is produced by proteolytic cleavage from amyloid precursor protein (APP) which is expressed in normal cells of various organs [16, 57, 67]. In early-onset FAD with a certain point mutation of APP gene linked to the chromosome 21, the mutation was inherited to the posterity and the patients had much more accumulation of Aβ in the brain [46, 49, 57]. Apolipoprotein E (apo E) is also deposited in senile plaques and cerebrovascular amyloid as well as Aβ [29, 72], and the ε4 gene of apo E involved in the chromosome 19 is considered as one of the risk factors in late-onset and FAD [5, 37, 47]. Recently, AD3 (S182) gene on the chromosome 14 and STM2 gene on the chromosome 1 were confirmed as genetic risk factors in early-onset FAD, although physiological function of these genes' products in the brain were unknown [19, 20, 40]. Since genetic factors are associated with Alzheimer's disease, animal models of the disease are required for the data base of animal's pedigree and genetic background.

Before starting genetical analysis of APP, Apo E, AD3 and STM2 genes in the animal models, it is important to know histopathological features of senile plaques and cerebrovascular amyloid in the brain of the animals. In the present study, I examined firstly laboratory bred cynomolgus monkeys having clear

breeding and clinical histories, which mainly belong to Tsukuba Primate Center for Medical Science (TPC), National Institute of Health, Japan. Then, detailed histological distribution and incidence of the senile plaques and cerebrovascular amyloid were studied in cynomolgus monkeys with wide variety of ages; especially about the characteristics of the various types of senile plaques, the relation between age and senile plaque occurrence, and the topographical localization of senile plaques.

## Materials and methods

### *Animals*

We examined the cerebrum of 49 cynomolgus monkeys including 33 derived from TPC, National Institute of Health, Japan, and 8 from the Laboratory Animal Science and Toxicology Laboratories of Sankyo Co. LTD., 5 from the Experimental Technology Research Center of Daiichi Pharmaceutical Co. LTD., and 3 from School of Medicine, Tsukuba University. They included 17 aged monkeys which are 20 to 29 years old, and 32 young or middle-age animals (Table 1). Since the TPC-originated old monkeys were registered on their profile such as age, sex, and detailed breeding and clinical histories, these records were listed in Table 2.

### *Histopathology*

Tissue samples from the cerebrum were fixed in 10% neutral buffered formalin. Paraffin sections (4- to 6-μm-thick) were

stained with hematoxylin and eosin (HE), periodic acid methenamine silver (PAM) for detecting senile plaques, and alkaline Congo red for amyloid deposits.

*Immunohistochemical examination*

Four μm-thick paraffin sections were immunostained through ABC method following the treatment with 90% formic acid for 5 min, as described previously [17]. Antibodies used in this study were as follows; rabbit antiserum against synthetic peptide of Aβ 1-40 (anti-$A\beta_{1-40}$, Boehringer Mannheim Biochemica, Germany, used at 1:5 dilution), and mouse monoclonal antibody against synthetic peptide of Aβ 8-17 (anti-$A\beta_{8-17}$, Dako, Carpinteria, CA, U.S.A., 1:50) as primary antibodies, and biotinylated goat anti-rabbit and anti-mouse Ig G (Kirkegaard & Perry Lab., Inc., Gaithersburg, MD, 1:100) as secondary antibodies. The sections were, then, treated with avidin-biotin-peroxidase complex (ABC, Vectastain ABC kit, Vector Lab., Burlingame, CA) and finally visualized in 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO) plus hydrogen peroxide solution.

*Topographical analysis*

I surveyed systematically the localization of senile plaques in three aged cynomolgus monkeys of which whole cerebrum including both right and left hemispheres was sampled (case No. 24, 29, and 35 shown in Table 1; 26, 24, and 20 years old, respectively). The cerebrum was grossly cut into 26 coronary parts from frontal to back end. Each cut-part embedded in paraffin was sectioned into 4μm-thickness. The sections were then stained

with HE, PAM, and alkaline Congo red. Lightmicroscopically examined items were as follows; 1) total number of senile plaques in each section, 2) number of each type of senile plaque according to morphological classification [52, 70], and 3) coronal and lateral topograph on the localization of these plaques. For 1) and 2), I composed the graphs showing the number of all senile plaques in each section and that of different types of senile plaques. In the graphs, the anatomical area in the lateral view of the cerebrum was expressed serially from 1 to 60 corresponding to the frontal to back end. The neuroanatomical portions of the brain in cynomolgus monkey were referred to the brain atlas written by Szabo *et al*. [51].

## Results

### *Histopathological distributions of senile plaques and cerebrovascular amyloid in cynomolgus monkeys*

Senile plaques: Senile plaques were found in the cerebral cortex of 13 out of 17 aged monkeys which were more than 20 years old (Fig. 1, and Table 1). The senile plaques were classified into 2 subtypes according to the category of human senile plaques [38, 52, 70]. One type was mature plaque with swollen neurites, which is further divided into classical plaque with central amyloid core (Fig. 2-a) and primitive plaque without one (Fig. 2-b). Amyloid deposition was observed in all classical plaques, but it was not observed in a small number of primitive plaques by Congo

red stain. The other type was diffuse plaque which showed amorphous form without Congophilic amyloid deposition (Fig. 2-c). Mature plaques including classical and primtive ones were more frequently seen than immatured diffuse plaques. Among 13 monkeys, the total number of plaques was tended to increase with aging, except for the oldest monkey (29 years old, case No. 1) which was shown to have few number of senile plaques. No senile plaque was found in cynomolgus monkeys under 20 years old.

Cerebrovascular Amyloid: Cerebrovascular amyloid was mostly observed in the parenchymal capillaries and arterioles of the cerebral cortex in 7 of 13 aged monkeys with senile plaques (Fig. 1 and Table 1). Most of vascular amyloid localized in the area where senile plaques were distributed densely. In these area, it was often seen that amyloid deposition to blood vessels was very adjacent to mature plaque (Fig. 3-a). Vascular amyloid of case No. 24. was one exception, in which amyloid was deposited in meningeal arterioles (Fig. 3-b and Table 1). No cerebrovascular amyloid was seen in cynomolgus monkeys less than 21 years old.

Immunohistochemical findings: By Immunohistochemical analysis, all types of the senile plaques of 13 cases positively reacted to anti-$A\beta_{1-40}$ (Fig. 4-a, b). Diffuse plaques which were not stained with PAM could be detected by immunohistochemical examination using anti-$A\beta_{1-40}$. All diffuse plaques and one-third of primitive plaques did not immunoreact to anti-$A\beta_{8-17}$. In the case of cerebrovascular amyloid, both antibodies against Aβ were mostly

detected in parenchymal capillaries and arterioles (Fig. 4-a), and they were found in meningeal arterioles only in one case No. 24 (Fig. 4-c). Aβ-deposit on blood vessels was more sensitively detected by immunostaining to anti-$A\beta_{1-40}$ than by that to anti-$A\beta_{8-17}$ and Congo red staining. Most of vascular Aβ located in the area where senile plaques existed densely as well as the result with Congo red staining.

The other lesions: Spheroid and polyglucosan bodies were found in the cerebrum of cynomolgus monkeys which were more than 15 years old. Neurofibrillary tangle was not found in all the monkeys in this study.

*Topographical studies on senile plaques in three aged cynomolgus monkeys*

Total number of senile plaques: Total number of senile plaques was counted in three monkeys of different age. The youngest monkey (case No. 35) had the smallest number of senile plaques, whereas the oldest one (case No. 24) had the largest number of plaques in three monkeys. Eventually, the total number of senile plaques in each monkey tended to increase with aging (Fig. 5-a). In respective type of senile plaques, classical and primitive plaques were frequently found in the area of lateral view corresponding to the sections 15 to 30 of both hemispheres in three animals (Figs. 5-a and b). However, diffuse plaques were distributed densely in the area of left hemisphere corresponding to the sections 30 to 40 in only one animal (case No. 24) (Fig. 5-b). The number of

classical and primitive plaques tended to increase with aging in wide area of all monkeys, whereas diffuse plaques were distributed densely in narrow area of the oldest animal (case No. 24) (Fig. 5-b). Furthermore, senile plaques were distributed asymmetrically between right and left hemispheres in all three cases.

Topographical analysis on coronal sections: To plot localization of senile plaques, the lesions were drawn on cerebral topographs in each section of all cases (Figs. 6 and 7). Classical and primitive plaques were distributed densely in the cerebral cortex along temporal lobes, especially superior and inferior gyri in all cases, and amygdala in cases No. 24 and 29 in both hemispheres, and case No. 35 in left hemisphere (Fig. 6). In frontal lobes, classical and primitive plaques were sporadically found in both hemispheres of cases No. 24 and 29 (Fig. 6). On the other hand, diffuse plaques were densely observed in intraparietal gyri only in one case No. 24. A small number of mature plaques was found in occipital lobes. In the lateral view, dense area of the senile plaques located absolutely in temporal lobes along lateral fissure of Sylvius in all cases (Fig. 7).

## Discussion

Podlisny *et al*. [31] reported that senile plaques and cerebrovascular amyloid consisted of Aβ were observed in two out of three cynomolgus monkeys of 19 years old. In the study, diffuse plaques were more frequently detected than mature plaques,

and it was speculated that the tendency of abundant diffuse plaques was due to younger age of animals. Generally speaking, it is considered that the type of senile plaque changed from diffuse to mature type with aging [58, 59]. In the present study, I examined 49 cynomolgus monkeys and found senile plaques in 13 aged monkeys which were more than 20 years old. The senile plaques were classified into classical, primitive, and diffuse plaques similar to human cases [38, 52, 70], and I found classical and primitive plaques more frequently than diffuse plaques in cynomolgus monkeys of 20 to 29 years old. The youngest monkey (20 years old, case No. 22) had only mature plaques. In addition, three cases examined in detail had often mature plaques. This finding was opposed to the speculation of Podlisny *et al.* [31].

In general, as for the total number of senile plaque, an age-dependent increase was reported in humans [58, 59]. Similar tendency was also found in rhesus monkeys [48, 65] as well as in cynomolgus monkeys in this study. However, two aged monkeys showed some exceptions as follows. Namely, the second oldest monkey (26 years old; case No. 24) demonstrated numerous diffuse plaques and the oldest one (29 years old; case No. 1) had few number of plaques. It suggested that an appearance of senile plaques in aged cynomolgus monkeys had a big diversity.

Senile plaques were mainly distributed in the cortex of temporal lobe or amygdala in squirrel and rhesus monkeys [66]. In this study, senile plaques in aged cynomolgus monkeys located in the cerebral cortex of temporal lobes especially in the superior and inferior gyri, and amygdala. This feature, i.e. dense distribution of senile plaques in temporal lobes, was not recognized

in the other animals examined such as dogs, cats and a camel in Chapter 1. Since neurofibrillary tangles or severe neuronal loss were not found in cynomolgus monkeys contrary to humans [18], this feature suggested that pure effects of senile plaques on brain function might be clearly estimated by surveying the function of temporal lobe between young and aged cynomolgus monkeys.

All the lesions of senile plaques reacted to anti-$A\beta_{1-40}$, though all diffuse plaques and a part of primitive plaques did not react to anti-$A\beta_{8-17}$ in the present study. In the previous study, Podlisny *et al.* [31] used two antisera against synthetic peptide of Aβ 1-38 and purified Aβ from cerebral extract of Alzheimer's patients. Although almost senile plaques reacted to these two antibodies, a part of silver-stained senile plaques did not react. Because it was not referred to what type of the plaque was negative [31], it was not clear that both results meant the same phenomenon. It was reported that if the activity of the antibody recognizing whole Aβ was partially blocked by saturation with synthetic Aβ 17-24 peptide, then it stained only amyloid core [71]. Recently, monoclonal antibodies which specifically recognize Aβ 1-40 and 1-42(43), respectively, were developed and they showed different reactivity to senile plaques [13]. Therefore, difference of Aβ epitope recognized by antibody might effect on a staining profile.

