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Demyelination in natural canine distemper encephalomyelitis : An immunohistochemical study of myelin basic protein, myelin associated glycoprotein and glial fibrillary acidic protein in the lesion of demyelination*

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홍역이환개에서 발생한 수초탈락성 뇌척수염 : 수초탈락부위에서 MBP, MAG 및 GFAP의 면역조직학적 관찰

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초록 : 홍역이환개의 중추신경계에서 발생한 수초탈락을 수반하는 뇌척수염을 병리조직학적으로 관찰하고 조직반응과 수축탈락의 과정을 알아보기 위하여 중추신경계 조직을 파라핀 포매, 절편을 만들고 병소가 빈발하는 소뇌와 시신경로는 수초의 주요 구성단백인 MBP와 MAG 그리고 별아교세포의 Marker인 GFAP의 항혈청을 반응시켜 면역세포학적으로 관찰하였다. 조직학적으로는 대뇌에서 신경세포의 일부괴사, 신경아교세포의 결절형성, 소뇌에서는 4뇌실에 인접하여 백질부에서 수초의 공포변성, 수초탈락과 염증세포의 침윤이 현저하였고 피질부의 과립층에 인접한 부위와 시신경로에서도 수초탈락이 인정되었다. MBP와 MAG를 면역반응시킨 결과 수초의 공포화와 수초탈락이 현저한 부위에서는 MBP와 MAG가 동시에 소실되었고 부위에 따라서는 MBP 또는 MAG가 먼저 소실되기도 하였다. 동시에 별아교세포의 반응은 수초탈락 초기에는 GFAP양성의 섬유와 세포가 크게 증가한 반면 수초탈락이 심하게 진행된 부위에서는 오히려 소실되는 경향이었다. 따라서 홍역이환 개에서 발생되는 수초탈락은 일차적으로 수초가 공격을 받아 파괴됨을 알 수 있었고 동시에 부위에 따라 다른 소견을 요인별로 비교하였다.

Key words : canine distemper, demyelination, encephalomyelitis. MAG, MBP. GFAP.

Introduction

Canine distemper has been known as an important disease in dogs and proposed as a further model of human demyelinating diseases[1]. Certain strains of canine distemper virus(CDV) induce encephalomyelitis with primary demyelination in the central nervous system(CNS)[2~4]. In studying the pathogenesis of natural and ex-

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perimental CNS demyelinating diseases[5~8], immunocytochemistry of myelin proteins has been applied as a marker for the study of demyelination in the CNS.

In natural and experimental CDE, microscopic and ultrastructural changes in demyelination around the fourth ventricle were well evaluated by several investigators.[9~13]. However, studies on the behavior of MBP and MAG with astroglial reactions in the demyelinating lesion of canine distemper encephalomyelitis have been rarely reported.

This paper describes histopathological changes of natural CDE and an immunohistochemical localization of myelin proteins and GFAP in a demyelinating lesion.

Materials and Methods

The materials used in this study were obtained from a 3-month-old, Dosa dog and a 3-month-old Doberman dog. These dogs suffered from anorexia, diarrhea and salivation. Canine distemper was diagosed in these dogs by clinical and histopathological observations. Intracytoplasmic and intranuclear inclusion bodies were found in the gastric epithelia, in the epithelia of renal pelvis and urinary bladder(data not shown) and in the brain.

Brains including optic tracts were chosen for this study. In CDE, the demyelinating lesions have been recognized in the subpial or periventricular predilection areas including the cerebellar white matter, and the white matter in the vicinity of the fourth ventricle.[12] Tissues were fixed in 10% buffered formalin, dehydrated with graded eathanol and embedded in paraplast. Five micron-thick sections were cut serially and mounted on glass slides for immunocytochemistry.

Serial sections were processed for immunohistochemistry using extravidin-biotin complex technique with a commercial kit (Zymed, CA, USA). Primary antisera were anti-MBP(1 : 500), anti-MAG(1 : 100~1 : 300) and anti-GFAP(1 : 500, Dako corp.). Anti-MBP and anti-MAG antisera were kindly donated by Dr. S. U. Kim[14](University of British Columbia, Vancouver, B.C., Canada), and has been routinely used in this laboratory in cultured brain cells.[14,15]. After immunoreaction, sections were counterstained with hematoxylin and then mounted with GVA media. For negative control, the primary antiserum was substituted with normal rabbit sera or omitted.

