REVIEW

Pathologic mechanisms of influenza encephalitis with an abnormal expression of inflammatory cytokines and accumulation of mini-plasmin

Dengfu Yao[1,2] Masamichi Kuwajima[3] and Hiroshi Kido[1]

[1]*Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Tokushima, Japan ;* [2]*Department of Gastroenterology, Affiliated Hospital, Nantong Medical College, Nantong 226001, China ; and* [3]*Department of Laboratory Medicine, The University of Tokushima School of Medicine, Tokushima, Japan*

Abstract: The pathogenesis of influenza encephalopathy or encephalitis is poorly understood. This review summarizes our recent studies of the roles played by inflammatory cytokines, inducible nitric oxide synthase (iNOS), adhesion molecules and mini-plasmin in influenza encephalitis. After the intranasal infection of newborn mice with the non-neurotropic strain of influenza A virus (IAV) Aichi/2/68/H3N2, encephalitis and severe brain edema were observed within 3-5 days. IAV-RNA and abnormalities in the blood-brain barrier permeability were detected in association with an increase in the mRNA expressions of endothelin-1, iNOS, and tumor necrosis factor-α. Furthermore, the accumulation in the brain capillaries of mini-plasmin, which proteolytically induces the viral envelope fusion activity and allows the virus to enter the cells, changes the brain from non-susceptible to susceptible to non-neurotropic IAV multiplication. The accumulation of mini-plasmin was markedly greater in newborn mice with an impaired mitochondrial fatty acid metabolism. These inflammatory mediators and the accumulation of mini-plasmin in the brain may play an important role in the onset and progression of IAV encephalitis. J. Med. Invest. 50 : 1-8, 2003

Keywords **:** *influenza encephalitis ; cytokines ; nitric oxide synthase ; mini-plasmin*

INTRODUCTION

The influenza A virus (IAV) is one of the most common infectious pathogens in human, causing considerable morbidity and mortality in infants and older persons (1). IAV readily infects and replicates in the airway epithelial cells, though occasionally replicates in the central nervous system, particularly in children younger than 6 years of age. There appears little doubt as to the encephalitis of patients infected with a neurovirulent strain of IAV, such as the 1918/1919 pandemic (2). There are also rare, though often fatal, cases of encephalopathy due to infection by non-neurovirulent IAV in Reye's syndrome children treated with antipyretics (3-7). A systemic disorder of mitochondrial β-oxidation and degeneration of the liver with fatty infiltration are suspected to be involved in acute encephalitis or encephalopathy of patients suffering from Reye's syndrome. However, the pathological roles of an impaired mitochondrial β-oxidation in the influenza encephalitis and the mechanism of cerebral IAV invasion remain unclear. This review focuses on the pathogenesis of influenza-virus-associated en-

Received for publication November 14, 2002 ; accepted December 9, 2002.

Address correspondence and reprint requests to Hiroshi Kido, M.D., & Ph.D., Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Kuramoto-cho 3-18-15, Tokushima 770-8503, Japan and Fax : +81-88-633-7424.

cephalitis or encephalopathy, and discusses the roles of inflammatory cytokines, inducible nitric oxide synthase (iNOS) and mini-plasmin in the development of encephalitis.

INFLUENZA A VIRUS AND ITS PATHOGENESIS

Influenza viruses are enveloped, negative-stranded RNA viruses, which belong to the family of *Orthomyxoviridae*, and are subclassified into A, B and C, of which the A viruses are the most important pathogens (8). IAV-RNA is composed of eight segmented genes, which encode for ten different proteins, including envelope glycoproteins hemagglutinin (HA) and neuraminidase (NA), matrix protein M1, nucleoprotein, three polymerases (PB1, PB2 and PA), ion channel protein M2, and nonstructural proteins (NS1 and NS2). Anti-HA antibodies inhibit viral attachment, and are classified (H1-H15) according to their antigenicity. Anti-NA antibodies restrict the viral replication and are distinguished as nine (N1-N9) subtypes. Viruses with HA types, H1, H2 and H3, and NA types, N1 and N2, have been identified in human and H3N2 subtype of IAV is predominant in Japan (4, 5, 9). During the years 1989-1999, H3N2 and H1N1subtypes of IAV and B type influenza viruses were co-circulating in Chinese populations (Fig. 1), while IAV H3N2 was the predominant strain (76%), associated with a morbidity as high as 10% in the Beijing area (10).