Aβ deposition on the cerebral blood vessels was observed in aged rhesus and squirrel monkeys [39, 66] as well as in cynomolgus monkeys [31]. As compared with rhesus monkeys, squirrel monkeys showed Aβ deposition more frequently in the cortical blood vessels than in the senile plaques [66]. In cynomolgus monkeys in the present study, cerebrovascular amyloid was more

rarely found than the senile plaque, when compared with squirrel monkeys. Aβ in blood vessels reacted more sensitively to anti-$A\beta_{1-40}$ than to anti-$A\beta_{8-17}$ and Congo red. The result suggests that soluble or slightly filamentous Aβ was detected by anti-$A\beta_{1-40}$ [50]. Mature plaques and cerebrovascular amyloid in capillaries frequently located at adjoining positions each other. They were detected by both Congo red stain and by immunostaining, and were demonstrated in the area where senile plaques localized densely. These morphological findings indicate that amyloid deposition on mature plaques and capillaries had an intimate relationship in cynomolgus monkeys.

In FAD, a certain point mutation of the APP gene was inherited to the posterity and the patients had much more accumulation of Aβ in the cerebrum [46, 49]. Other than APP gene, it was predicted that genetic risk factors such as Apo E ε4 [5, 37], AD3 (S182) [40], and STM2 [19, 20] genes were linked to FAD with Aβ deposition. Thus, genetical back ground was important to investigate pathogenesis of cerebral amyloidosis when using animal model. However, previous studies on beta amyloidosis in animals did not describe about their pedigree and histories. In the present study, I used cynomolgus monkey having an obvious profile including breeding and clinical histories and known pedigree. Previous studies described that amino acid sequence of cynomolgus monkey $APP_{695}$ was completely homologous to that of human [31] and apo E of cynomolgus monkey had also 93% homology to that of human [22]. Therefore, this animal model was very useful for not only histopathological analysis but also genetic analysis of amyloid deposition. Since physiological functions of AD3 and

STM2 gene-products have not been clarified yet, this animal model will be possible to research the role of these gene products in the pathogenesis of FAD.


## Abstract

Amyloid β protein (Aβ) deposition in the brain is closely related with some genetic risk factors. To establish an animal model for cerebral amyloidosis, the data base of animal's pedigree and genetic background is required. Therefore, I examined mainly laboratory bred cynomolgus monkeys having clear breeding and clinical histories.

Senile plaques were detected histopathologically and immunohistochemically in the cerebrum of 13 out of 17 aged cynomolgus monkeys, more than 20 years old. Morphologically, mature plaques including classical and primitive ones were more frequently observed than diffuse one. Furthermore, senile plaques were often seen in the cortex of temporal lobes especially in the superior and inferior gyri. On the other hand, cerebrovascular amyloid was recognized in 7 aged cynomolgus monkeys. Since amyloid deposition in capillaries was frequently seen adjoining mature plaques, the close relation between mature plaque and cerebrovascular amyloid was suggested. All senile plaques and cerebrovascular amyloid were detected by anti-$A\beta_{1-40}$. However, all diffuse plaques and one-third of primitive plaques were not detected by anti-$A\beta_{8-17}$. Although the morphology of senile plaques in cynomolgus monkeys was consistent with that in humans and

dogs, localization of senile plaques and morphological correlation between senile plaques and cerebrovascular amyloid in cynomolgus monkeys were different from those in humans.

Since detailed histopathological distribution and incidence of senile plaques and cerebrovascular amyloid with aging were clarified in cynomolgus monkeys having evident pedigree and histories, cynomolgus monkeys would be useful to investigate pathogenesis of cerebral amyloidosis by pathological and genetical analyses.

**Table 1 Distribution of senile plaque and amyloid angiopathy in the cerebral cortex of cynomolgus monkeys stained by PAM and Congo red, respectively.**

| Case No. | Age | Sex | Source | Senile plaques by PAM | | | Amyloid angiopathy by Congo red | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | CP [a] | PP [b] | DP [c] | CAP [d] | PA [e] | MA [f] |
| 1 | 29y8m | Male | TPC [1] | - | + | - | - | - | - |
| 2 | 22y3m | Female | TPC | + | + | + | + | + | - |
| 3 | 23y1m | Female | TPC | + | + | + | + | - | - |
| 4 | 20y10m | Female | TPC | - | - | - | - | - | - |
| 5 | 22y8m | Female | TPC | + | + | - | + | + | - |
| 6 | 23y4m | Male | TPC | + | + | - | + | + | - |
| 7 | 11y5m | Female | TPC | - | - | - | - | - | - |
| 8 | 11y | Female | TPC | - | - | - | - | - | - |
| 9 | 16y4m | Female | TPC | - | - | - | - | - | - |
| 10 | 8y10m | Female | TPC | - | - | - | - | - | - |
| 11 | 11y10m | Female | TPC | - | - | - | - | - | - |
| 12 | 10y11m | Female | TPC | - | - | - | - | - | - |
| 13 | 8y2m | Female | TPC | - | - | - | - | - | - |
| 14 | 13y2m | Female | TPC | - | - | - | - | - | - |
| 15 | 12y2m | Female | TPC | - | - | - | - | - | - |
| 16 | 8y2m | Female | TPC | - | - | - | - | - | - |
| 17 | 13y4m | Female | TPC | - | - | - | - | - | - |
| 18 | 10y2m | Female | TPC | - | - | - | - | - | - |
| 19 | 8y8m | Female | TPC | - | - | - | - | - | - |
| 20 | 10y10m | Female | TPC | - | - | - | - | - | - |
| 21 | 8y8m | Female | TPC | - | - | - | - | - | - |
| 22 | 20y | Male | SCL [2] | + | + | - | - | - | - |
| 23 | 16y | Male | SCL | - | - | - | - | - | - |
| 24 | 26y | Male | SCL | + | + | + | + | + | + |
| 25 | 15y | Female | SCL | - | - | - | - | - | - |
| 26 | 18y | Female | SCL | - | - | - | - | - | - |
| 27 | 22y | Female | SCL | - | + | - | - | - | - |
| 28 | 23y | Female | SCL | + | + | - | + | - | - |
| 29 | 24y | Female | SCL | - | + | - | - | - | - |
| 30 | 21y | Female | TPC | - | + | - | - | - | - |
| 31 | 18y | Female | TPC | - | - | - | - | - | - |
| 32 | 13y3m | Female | TPC | - | - | - | - | - | - |
| 33 | 8y3m | Female | TPC | - | - | - | - | - | - |
| 34 | >20y [A] | Female | TPC | - | - | - | - | - | - |
| 35 | >20y [A] | Female | TPC | + | + | - | - | - | - |
| 36 | 20y2m | Female | TPC | - | - | - | - | - | - |
| 37 | 17y3m | Male | TPC | - | - | - | - | - | - |
| 38 | 15y11m | Male | TPC | - | - | - | - | - | - |
| 39 | 18y | Male | TPC | - | - | - | - | - | - |
| 40 | 21y7m | Female | TPC | - | - | - | - | - | - |
| 41 | 2y | Male | TUV [3] | - | - | - | - | - | - |
| 42 | 2y | Male | TUV | - | - | - | - | - | - |
| 43 | 2y | Female | DCL [4] | - | - | - | - | - | - |
| 44 | 4y | Female | DCL | - | - | - | - | - | - |
| 45 | 2y | Female | DCL | - | - | - | - | - | - |
| 46 | 2y | Female | DCL | - | - | - | - | - | - |
| 47 | 2y | Female | DCL | - | - | - | - | - | - |
| 48 | 22y3m | Female | TPC | + | + | - | + | - | - |
| 49 | 3y | Male | TUV | - | - | - | - | - | - |

[a] Classsical plaques [b] Primitive plaques [c] Diffuse plaques [d] Capillaries [e] Parenchymal arterioles [f] Meningeal arterioles +: Positive -: Negative [A] Precised age was unknown, at least more than 20 years old (wild originate)

[1] Tsukuba primate center for medical science

[2] Laboratory animal science and toxicology laboratories of Sankyo Co. LTD.

[3] Experimental technology research center of Daiichi pharmaceutical Co. LTD.

[4] School of medicine, Tsukuba University

**Table 2 Clinical course of 8 aged cynomolgus monkeys with senile plaques which are derived from TPC.**

| Animal No. (Origin) | Sex | Age | Breeding history | Anamnesis |
|---|---|---|---|---|
| NO.1 (Cambodia) | Male | 29Y 8M | 41/147*<br>Final breeding age: 24Y 9M | Diabetes Mellitus<br>Vesicular stomatitis, rash on hypogastrium<br>Conjunctivitis<br>Atrophy of testis, spleen and thyroid gland |
| NO.2 (Cambodia) | Female | 22Y 3M | 1/5**(stillbirth)<br>Final delivery age; 6Y 11M<br>Final menses: 3 month before deceased | Fatty degeneration of liver<br>Endometriosis<br>Pulmonary edema<br>Atrophy of glomerulus |
| NO.3 (Philippines) | Female | 23Y 1M | 0/10**<br>Final menses: at deceased | Diabetes mellitus<br>Diarrhea, anorexia<br>Septicemia |
| NO.5 (Cambodia x Vietnam) | Female | 22Y 8M | 6/16** (1 time partus caesarius)<br>Final delivery age: 13Y 2M<br>Final menses: 3 month before deceased<br>(menoxenia for the last 1 year) | Sporadic diarrhea<br>Rupture of Endometriosis |
| NO.6 (Vietnam) | Male | 23Y 4M | 21/48*<br>Final breeding age: 16Y 4M | Inguinal hernia<br>Focal infection of gingival abscess<br>Septicemia |
| NO.30 (Philippines x Vietnam) | Female | 21Y | 1/8** ,1time abortion<br>Final pregnance age: 6Y 8M<br>Final menses: 9 month before deceased | Endometritis<br>Stenosis of ureter by adhesion |
| NO.35 (Malaysia) | Female | >20Y*** | 3/5**<br>Final delivery age: 9Y<br>Final menses: 5 month before deceased | Diabetes mellitus<br>Endometriosis<br>Hydronephrosis |
| No.48 (Malaysia) | Female | 22Y 3M | 4/4**<br>Final pregnance age: 9Y 8M<br>Final menses: 20y2m | Dibetes mellitus<br>Lung abscess |