Results

Histopathology of CNS tissues : In the grey and white matter of the cerebrum, there was diffuse and focal microgliosis with occasional neuronal degeneration and hypertrophy of a few astrocytes. Areas of sponginess and vacuolation indicating a demylinating process were seen in the subependymal white matter of the fourth ventricle and the cerebellar white matter below the granular cell layer. Hypertrophic astrocytes were commonly identified in the well developed demyelinating lesion and adjacent vaculoar lesion. A few inflammatory cells including macrophages and lymphocytes were seen around some vessels and along the subependymal layer of the fourth ventricle. Perivascular cuffings were occasionally found within the demyelinating lesion. However, the area of myelin loss is not directly related to blood vessels and inflammatory cells. Periventricular white matter lesions in general were similar to previous observations by Summers and Appel[16] and Summers et al.[11]

Optic tract lesions were also characterized by sponginess and vacuolar changes leading to demyelination. Some reactive astrocytes had hypertrophic and swollen cytoplasm. Intranuclear inclusions were commonly identified in astorcytes. Expansion of demyelination lesions appeared to develop by ballooning changes at the margin of demyelinating foci.

Immunocytochemical localization of MBP, MAG and GFAP in demyelination : In the cerebellum(Figs 1~4) and optic tracts(Figs 5, 6)in natural CDE, there was an uniform disappearance of MBP(Figs 1, 5A) and MAG (Figs 2, 5B) at well-demarcated demyelinating lesions and at the earlier stage of myelin vacuolation. At the margin of demyelination, the immunoreactivity of MAG is in part weaker than that of MBP, showing that MAG loss in part precedes myelin vacuolation(Figs 5B). Some phagocytosed MBP-immunoreactive myelin figures were found in the activated microglial cells which the nuclei were rod-shaped. At a lesion with infiltration of cells in the optic tract, MBP disappeared earlier than MAG(Figs 6 A, B). There were loss of MBP immunoreaction without myelin vacuolation, presence of MAG immunoreactivity, infiltration of mononuclear cells and hypertrophy of astrocytes.

Astrocytic processes were prominent in the demyeli-

nating lesion of cerebellum and optic tract by immunohistochemical localization of GFAP(Figs 3, 5C). A few GFAP-positive astrocytes contained two nuclei. Loss of GFAP immunoreactive fibers is recognized in the middle of the well-demyelinated lesion in the cerebellum (Fig 4). GFAP-positive fibers and hypertrophic astrocytes were commonly seen in the white matter and the granular cell layer as well as in the adjacent demyelinating lesion(Figs 3, 4).

In the optic tract, GFAP-positive astrocytes are lined along the sub-pial layer and in the interfascicular layer (Fig 5C). Partial destruction of glial limiting membrane was recognized in the subpial layer. In well-developed demyelinating lesions, some astrocytes were hypertrophied and contained viral intranuclear inclusions. GFAP-positive fibers were increased in the demyelinating lesion (Fig 5C). No evidence of astroglial mitosis was found in this case. Hypertrophic astrocytes with intranuclear inclusions were commonly identified in the interfascular layer without vacuolar changes and demyelination.

Discussion

The pathogenesis of CDV-induced demyelination is still unsolved. In this study of natural demyelinating encephalomyelitis in two dogs, we found largely non-inflammatory demyelination in the cerebellar white matter and in the optic tract, and examined the immunohistochemical distribution of MBP, MAG and GFAP in the demyelinating lesion. From the histological and immunohistochemical findings, the present case was identified as primary demyelination in natural CDE in dogs.[11]

In the pathogenesis of demyelinating diseases, the relative distribution of MAG and MBP loss in the white matter has been shown to be a reflection of primary attack of oligodendrocytes versus primary myelin attack.[5,7,12,17] MAG appears to be associated with the oligodendroglial and Schwann cell membrane components of the myelin sheaths. The earlier disappearance of MAG rather than MBP has been recognized in progressive multifocal leukoencephalopathy, in which the oligodendrocytes is a primary target.[18] In EAE, in which myelin appears to be the primary target, there is a parallel loss of MBP and MAG.[5]

In the cerebellar white matter of natural CDE in this study, we observed that the concurrent disappearance of MBP and MAG occurred in the demyelinating lesion. This supports the hypothesis of a direct attack on the myelin sheath rather than primary viral cytolytic infection on oligodendrocytes as the main mechanism of demyelination. The involvement of oligodendrocytes in myelin loss in CDE is still unclear. Glaus et al[19] found that a few oligodendrocytes contained CDV antigens in a brain cell culture study, and Blakemore et al[20] also confirmed uncommon virus-infected oligodendrocytes *in vivo* in experimental CDE. Although oligodendrocytes may contain CDV antigens in the demyelinating lesions, they are sporadic in both *in vivo* and *in vitro* studies in CDE. The present observation which MAG in part disappear earlier than MBP in this natural CDE supports the previous ultrastructural and immunocytochemical observations *in vivo*[20] and *in vitro*.[19]