Infection with IAV affects the upper respiratory tract first, followed by an acute and diffuse inflammation of the broncho-alveolar tract. Clinically, IAV infections are accompanied by the production of endogenous pyrogens, high fever, malaise and pulmonary complications. The pathogenesis and organ tropism of the virus are primarily determined by genetic polymorphisms of its subtypes, and by organ-specific trypsin-type processing protease(s) for viral envelope glycoprotein HA, *i.e.* tryptase Clara, ectopic anionic trypsin and mini-plasmin in the airways, which induce the membrane fusion activity of a virus *in vivo* and allow the viral genome to enter the cells (11-14). In influenza encephalitis, the heavy immunoreactive deposits of both virus antigen and mini-plasmin, which amplify the multiplication of IAV and destructs the blood-brain barrier (BBB) *in vivo*, have been exclusively detected in the brain capillaries of newborn mice infected by IAV. These changes, as well as cerebral edema, were more prominent in newborn juvenile visceral steatosis (JVS) mice, which have an inherited defect of the sodium-dependent carnitine transporter,

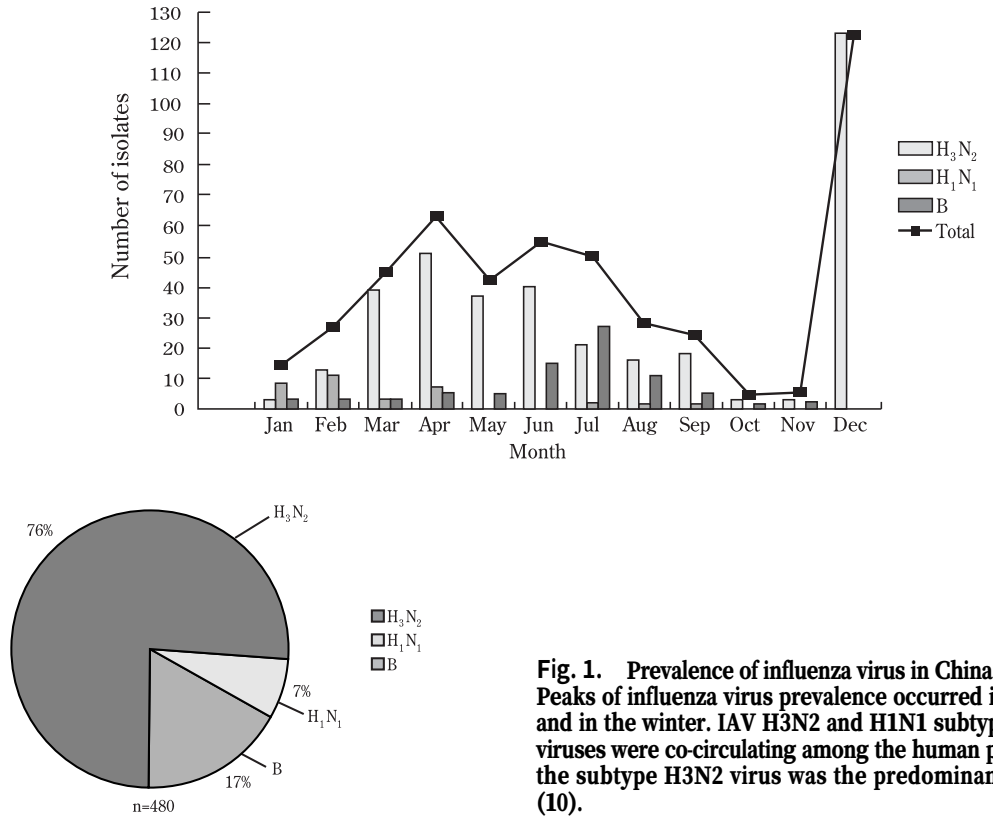


Fig. 1. **Prevalence of influenza virus in China in 1998. Peaks of influenza virus prevalence occurred in the summer and in the winter. IAV H3N2 and H1N1 subtypes and B type viruses were co-circulating among the human population, and the subtype H3N2 virus was the predominant strain (76%) (10).**

OCTN2, in the plasma membrane, than in newborn wild type (WT) mice. Large amounts of mini-plasmin are generated from plasmin or plasminogen by granulocyte elastase in pulmonary inflammatory loci, along with an infiltration of granulocytes and monocytes (14). Since there was little infiltration of inflammatory cells in the brain capillaries even after IAV infection, and mini-plasmin was not detected in the capillaries before infection, accumulated mini-plasmin in the brain capillaries might be transferred from lungs with pneumonia. Mini-plasmin accumulated in the brain capillaries may increase the permeability of endothelial cells by its proteolytic activity, as well as transform capillary endothelial cells from non-susceptible to susceptible to the multiplication of IAV (Yao *et al*., manuscript submitted for publication). Studies of the molecular mechanisms of mini-plasmin accumulation in the brain capillaries of mice with an impaired mitochondrial fatty acid metabolism are currently in progress.