* : Number of females impregnated/matings

**: Number of pregnancies/matings

***: See Table 1

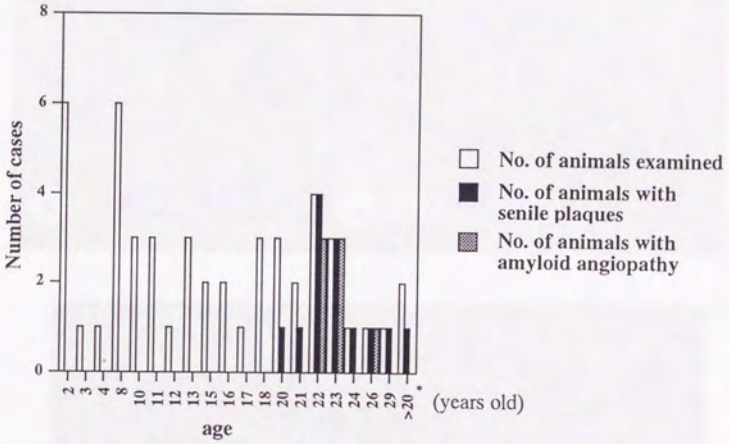



**Fig. 1 Relationship between aging and occurrence of senile plaques or amyloid angiopathy**

* See Table 1

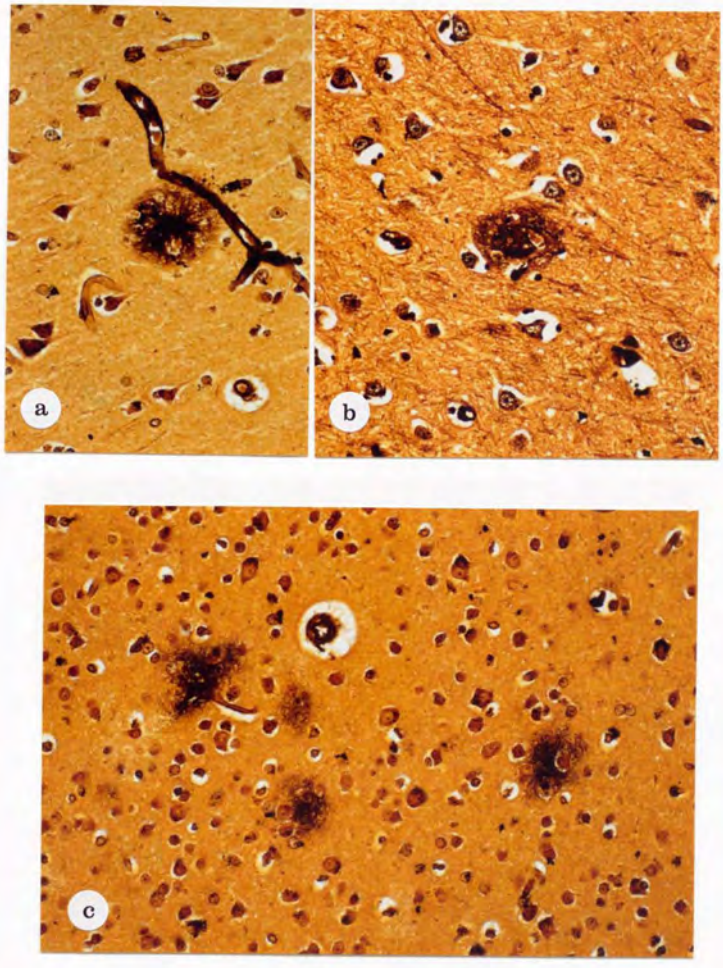



Fig. 2 Senile plaques in the cerebral cortex of aged cynomolgus monkeys. (a) A classical plaque. PAM. x320. (b) A primitive plaque. PAM. x320. (c) Diffuse plaques. PAM. x320.

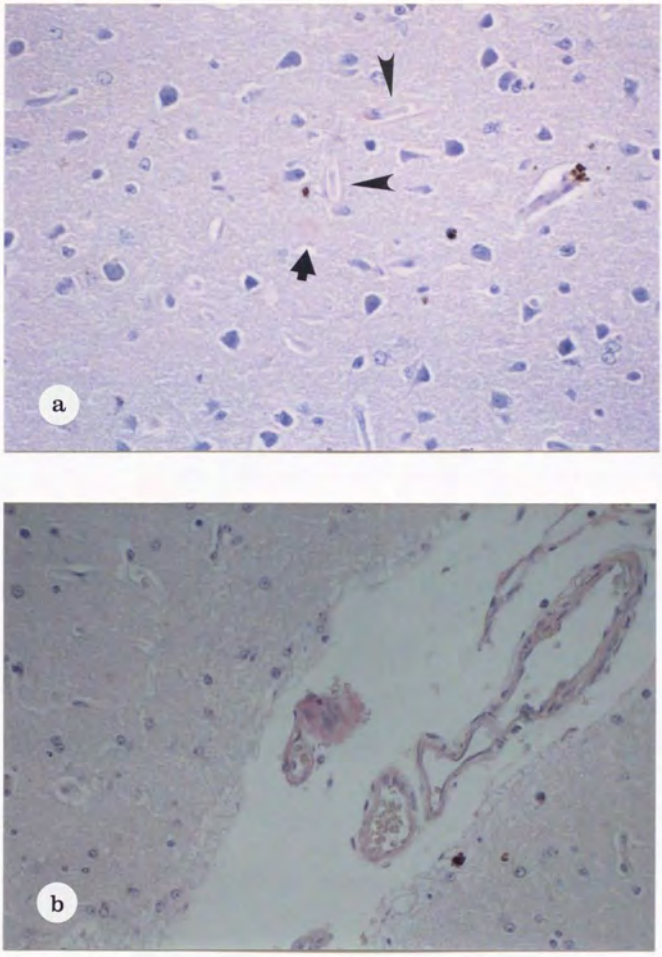



Fig. 3 Amyloid deposition to a classical plaque and cortical blood vessels in aged cynomolgus monkeys. Congo red. x . (a) A classical plaque (arrow) and capillaries (arrowheads). Congo red. x320. (b) Menigeal arterioles. Congo red. x160.

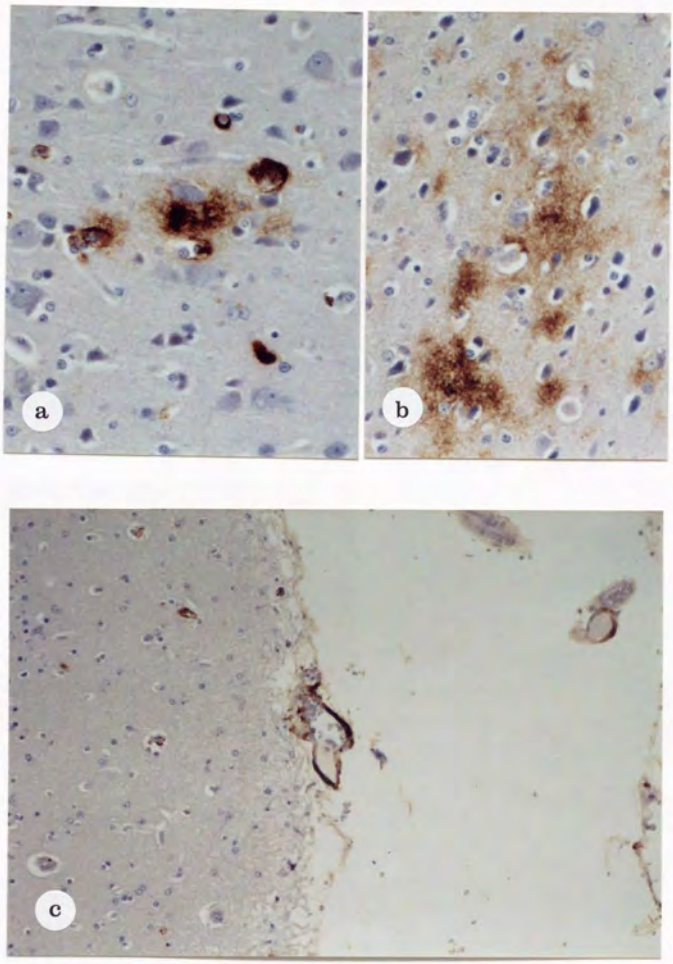



Fig. 4 Immunostainings of senile plaques and cerebrovascular amyloid to anti-$A\beta_{1-40}$ in aged cynomolgus monkeys. (a) A classical plaque and capillaries. x320. (b) Diffuse plaques. x320. (c) Menigeal arterioles. x160.

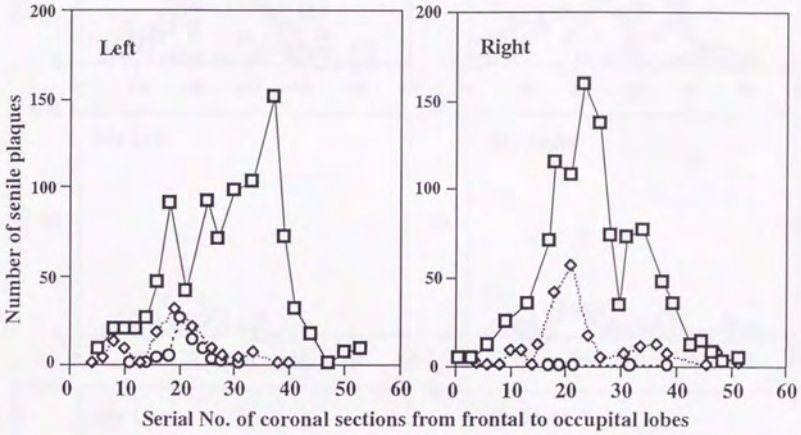



Fig. 5-a. Total number of senile plaques in the serial coronal sections in aged monkeys

—□— Case No. 24
·····◇····· Case No. 29
----○---- Case No. 35

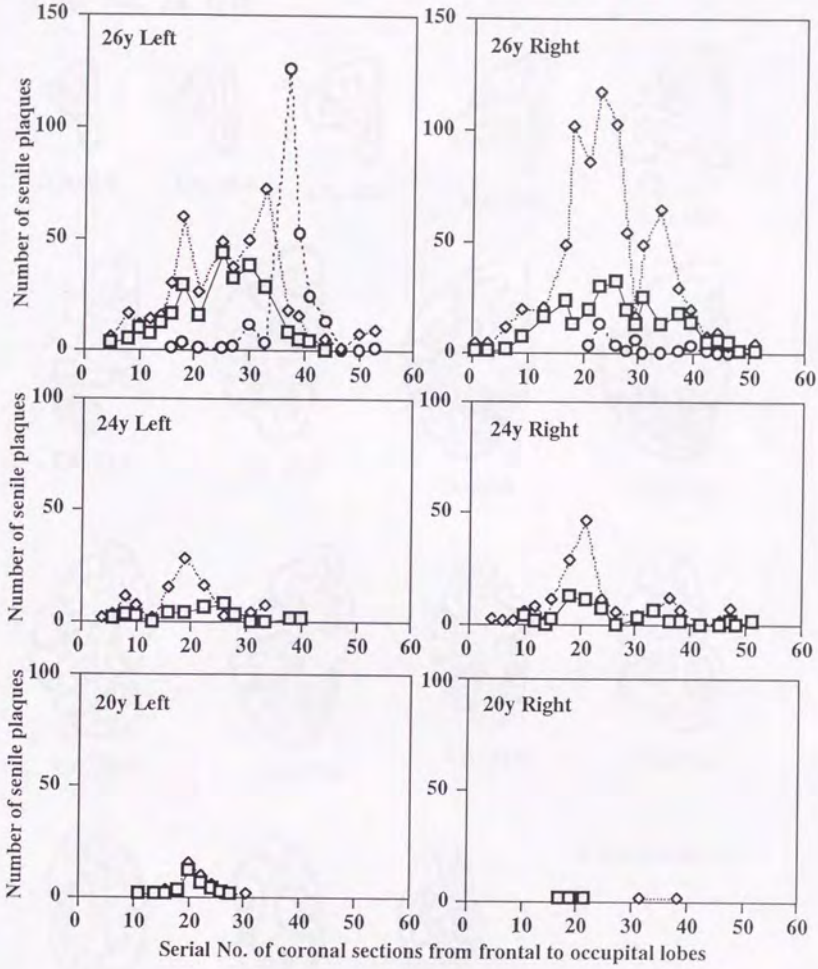



Fig. 5-b Number of differnt type senile plaques in the serial coronal sections in aged monkeys

## Case No. 24, left

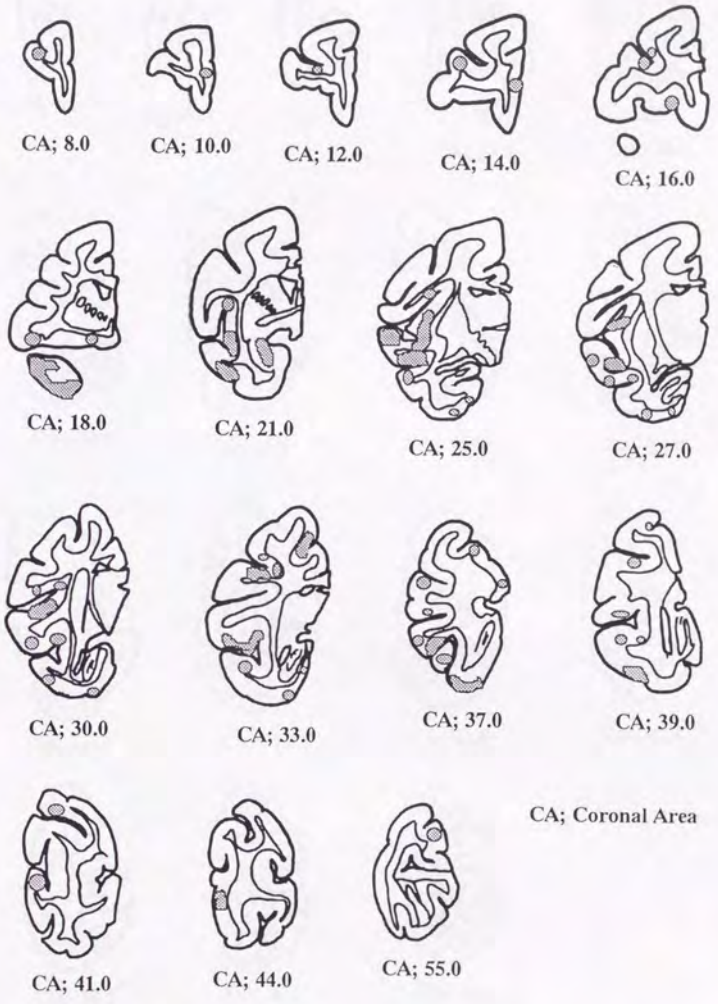



**Fig. 6-a** Topograph of senile plaques which were frequently distributed in superior and inferior gyri, and amygdala in the whole brain of the coronal section.