Astrogliosis is a process of brain repair[21,22] and plays an important role in persistent viral infection in the CNS.[16,23] In EAE, astrogliosis is characterized by hypertrophy of astrocytes and up-regulation of GFAP molecules.[21,22] In the well developed demyelinating lesions in this study, the increased number of GFAP-positive fibers may also result from hypertrophy of astrocytes and increased regulation of GFAP molecules as the number of GFAP positive cell bodies did not appear to increase. An uncommon finding was that the subpial glial limitans was destroyed. However, there is no evidence of inflammatory cell movement through damaged glial limitans, contrary to the finding of Theiler's virus infection.[7] In unaffected lesions, many astrocytes with intranuclear viral inclusions were also hypertrophied. These findings support the hypothesis that sponginess or vacuolation leading to demyelination is preceded by astroglial activation as proposed by Summers and Appel.[16]

In conclusion, this is the first study of primary demyelination and astroglial reactions in natural CDE investigated using immunocytochemistry of two myelin proteins and GFAP. Concurrent loss of MBP and MAG suggest that the myelin sheath is the target in the demyelinating process in CDE. The ultrasturctural localizatio of MBP and MAG in the process of CDE demyelination needs further study.

Summary

Central nervous system of two dogs with natural canine distemper was investigated histopathologically and immunocytochemically with antisera to MBP, MAG and GFAP. Histopathologically, there were neuronal degeneration and diffuse gliosis in the cerebrum, vacuolar degeneration, hypertrophy of astrocytes and demyelination in cerebellar white matter adjacent to the 4th ventricle and optic tracts showing non-inflammatory demyelinating encephalomyelitis(Summers and Appel, 1987). Immunohistochemically, there was a concurrent disappearance of MBP and MAG in the well developed demyelinating lesion in the cerebellar white matter. At the margin of demyelination, Loss of both MBP and MAG varied on the stage of demyelinating process. GFAP-positive astrocytes were hypertrophied and contained canine distemper virus intranuclear inclusions. GFAP-positive fibers were increased at the early stage of demyelination, and then were not immunoreacted at the well developed demyelination. Hypertrophic astrocytes with intranuclear inclusions were commonly identified in the interfascular layer without myelin vacuolation and demyelination.

This is the first study of primary demyelination and astroglial reactions in natural CDE investigated using immunocytochemistry of two myelin proteins and GFAP. Concurrent loss of MBP and MAG suggest that the myelin sheath is the target in the demyelinating process in CDE.

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Legends for figures

Fig 1. A lesion in the cerebellar white matter of a 3-month old dog with natural CDE. Marked demyelination. Anti MBP-Hematoxylin, Original magnification × 80. Inset : Unaffected myelin reacted with anti-MBP antisertm.

Fig 2. Adjacent section to Fig 1. MAG is still present in the lesion of MBP loss in Fig 1. Anti-MAG-Hematoxylin. Inset : Unaffected myelin reacted with anti-MAG um. × 80.

Fig 3. A cerebellar white matter lesion prior to demyelination. GFAP-positive fibers and cells are conspicous in the lesion. Anti-GFAP-Hematoxylin. × 80.

Fig 4. Demyelination in the cerebellar white matter. Loss of GFAP immunoreactive fibers is recognized in the middle of the lesion. Some GFAP-positive astrocytes are seen around the lesion. Anti-GFAP-Hematoxylin. × 80.

Fig 5. A demyelinating lesion in the optic tract of a dog with natural CDE. Serial sections were reacted with anti-MBP(A), anti-MAG(B) and anti-GFAP(C) antisera. × 80.

Fig 6. Serial sections of a demyelinating lesion with cellular infiltration in the optic tract adjacent Fig 5. MAG(B) is still present in the loss of MBP(A). A : anti-MBP-Hematoxylin, B : anti-MAG-Hematoxylin. × 80.

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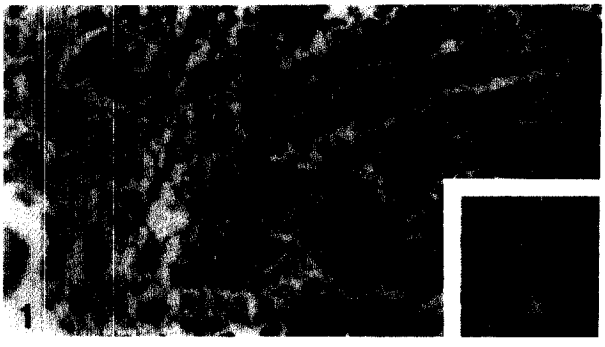

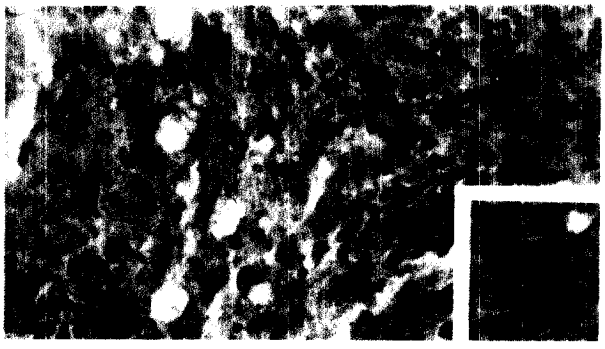

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