INFLUENZA-ENCEPHALITIS AND REYE'S SYNDROME

Reye's syndrome is characterized by encephalitis and fatty degeneration of the liver due to an impaired free fatty acid metabolism and β-oxidation in mitochondria in children treated with aspirin, ibuprofen and diclofenac, and, in over 85% of cases, infected with influenza or varicella (15, 16). To determine whether a disorder of mitochondrial β-oxidation is a risk factor for influenza encephalopathy or encephalitis, we infected newborn JVS mice and acquired carnitine deficiency mice with non-neurotropic IAV/Aichi/2/68 (H3N2), which has been classified as a dominant and epidemic influenza subtype since 1968. Carnitine is an obligatory amino acid for the transfer of long-chain fatty acids from the cytosol to mitochondria. These fatty acids contribute the major source of energy in the mitochondria, particularly in patients with high fever, vomiting and anorexia during the newborn/suckling periods. Antipyretics, such as aspirin, ibuprofen and diclofenac, have potent anti-inflammatory effects, though impair the mitochondrial fatty acid metabolism and generation of ATP. Furthermore, influenza virus proteins, such as M protein, PB2 and PB1-F2, also cause mitochondrial damage and inhibition of β-oxidation (17, 18). Therefore influenza virus infection in combination with antipyretic treatment may cause a systemic disorder of fatty acid metabolism, particularly in the newborn/suckling period (19). Newborn JVS mice have significantly higher numbers of virus-genome in the brains, accumulation of virus antigen in the capillaries, and an increased blood-brain barrier permeability after intranasal infection with non-neurotropic IAV. Mini-plasmin was prominently accumulated with virus antigen in the brain capillaries of JVS mice, but only mildly in WT mice. Although the mechanisms of IAV-associated encephalopathy by antipyretics have not been clarified, Funato *et al*. have recently described a single-base mutation of the CYP2C9 gene, the major cytochrome P450 gene product that catalyzes diclofenac in human liver, in one of thirty healthy subjects (20). This mutation in the CYP2C9 gene may be related to diclofenac-induced influenza-virus-associated encephalitis or encephalopathy.

AVIAN IAV INFECTION AND ENCEPHALITIS

It is noteworthy that, during the influenza surveillance of 1998-1999 in China, five strains of avian IAVs were isolated from patients with influenza-like illness (10). To examine the infectivity of avian influenza virus in mammalian species, 4-week old ddY mice were infected intranasally with the H5N1 A/Hong Kong/156/97 (HK156) and A/Hong Kong/483/97 (HK483) influenza viruses isolated from humans. HK156 and HK483 required 200 and 5 plaque forming units of virus, respectively, to administer a 50% lethal dose to the mice in a 10 μl volume of inoculum. Both viruses caused encephalitis and severe bronchopneumonia (21). While the severity of lung lesions caused by the viruses was similar, lesions in the brain caused by HK483 were more extensive than those caused by HK 156. This was concordant with the measurements of brain homogenates virus titers, which were over 100-fold higher in HK483- than in HK156-infected mice, while virus titers in lung homogenates were nearly identical. Both viruses were detected in heart, liver, spleen and kidney homogenates, and in the blood of the infected mice. Virus antigen was sporadically detected by immunohistochemical staining, though was associated with no degenerative changes in the heart and liver. The antigen was not detected in the thymus, spleen, pancreas, kidney or gastrointestinal tract. In contrast, it was found regularly in adipose tissue attached to these organs. The adipose tissue showed severe degenerative changes, and contained high virus titers, similar to those measured in

lungs. Thus, infection with HK156 and HK483 in this mouse model was pneumo-, neuro- and adipo-tropic, not pantropic. Furthermore, the neurotropism of HK483 was higher than that of HK156, which may explain its higher lethality.