: Portion distributed with the senile plaques densely

## Case No. 24, right

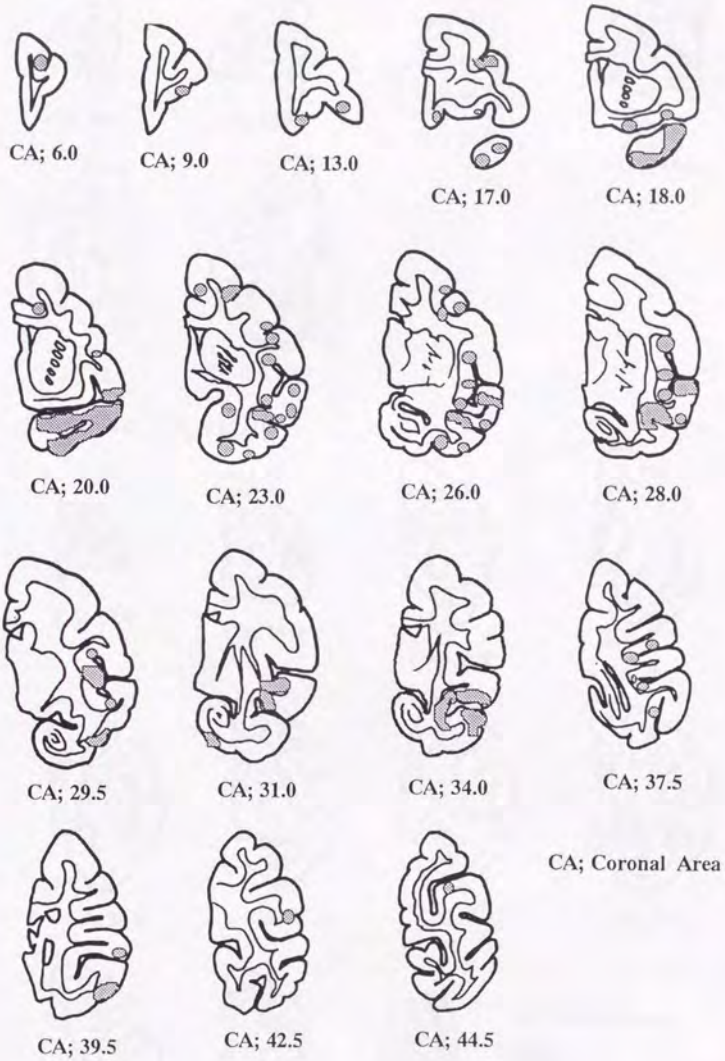
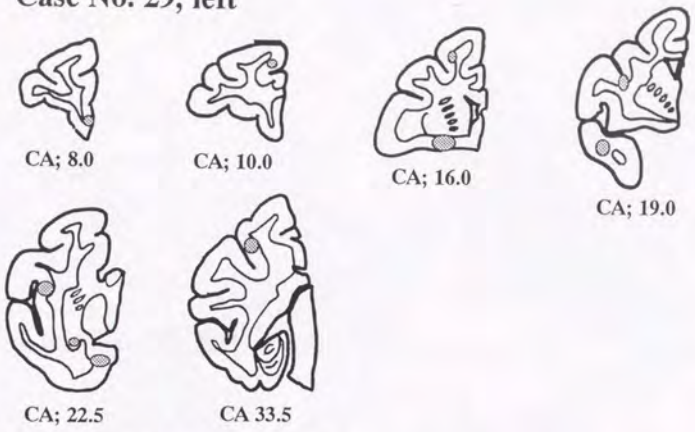



**Fig. 6-b** Topograph of senile plaques which were frequently distributed in superior and inferior gyri, and amygdala in the whole brain of the coronal section.

: Portion distributed with the senile plaques densely

## Case No. 29, left




## Case No. 29, right

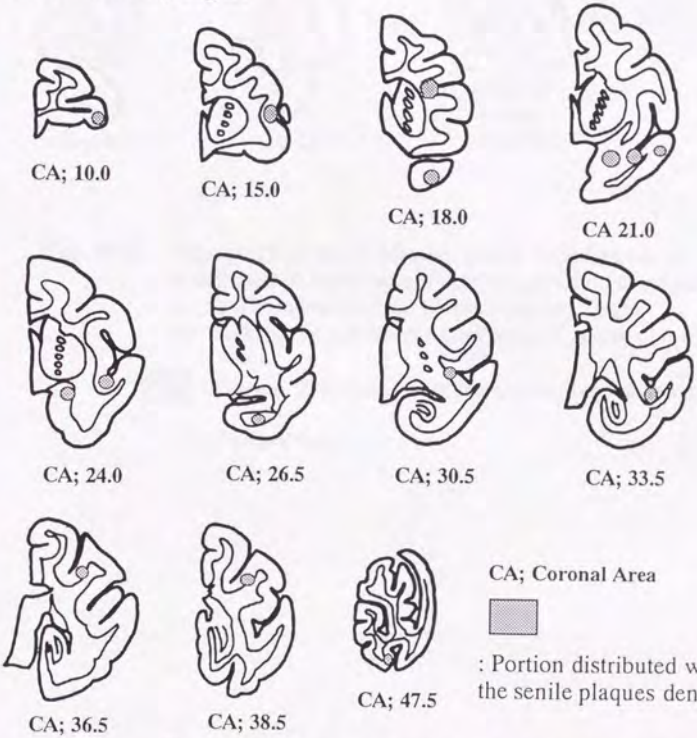



**Fig. 6-c** Topograph of senile plaques which were frequently distributed in superior and inferior gyri, and amygdala in the whole brain of the coronal section.

## Case No. 35, left


**Fig. 6-d** Topograph of senile plaques which were frequently distributed in superior and inferior gyri, and amygdala in the whole brain of the coronal section. Right hemisphere did not show dense area of senile plaques.

▨ : Portion distributed with the senile plaques densely

**CA; Coronal Area**

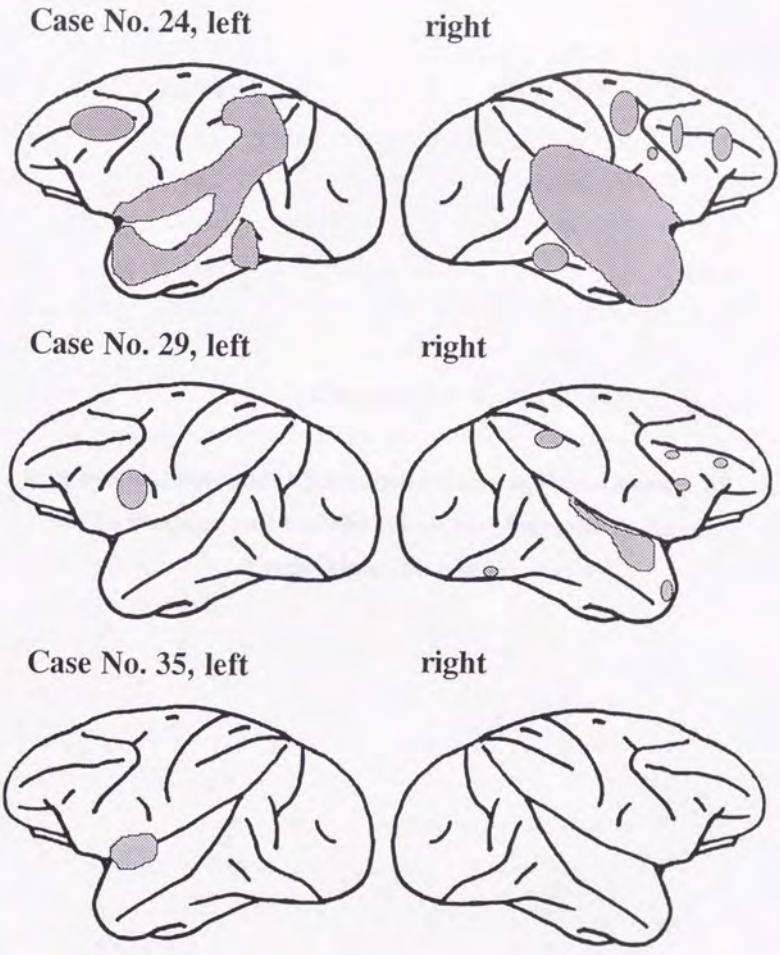



Fig. 7 Topograph of the whole brain from temporal view.
Senile plaques were frequently observed along superior.
Superior gyri (arrows).

: Portion distributed with the senile plaques densely

## Chapter 3.

# Immunohistochemical Studies on Several Constituents of Senile Plaques and Cerebrovascular Amyloid in Aged Cynomolgus Monkeys

## Introduction

Senile plaques and cerebrovascular amyloid are consisted of amyloid β protein (Aβ), which contains 39-43 amino acids and is produced by proteolytic cleavage from amyloid precursor protein (APP) [11, 16, 25, 26]. Aβ deposited in the brain of humans includes two major subtypes that have different carboxyl termini: Aβ1-40 (Aβ40) and Aβ1-42(43) [Aβ42(43)] [27, 35, 36]. Several studies have demonstrated that Aβ42(43) initiates to form amyloid fibrils [14, 54]. Recently, two carboxyl end-specific monoclonal antibodies that differentiate Aβ40 and Aβ42(43) have been identified and well characterized [13, 50].

All types of senile plaques and cerebrovascular amyloid consisting of Aβ are immunoreactive to apolipoprotein E (apo E) [29, 34, 37, 47, 72]. *In vitro* studies have indicated that apo E accelerates the formation of amyloid fibrils from Aβ [21]. Recently, it has been reported that inclusion body myositis consisting of Aβ was also immunoreactive to apo E [3]. On the other hand, systemic amyloid consisting of AA amyloid but not of Aβ amyloid, is immunoreactive to apo E [29]. These studies support the prediction that a close correlation exists between certain types of amyloid and apo E deposition.

In aged humans, alpha-1-antichymotrypsin (αACT) as a protease inhibitor is found in all types of senile plaques and cerebrovascular amyloid corresponding to Aβ and apo E deposition [1, 44]. On the other hand, swollen neurites in senile plaques of humans contain APP, ubiquitin (Ub), microtubule-associated protein-2 (MAP-2), and tau [7, 8, 43, 45, 60]. Reacted astrocytes

and its processes containing glial fibrillary acidic protein (GFAP) are observed around mature plaques in aged humans [12]. These findings have been also described in aged monkeys [2, 9, 23, 32] and dogs [30, 62, 63].

Podlisny *et al.* [31] described that Aβ, αACT, and complement factors deposited in senile plaques and cerebrovascular amyloid in middle-aged cynomolgus monkeys of 19 years old. However, the other constituents in senile plaques and cerebrovascular amyloid have not been reported in aged cynomolgus monkeys more than 20 years old. In Chapter 2, I described the incidence and distribution of senile plaques and cerebrovascular amyloid in aged cynomolgus monkeys more than 20 years old. To clarify the characteristics of senile plaques and cerebrovascular amyloid, I performed immunohistochemical examination on the several constituents of senile plaques and cerebrovascular amyloid in aged cynomolgus monkeys. The items discussed in this chapter are as follows; 1) characterization of various constituents of senile plaques and cerebrovascular amyloid and 2) distribution of Aβ subtypes, Aβ40 and Aβ42(43), and APP, precursor protein of Aβ.

## Materials and methods

### *Animals*

I examined the cerebrum of aged cynomolgus monkeys (*Macaca fascicularis*) more than 20 years old. Animals used in this study were listed in Table 1 and 2 for the studies on the

several constituents and on Aβ subtypes and APP, respectively.

*Histopathology*

Tissue samples of the brains were fixed in 10% neutral buffered formalin. Four to 6 μm-paraffin sections were stained with hematoxylin and eosin (HE), periodic acid methenamine silver (PAM) for senile plaques, and alkaline Congo red for amyloid.

*Antibodies*

For the detection of several constituents in senile plaques and cerebrovascular amyloid, polyclonal rabbit antibodies against synthetic Aβ1-40 (anti-$A\beta_{1-40}$; Boehringer Mannheim Biochemica, Germany, used at 1:5 dilution), human-αACT (anti-αACT, Dako, Carpinteria, CA, U.S.A., 1:100), cow-Ub (anti-Ub, Dako, Carpinteria, CA, U.S.A., 1:100), chicken embryo-tau (anti-tau, Sigma, St. Louis, MO, 1:50), and cow-GFAP (anti-GFAP, Dako, Carpinteria, CA, U.S.A., 1:100), a polyclonal goat antibody against human recombinant apoE (anti-apoE, Chemicon, Temecula, CA, 1:800), and a mouse monoclonal antibody against rat brain MAP-2 (anti-MAP-2, Sigma, St. Louis, MO, 1:50) were used as primary antibodies in this experiment. Secondary antibodies were as follows; biotinylated goat antibodies against rabbit and mouse Ig G, and biotinylated rabbit antibody against goat Ig G (Kirkegaard & Perry Lab., Inc., Gaithersburg, MD, 1:100).

For the detection of Aβ subtypes and APP, primary antibodies used were mouse monoclonal antibodies against recombinant APP

(anti-APP; Boehringer Mannheim Biochemica, Germany, used at 1:5 dilution), synthetic Aβ35-43 (BC05; Aβ42 end-specific, generously provided by A. Tamaoka, Department of Neurology, Institute of Clinical Medicine, University of Tsukuba, dilution at 0.18 μg/ml) and synthetic Aβ1-40 (BA27; Aβ40 end-specific, generously provided by A. Tamaoka, 2.0 μg/ml) [13], and a polyclonal rabbit antibody against synthetic Aβ1-40 (α1280; generously provided by D. J. Selkoe, Harvard Medical School, Boston, MA, 1:1000) [53]. α1280 was used as a standard for positive reactions, because it recognizes both synthetic Aβ40 and Aβ42(43) peptides (unpublished data by A. Tamaoka).

*Immunohistochemical examination*

Four μm-paraffin sections were immunostained through ABC method following the treatment with 90% formic acid for 5 min for BC05, BA27, α1280 and anti-$A\beta_{1-40}$, and 0.1% pepsin for 30 min at 37℃ for anti-αACT, and hydrated by microwave for 5 min for anti-tau and anti-APP as described previously [17, 42]. The sections were, then, treated with avidin-biotin-peroxidase complex (ABC, Vectastain ABC kit, Vector Lab., Burlingame, CA) and finally visualized in 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO) plus hydrogen peroxide solution.

## Results

*Immunohistochemical findings of constituents of senile plaques*

Classical and primitive plaques and cerebrovascular amyloid with Congophilic amyloid deposition reacted strongly to anti-$A\beta_{1-40}$ and anti-apo E (Figs. 1-a and 2-a, and Table 3). However, swollen neurites involved in the lesion of classical and primitive plaques did not react to either antibodies. Thus, when classical and primitive plaques were examined for the immunoreactivity to these two antibodies, the results of staining profiles were similar each other. In contrast, diffuse plaques were stained strongly by anti-$A\beta_{1-40}$, whereas the immunoreactivity to anti-apo E was weak (Figs. 1-b and 2-b, and Table 3). The cortical blood vessels positively stained by anti-$A\beta_{1-40}$ were immunostained by anti-apo E (Figs. 1-a and 2-a and Table 3). About twice the number of diffuse plaques reacted to anti-$A\beta_{1-40}$ when compared 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.

Anti-αACT, anti-Ub, anti-MAP-2, and anti-GFAP reacted to classical and primitive plaques (Table 3). In detail, αACT deposited at extracellular portions such as cores in classical plaques and granular or radial structures in primitive plaques corresponding to Aβ deposition (Fig. 3), whereas αACT did not deposit in diffuse plaques. Ub and MAP-2 were contained in swollen neurites of classical and primitive plaques (Figs. 4 and 5, respectively). Tau was not found in any portion in all types of senile plaques, while degenerative neurons reacted to anti-tau (Fig. 6). Astrocytes and their processes containing GFAP were observed around classical

and primitive plaques (Fig. 7) but not around diffuse plaques.

On the other hand, the lesions in capillaries of cerebrovascular amyloid reacted to anti-αACT (Fig. 3). The number of cerebrovascular amyloid lesions showing reactivity to anti-αACT was about three times less than that to anti-$A\beta_{1-40}$ and anti-apo E. Anti-Ub, anti-MAP-2, anti-tau, and anti-GFAP did not react to any portion of the lesion with cerebrovascular amyloid.

*Immunohistochemical differentiation of Aβ in senile plaques and cerebrovascular amyloid in aged cynomolgus monkeys*

The polyclonal antibody, αl280 and monoclonal antibody, BC05 stained all senile plaques and cerebrovascular amyloid, whereas monoclonal antibody, BA27 stained more selectively. All types of senile plaques, including diffuse plaques that could not be detected by PAM, also reacted to BC05. However, only about one third of the classical and primitive plaques and none of the diffuse plaques were stained by BA27. Figs. 8-a~f and Table 3 summarized the results of staining: (1) BC05 stained all parts of the classical plaques, including the central core and the halo, whereas BA27 stained only the core, (2) BC05 evenly stained the entire area of the primitive plaques, whereas BA27 tended to stain only the granular structures in the plaques, and (3) BC05 stained all diffuse plaques that were consisted of amorphous and dense thin-fibrillar clusters, but BA27 did not stain any diffuse plaques.

In cerebrovascular amyloid, BC05 labeled the cortical parenchymal and meningeal arterioles, and demonstrated a particularly intense staining of capillaries (Fig. 8-g~i and Table

3). In contrast, BA27 strongly stained cortical parenchymal and meningeal arterioles, but labeling of capillaries was significantly reduced with BA27 as compared to BC05 (Fig. 8-g~i and Table 3). However, the staining of parenchymal and meningeal arterioles by BA27 was more intense than that by BC05.

Anti-APP reacted to swollen neurites in classical and primitive plaques, but not in diffuse plaques, as well as anti-MAP-2 and anti-Ub (Fig. 9-a). APP was also demonstrated in many normal or degenerative neurons (Figs. 9-a, b).

## Discussion

The constituents of senile plaques and cerebrovascular amyloid in cynomolgus monkeys were clarified in this study. Aβ was deposited in the extracellular portions, but not in the neurites of senile plaques. In addition, Aβ deposition was observed in cerebral blood vessels, as described previously [38]. As for other constituents, apo E and αACT were deposited in the same portion as Aβ in mature plaques, though diffuse plaques reacted weakly to anti-apo E, but not to anti-αACT in comparison with the strong reactivity to anti-$A\beta_{1-40}$. The amino acid sequence of apo E in cynomolgus monkey has about a 93% homology with that of human [22], and the Aβ sequence has 100% homology [31]. In cynomolgus monkeys, reactivity of diffuse plaques to anti-apo E was weaker than that to anti-$A\beta_{1-40}$. In human, the reactivity to anti-apo E was as the same as that to anti-$A\beta_{1-40}$ [34]. For the detection of apo E, I used an antiserum prepared against recombinant human

apo E. Therefore, the affinity of this anti-apo E in cynomolgus monkey might be less than that in human. Antiserum against αACT could detect all types of senile plaques in humans [44], but it could not detect any senile plaques in dogs [63]. Since the antiserum was prepared by immunizing rabbits with human αACT, these results suggest that antiserum against αACT has low affinity to αACT among animal species except for humans.

Because diffuse plaque ultrastructurally consists of a small number of amyloid fibrils, it contains fewer polymerized fibrils in comparison with mature plaques [73]. Consequently, I considered that the diffuse plaques remained in a non-Congophilic form until amyloid fibrils were well polymelized. In this study, diffuse plaques in cynomolgus monkeys reacted weakly to anti-apo E. This suggests that apo E in diffuse plaques plays a role in the polymerization of amyloid fibrils at the early stage of senile plaque formation. This finding supports the previous *in vitro* results showing that apo E accelerates Aβ polymerization [21]. A previous study reported that systemic amyloid and prion amyloid plaques without Aβ also reacted to anti-apo E in humans [29]. Moreover, I confirmed firstly that apo E also deposited in the diabetic islets of cynomolgus monkeys (data not shown). These results suggest that apo E is related with Congophilic amyloid formation in various types of amyloidosis.

On the other hand, swollen neurites in mature plaques reacted to anti-MAP-2, anti-Ub, and anti-APP. Since Aβ has neuronal toxicity [24, 74, 75] and swollen neurites contain MAP-2, Ub, and APP as previously described [7, 8, 43, 45, 60], I considered that the dendritic changes observed in mature plaques were caused by

Aβ toxicity. Swollen neurites in senile plaques reacted to anti-tau in humans [43], but not in cynomolgus monkeys. Tau is known as main component of neurofibrillary tangles [18], though the lesion was found neither in aged non-human primates nor in aged dogs [4, 31, 39, 48, 62, 66]. Since human life span is longer than life spans of non-human primates and dogs, accumulation of tau may be required a very long duration and may be a characteristic nature in humans. Aβ toxicity and neuritic changes of senile plaques induced a reaction of astrocytes and their processes in aged cynomolgus monkeys as in humans [12].

In order to investigate the characteristics of Aβ on senile plaques and cerebrovascular amyloid, I carried out immunohistochemical examinations by using carboxyl-end specific monoclonal antibodies, BC05 and BA27, which can differentiate Aβ42(43) and Aβ40, respectively [13]. In human studies, BC05 strongly labeled all senile plaques, and BA27 stained only a subset of BC05-positive plaques. On the other hand, BA27 reacted more strongly with lesions of cerebrovascular amyloid [13, 56]. For both BC05 and BA27, the immunoreactivity of senile plaques and cerebrovascular amyloid in aged cynomolgus monkeys was similar to those of aged humans, though there were two remarkable features. First, in cynomolgus monkeys, BA27 intensely stained not only the central cores of classical plaques but also granular structures of primitive plaques. The second difference was that BC05 immunoreactivity in the cortical capillaries was observed more frequently in monkeys. One reason for this may be that cerebrovascular amyloid of capillaries in cynomolgus monkeys contains higher amounts of Aβ42(43) than that of parenchymal

and meningeal arterioles. However, we found that cerebrovascular amyloid in the cortical parenchymal and meningeal arterioles reacted more strongly to BA27 than BC05. This finding suggests that Aβ40 is a relatively major component in the cortical and meningeal arterioles when compared with Aβ42(43), which is consistent with findings in humans [13].

A previous study revealed that most of the vascular amyloid was localized in the area where there was a dense distribution of mature plaques in aged cynomolgus monkeys. In this study, I found that very high amounts of Aβ42(43) were deposited not only in the senile plaques but also in the cortical capillaries in the area where mature plaques and cerebrovascular amyloid coexisted. Thus, the abundance of Aβ42(43) in these lesions is consistent with my consideration that mature plaques and vascular amyloid in capillaries may be closely related in their formation. I found that these lesions contained relatively small amounts of Aβ40 in aged cynomolgus monkeys, as demonstrated by BA27-positive classical and primitive plaques with granular patterns. As determined by biochemical [54-56] or immunohistochemical [13] analyses, Aβ42(43) may have an important role in the initial stage of senile plaque formation as a seed molecule, but accumulation of Aβ40 may be more important during the maturation of senile plaques. In addition, the presence of Aβ40 in primitive plaques of cynomolgus monkeys may aid the formation and maturation of senile plaques. Moreover, it was predicted that Aβ42(43) was produced by neurons, since 1) normal or degenerative neurons without neuritic changes contained APP and 2) diffuse plaques as an initial form of senile plaque without neuritic changes are

consisted of Aβ42(43). Swollen neurites in mature plaques also contained APP so that Aβ40 may be derived from the proteolysis of APP in the neurites.

These results suggest that aged cynomolgus monkeys are an appropriate model system to study senile plaque and cerebrovascular amyloid formation. In future studies, biochemical and molecular pathological analyses of Aβ are required to clarify the mechanism of Aβ deposition as well as maturation process of the senile plaques.

**Abstract**

In order to study the characteristics of senile plaques and cerebrovascular amyloid, several constituents of the lesions were examined immunohistochemically. Mature plaques and cerebrovascular amyloid showed intense immunoreactivity to both antisera prepared against amyloid β protein (Aβ) and apolipoprotein E (apo E), whereas diffuse plaques showed weak immunoreactivity to antiserum against apo E. In addition, αACT also deposited in mature plaques, but not in diffuse plaques. Ubiquitin (Ub), microtubule-associated protein-2 (MAP-2), and beta amyloid precursor protein (APP) were contained in swollen neurites of mature plaques. Reacted astrocytes and their processes containing glial fibrillary acidic protein (GFAP) were frequently observed around mature plaques.

In the examination of Aβ subtypes, all types of senile plaques were identified by BC05 (Aβ42(43)-specific). However, BA27

(Aβ40-spcific) did not label diffuse plaques and stained only about one third of mature plaques. As to cerebrovascular amyloid, lesions of cortical capillaries reacted to BC05, but rarely and weakly to BA27. On the other hand, lesions of parenchymal and meningeal arterioles were stained by both BA27 and BC05. Capillary Aβ reacted intensely to BC05 but only slightly to BA27, which was remarkable feature in cynomolgus monkeys when compared with humans.

Among the several constituents of senile plaques and cerebrovascular amyloid, it is suggested that apo E plays a very important role in Aβ deposition at the initial stage. Because apo E deposited constantly in diffuse plaques which are considered to be immature Aβ deposition. The feature of abundant Aβ42(43) in capillaries and mature plaques supports my consideration that both lesions may be closely related in their formation. To clarify these unique results, biochemical and molecular biological examinations using young and aged cynomolgus monkeys are required.

**Table 1 Clinical course and histopathological features of 5 cynomolgus monkeys for the study on the several constituents of senile plaques and cerebrovascular amyloid**

| Case No. | Age [a] | Sex | Source | Anamnesis | Senile plaques [b] | | | Vascular amyloid [c] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | DP [d] | PP [e] | CP [f] | CAP [g] | ART [h] |
| 2 | 22y3m | Female | TPC [1] | Fatty degeneration of the liver<br>Endometriosis<br>Pulmonary edema<br>Atrophy of glomerulus | - | + | + | + | - |
| 3 | 23y1m | Female | TPC | Diabetes mellitus<br>Diarrhea, anorexia | + | + | + | + | + |
| 6 | 23y4m | Male | TPC | Inguinal hernia<br>Focal infection of gingival abscess<br>Septicemia | - | + | + | + | + |
| 24 | 26y | Female | SCL [2] | Diabetes mellitus | + | + | + | + | + |
| 28 | 23y | Female | SCL | Diabetes mellitus | - | + | + | + | - |

[1] Tsukuba primate center for medical science, National institute of health, Japan

[2] Laboratory animal science and toxicology laboratories of Sankyo Co., Ltd.

[a] In years [b] Stained by PAM or Congo red [c] Stained by Congo red

[d] Diffuse plaques [e] Primitive plaques [f] Classical plaques [g] Capillaries [h] Arterioles

+: positive -: negative

**Table 2 Histopathologic features of senile plaques and cerebrovascular amyloid in the cerebrum of aged cynomolgus monkeys for Aβ subtypes**

| Case no. | Age [a] | Sex | Source | Senile plaques [b] | | | Vascular amyloid [c] | |
|---|---|---|---|---|---|---|---|---|
| | | | | DP [d] | PP [e] | CP [f] | CAP [g] | ART [h] |
| 5 | 22 | Female | TPC [i] | - | + | + | + | + |
| 24 | 26 | Male | SCL | + | + | + | + | + |
| 29 | 24 | Female | SCL [j] | - | + | + | + | - |
| 36 | 20 | Female | TPC | - | - | - | - | - |
| 48 | 23 | Female | TPC | - | + | + | + | - |

[a] In years [b] Stained by PAM or Congo red [c] Stained by Congo red
[d] Diffuse plaques [e] Primitive plaques [f] Classical plaques [g] Capillaries [h] Arterioles
[i] Tsukuba Primate Center for Medical Science
[j] Laboratory of Animal Science and Toxicology Laboratories of Sankyo Co., Ltd.
+; Positive -; Negative

**Table 3 Immunoreactivity of senile plaques and cerebrovascular amyloid by several antibodies**

| Antibodies | Senile plaques | | | Amyloid angiopathy | |
|---|---|---|---|---|---|
| | DP [a] | PP [b] | CP [c] | CAP [d] | ART [e] |
| Anti-A $\beta_{1-40}$ | + | + | + | + | + |
| Anti-apo E | +/- | + | + | + | + |
| Anti-$\alpha$ ACT | - | + | + | + | + |
| Anti-APP | - | + [f] | + [f] | - | - |
| Anti-MAP-2 | - | + [f] | + [f] | - | - |
| Anti-Ub | - | + [f] | + [f] | - | - |
| Anti-Tau | - | - | - | - | - |
| Anti-GFAP | - | + [g] | + [g] | - | - |
| $\alpha$ 1280 | + | + | + | + | + |
| BC05 | + | + | + | + | + |
| BA27 | - | + | + | +/- | ++ |

[a] Diffuse plaques [b] Primitive plaques [c] Classical plaques
[d] Capillaries [e] Arterioles
[f] Positive with swollen neurites
[g] Positive with reacted astrocytes and its processes
++; Strongly positive +; Positive
+/-; Weakly positive -; Negative

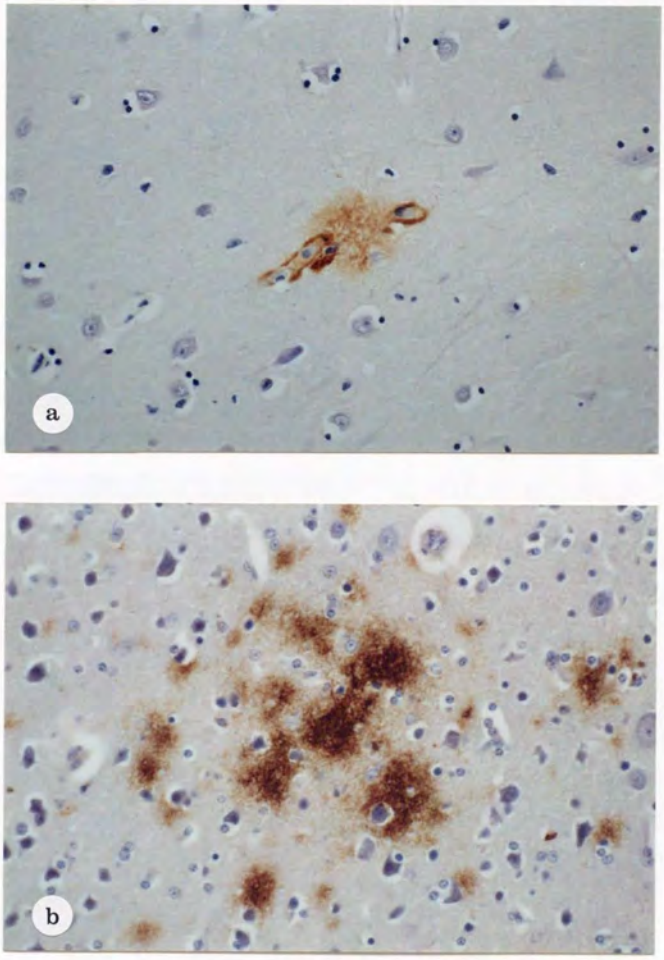



Fig. 1 Immunostainings of senile plaques and adojoining capillaries to anti-$A\beta_{1-40}$ in aged cynomolgus monkeys. (a) A primitive plaque and capillaries. x320. (b) Diffuse plaques. x320.

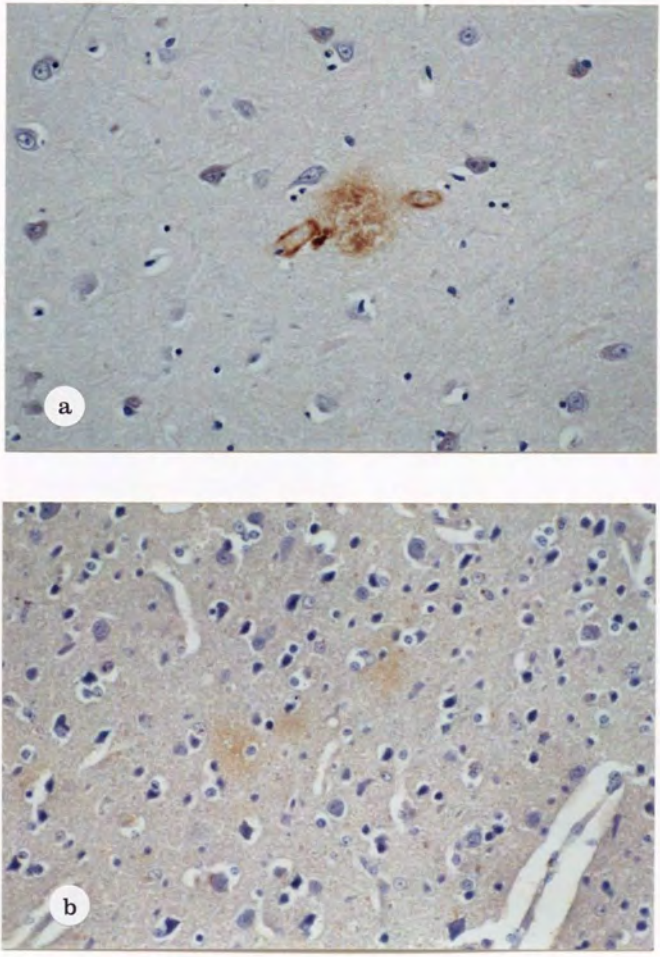



Fig. 2 Immunostainings of senile plaques and adojoining capillaries to anti-Apo E in aged cynomolgus monkeys. (a) A primitive plaque and capillaries. x320. (b) Diffuse plaques which was stained more slightly than the primitive plaque. x320.

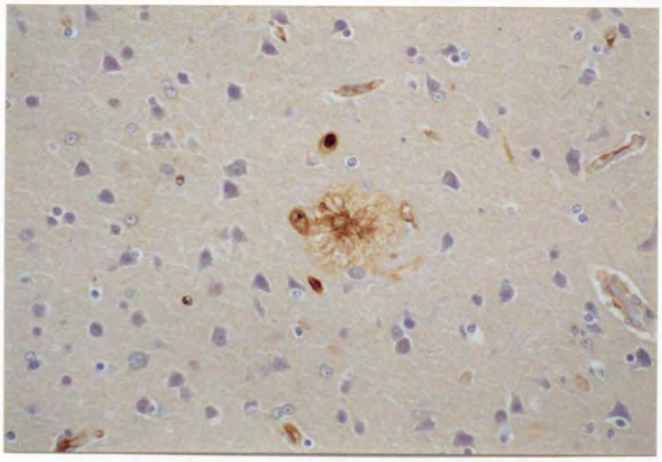


Fig. 3 Immunostaining of a classical plaques and adojoining capillaries to anti-αACT in aged cynomolgus monkeys. x320.

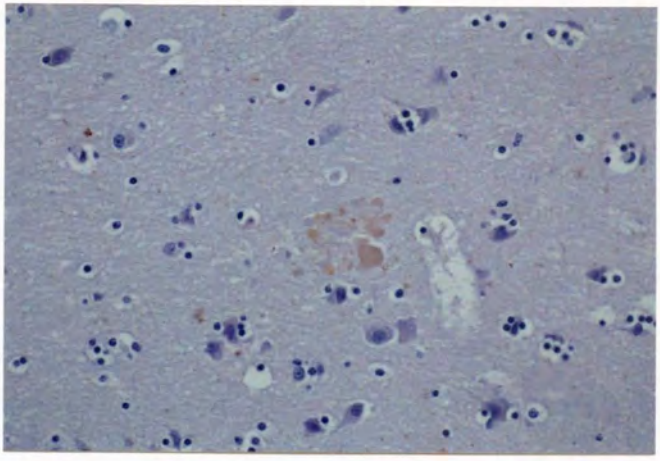


Fig. 4 Immunostaining of a primitive plaque to anti-Ub in aged cynomolgus monkeys. Swollen neurites in the plaque were positively to anti-Ub. x320.

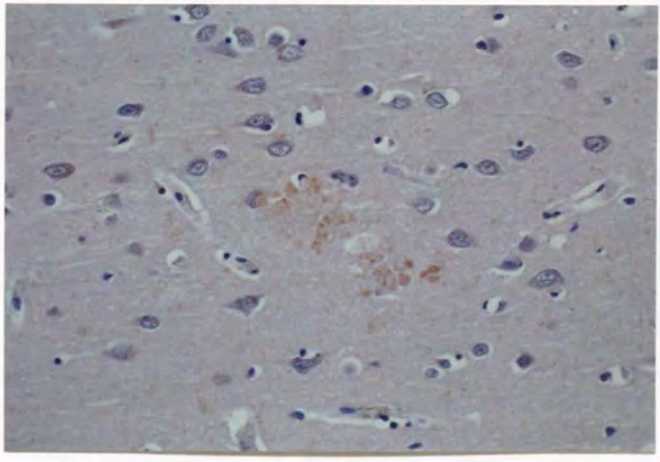


Fig. 5 Immunostaining of primitive plaques to anti-MAP-2 in aged cynomolgus monkeys. Swollen neurites of the plaques were positively to anti-MAP-2. x320.

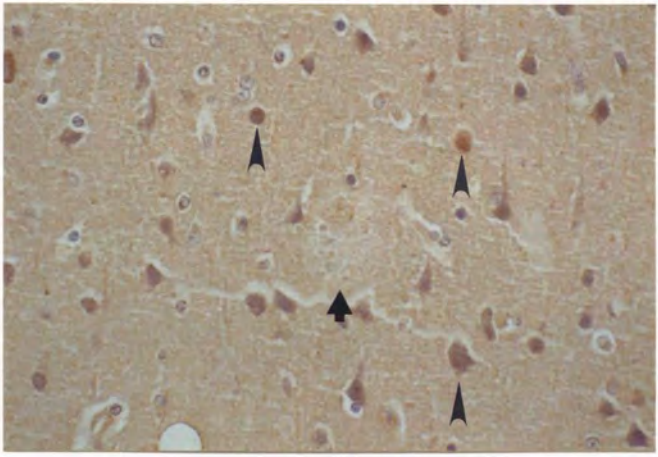


Fig. 6 Immunostaining of degenerative neurons and a primitive plaque to anti-tau in aged cynomolgus monkeys. Degenerative neurons (arrowheads) were positively to anti-tau, whereas swollen neurites in the plaques (arrow) were negative. x400.

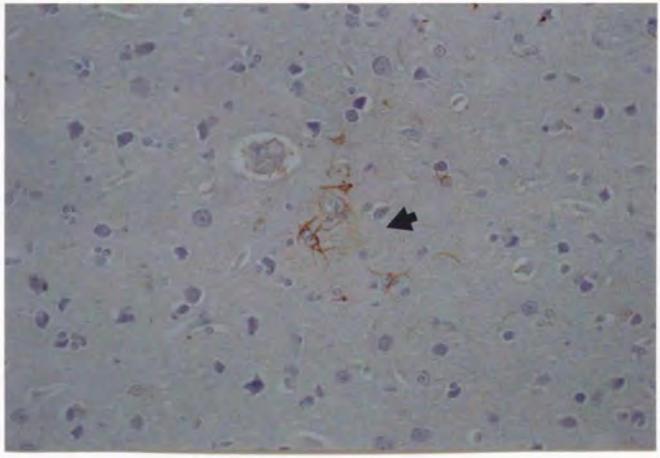
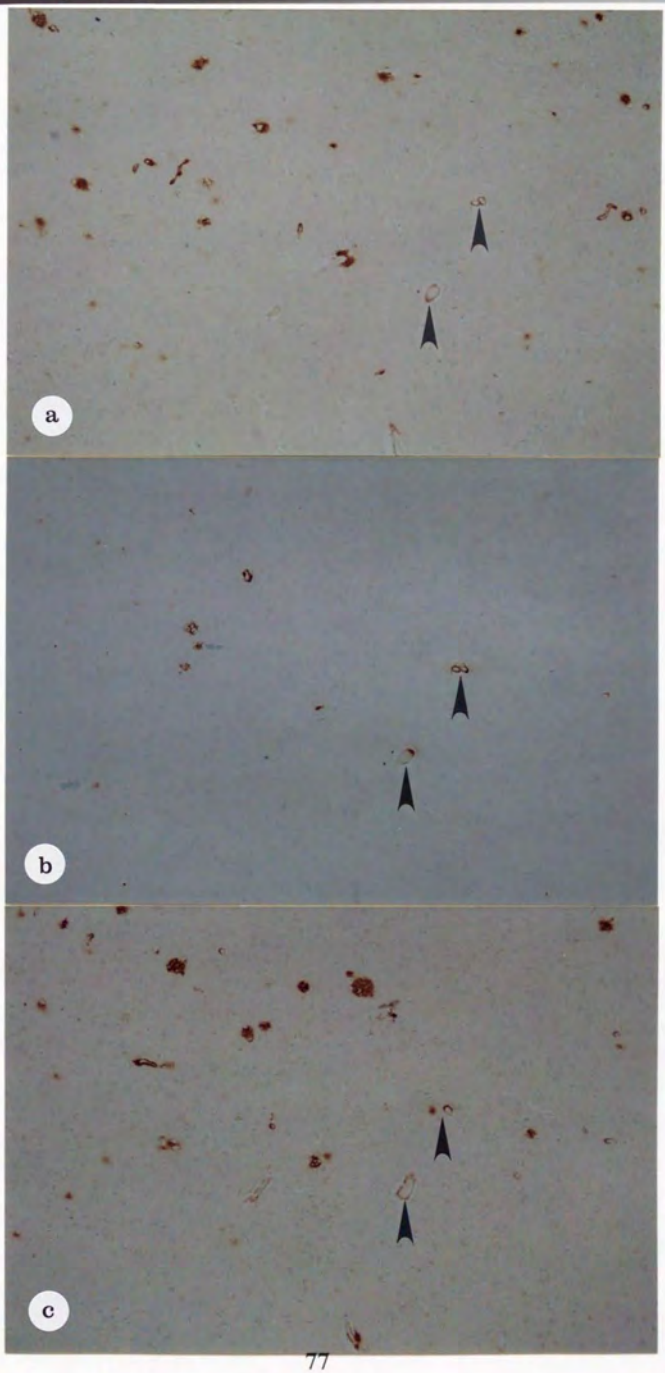


Fig. 7 Immunostaining of primitive plaques and reacted astrocytes and its processes to anti-GFAP in aged cynomolgus monkeys. Astrocytes and their processes (arrowheads) were positively to anti-GFAP, whereas the plaques (arrow) were negative. x265.

Fig. 8 Immunostainings of senile plaques and of cerebrovascular amyloid to BC05 (a, d, g), BA27 (b, e, h), and α1280 (c, f, i) in the cerebrum of aged cynomolgus monkeys. (a-c) All classical and primitive plaques were labeled by BC05 and α1280 and cerebrovascular amyloid associated with parenchymal arterioles (arrowheads) reacted to all antibodies. However, only about one third of the plaques reacted to BA27. x64. (d-f) Diffuse plaques and primitive plaques (arrowheads) were labeled by BC05 and α1280. However, no diffuse plaques and only two primitive plaques (arrowheads) were stained by BA27. x64. (g-i) The cortical capillary lesions reacted intensely to BC05, but rarely to BA27. On the other hand, BA27 labeled meningeal and parenchymal arterioles more intensely to BC05. All vascular lesions including meningeal and parenchymal arterioles and capillaries were stained by α1280. x64.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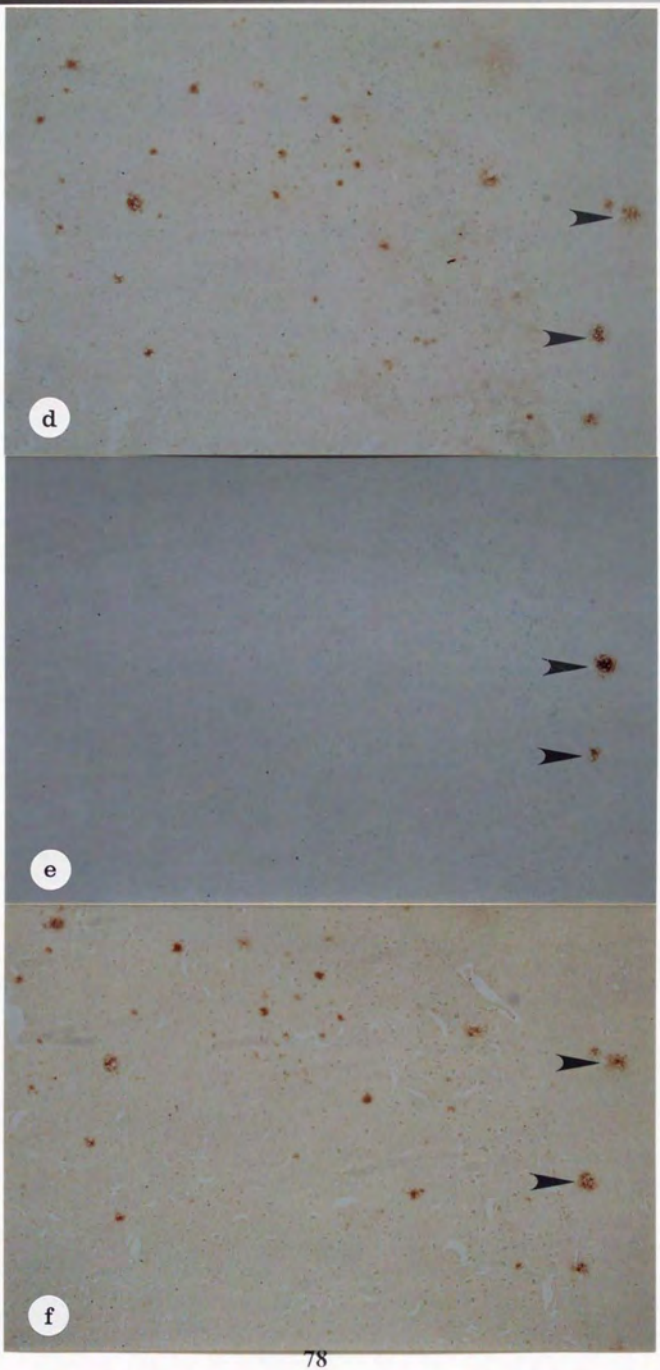
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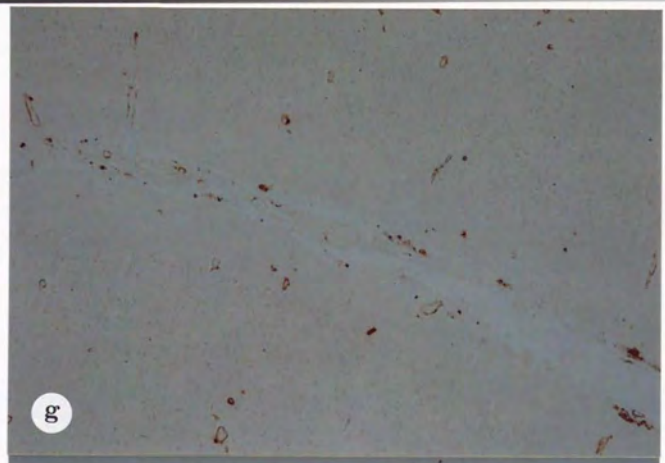
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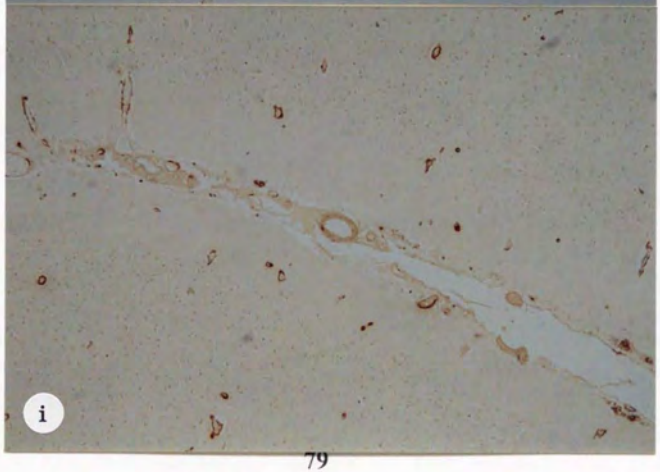
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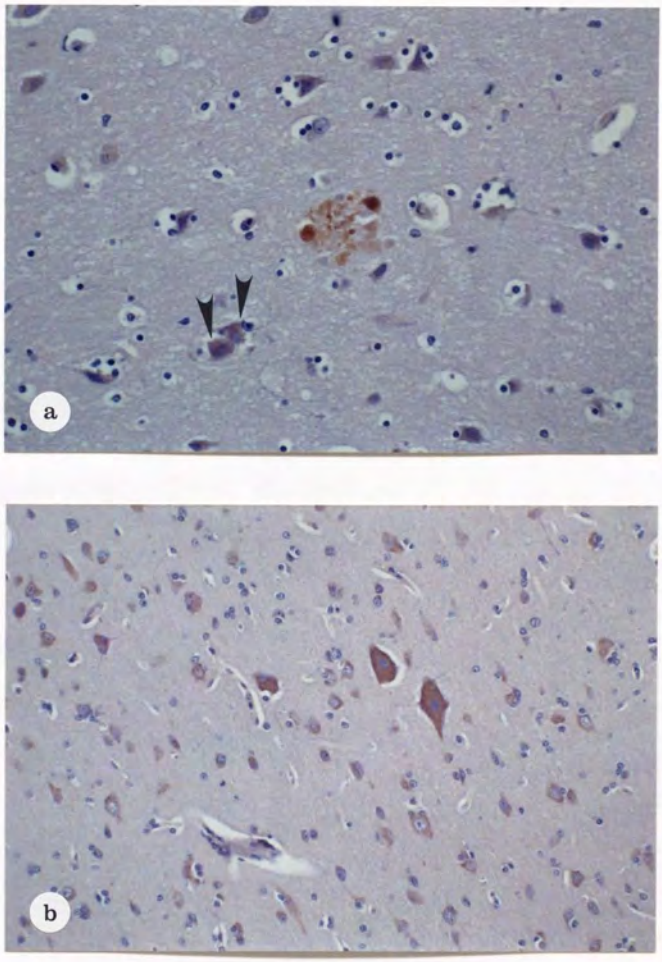


**Fig. 9 Immunostaining of a primitive plaque, and degenerative and normal neurons to anti-APP in aged cynomolgus monkeys. (a) Swollen neurites of the plaques and degenerative neurons (arrowhead) were positively to anti-APP. x 320. (b) Whereas, normal pyramidal neurons were also positive. x200.**

# Conclusion

In this study, histopathological and immunohistochemical characteristics of senile plaques and cerebrovascular amyloid in cynomolgus monkeys were clarified in detail. In addition, characteristics of the lesions in dogs, cats and a two-humped camel were also defined comparatively.

Morphology of senile plaques in cynomolgus monkeys and dogs was corresponded to that in humans as described previously. However, senile plaques in cats differed morphologically from any types of the plaques in other animal species reported previously. Furthermore, senile plaques in a camel seemed to be diffuse plaques, though they were compact and thin-fibrillar with clear border. These characteristics were different from diffuse plaques in other animal species as generally known. Thus, these features suggest that elucidation of amyloid β protein (Aβ) deposits will be possible by comparative studies on senile plaques and cerebrovascular amyloid in each animal species.

I examined cynomolgus monkeys bred in Tsukuba Primate Center for Medical Science, National Institution of Health, Japan, which had their own profiles including breeding and clinical histories and known pedigree. Senile plaques were found in the cynomolgus monkeys more than 20 years old and the lesions were frequently consisted of mature plaques containing Congophilic amyloid and swollen neurites. They were localized in the temporal lobes, especially in the superior and inferior gyri or amygdala. Since amyloid deposition to capillaries was often observed in the area where mature plaques localized densely, it was suggested that both lesions were closely correlated in the cerebral amyloid deposition. Immunohistochemically, Aβ and apolipoprotein E

(apo E) deposited at the extracellular portions in all types of senile plaques, whereas alpha-1 antichymotripsin (αACT) deposited only in mature plaques. Swollen neurites in mature plaques contained beta amyloid precursor protein (APP), microtubule-associated protein-2 (MAP-2), and ubiquitin (Ub). Tau was not observed in the swollen neurites of mature plaques in cynomolgus monkeys unlike the human cases. Therefore, presence of tau in swollen neurites may be a characteristic nature in humans. Reacted astrocytes and their processes around mature plaques were positive for glial fibrillar acidic protein (GFAP). Furthermore, Aβ with the different carboxyl terminus such as Aβ40 and Aβ42(43) in senile plaques and cerebral blood vessels was detected immunohistochemically. Aβ42(43) was frequently observed in mature plaques and neighboring capillaries which were agreed with histopathological findings as close relation of Congophilic amyloid deposits in both lesions. These results indicate that cynomolgus monkeys are very useful animal model to investigate the pathogenesis of Aβ deposition and senile plaque formation, especially to do correlation between senile plaques and cerebrovascular amyloid, because close location of the both lesions was confirmed in the histopathological and immunohistochemical studies.

Recently, several candidate genes associated with Alzheimer's disease were discovered, though it has not been known how the protein encoded by these genes are associated with senile plaque formation and cerebrovascular amyloid deposition. Since cynomolgus monkeys individually has clear pedigree and history, it is possible to carry out simultaneously both histopathological

and genetic analyses throughout posterity of the suffered animals. Therefore, this cynomolgus monkey model has an advantage to clarify the pathogenic relationships between Aβ deposits and candidate new genes.

## Acknowledgments

This work was performed in Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo (VPUT), and also in Tsukuba Primate Center for Medical Science (TPC), National Institute of Health, Japan. I wish to sincere gratitude to Dr. Yasuhiro Yoshikawa, the TPC, for guiding me to success each experiment. I also wish to express my thanks to Dr. Naoaki Goto, former professor of the VPUT, and now in Shin Nippon Biomedical Laboratories, for helpful advice throughout completion of this study. I wish to thank to Dr. Kunio Doi, professor of the VPUT, for leading to finish this manuscript. I am much indebted to Dr. Hiroyuki Nakayama, associate professor of the VPUT, for providing me technical help and new information of histopathology. Furthermore, thanks are tendered to Dr. Akira Tamaoka, Department of Neurology, Institute of Clinical Medicine, University of Tsukuba, for his guidance on neuroscience for humans and animals. I am grateful to Dr. Ippei Sakakibara, the TPC, to Dr. Fumiko Ono and to Rieko Kobayashi, Corporation and Production for Laboratory Primate, for offering technical help and clinicopathological data of cynomolgus monkeys. I am also grateful to Dr. Kazuyuki Uchida, Department of Veterinary Pathology, Faculty of Agriculture, Miyazaki University, for giving me new information in veterinary neuropathology. Further, my grateful thanks to Dr. Mitsunori Yasuda, Laboratory Animal Science and Toxicology Laboratories of Sankyo Co. Ltd., to Drs. Fujio Sekiguchi and Koichi Nakamura, Experimental Technology Research Center of Daiichi Pharmaceutical Co. Ltd., and to Dr. Teruhiko Matsushima, School of Medicine, University of Tsukuba for supplying cynomolgus monkeys. Finally, I am grateful

thanks to members of the VPUT, especially to Drs. Koji Uetsuka and Wijit Kiatipattanasakul, and of the TPC for collaboration and encouragement to my work.

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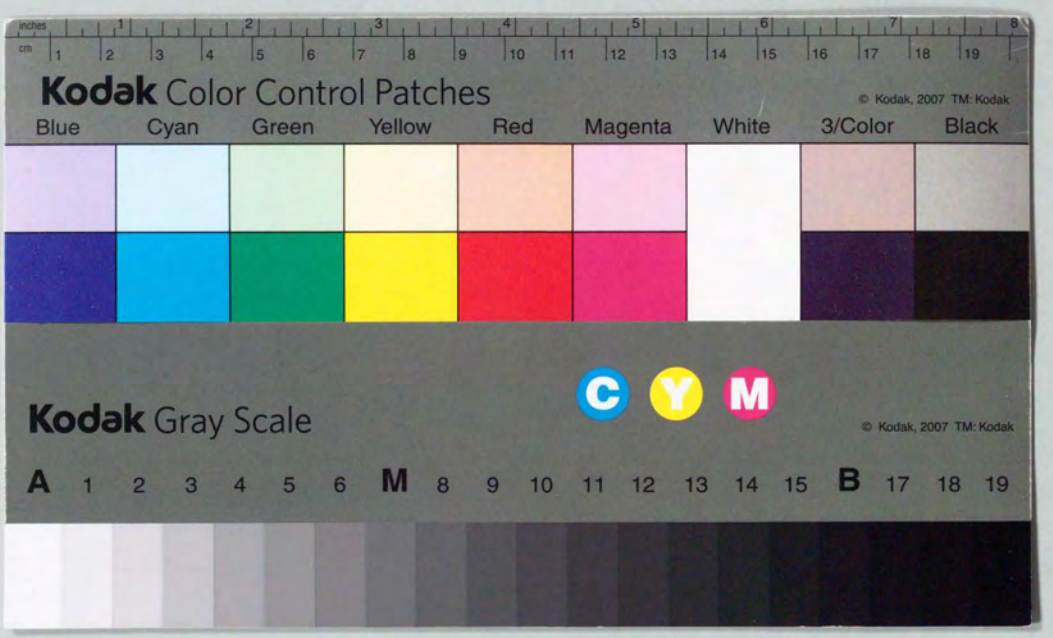
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