CYTOKINE LEVELS AFTER IAV INFECTION

Analyses of the host immune responses are required to study the molecular pathologic mechanisms of IAV. The infected epithelial cells and inflammatory leukocytes produce a variety of chemotactic, proinflammatory, and other immunoregulatory cytokines. The increase in expression of cytokine genes is associated with the activation of NF-κB, AP-1, STAT and IRF signal-transducing molecules in the infected cells (22-26). We found a significant increase in the expression of E-selectin, vascular cellular adhesion molecule-1 (V-CAM-1), macrophage inflammatory protein-2 (MIP-2), inducible nitric oxide synthase (iNOS) and endothelin-1 (ET-1) mRNAs, along with an increase in IAV-RNA in the brain of WT mice after IAV infection, whereas these mRNAs were undetectable in absence of infection (Fig. 2). Among the mRNAs tested, the greatest increases in the expressions of ET-1 and iNOS mRNAs were detected in the brain. These proteins have been reported to trigger the formation of edema in various tissues.

TNF-α is a pro-inflammatory cytokine that causes apoptotic tissue injury and a potent inhibitor of mitochondrial respiration. It prominently induces the mitochondrial permeability transition (MPT) in living cells, resulting in a necrotic and apoptotic cell death. Thus, an abrupt increase in TNF-α concentrations after virus infection in the systemic circulation induces systemic MPT in multiple organs, and MPT in cerebral capillary cells cause brain edema in acute IAV encephalopathy or encephalitis (27). We have found that the concentrations of TNF-α in the brain of IAV-infected WT newborn mice were significantly increased on day 5 after inoculation, and that treatment with diclofenac further increased these concentrations (Fig. 3). Since some cytokines, such as IFN-α/β, IFN-r, and IL-2, are protective against influenza infection, whereas others, such as IL-1, TNF-α and IL-6, are involved in the progression of inflammation (26), further studies of the concentrations of other cytokines in the brain, and studies of their mutual interactions are required to elucidate the pathogenesis of IAV encephalitis.

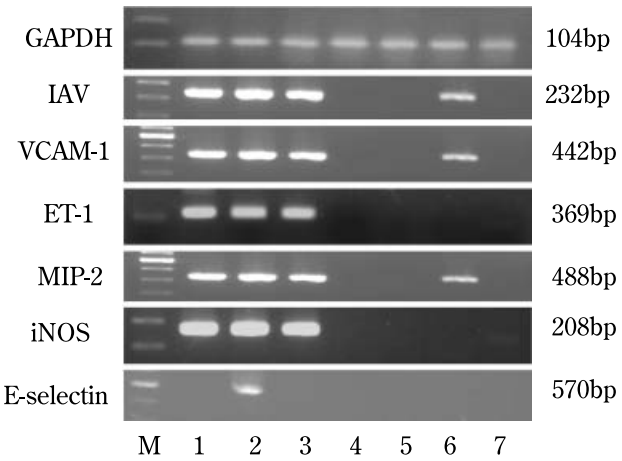


Fig. 2. Expression of IAV HA gene and mRNAs of ET-1, iNOS, MIP-2, E-selectin, and V-CAM-1 in the brains of newborn mice analyzed by RT-PCR.
IAV, influenza A virus ; iNOS, inducible nitric oxide synthase ; ET-1, endothelin-1 ; E-selectin, endothelium selectin ; V-CAM-1, vascular cellular adhesion molecular-1 ; MIP-2, macrophage inflammatory protein-2 ; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Total RNA was extracted from the brains of newborn WT mice on day 5 after inoculation. IAV replication was measured directly by genome amplification, and the expressions of mRNAs of ET-1, iNOS, MIP-2, E-selectin, and V-CAM-1 were analyzed by RT-PCR using random primers and total RNA (1 μg/μl).

ROLE OF NITRIC OXIDE

Nitric oxide (NO) has complex and diverse functions in physiologic and pathophysiologic processes *in vivo*. NO and oxygen radicals appear to be key molecules explaining the pathogenesis of vari-

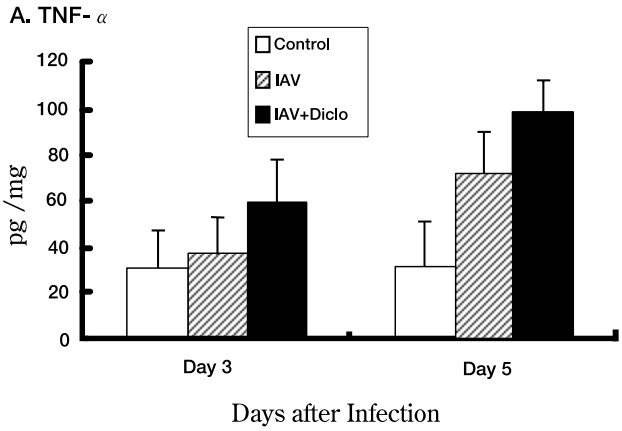
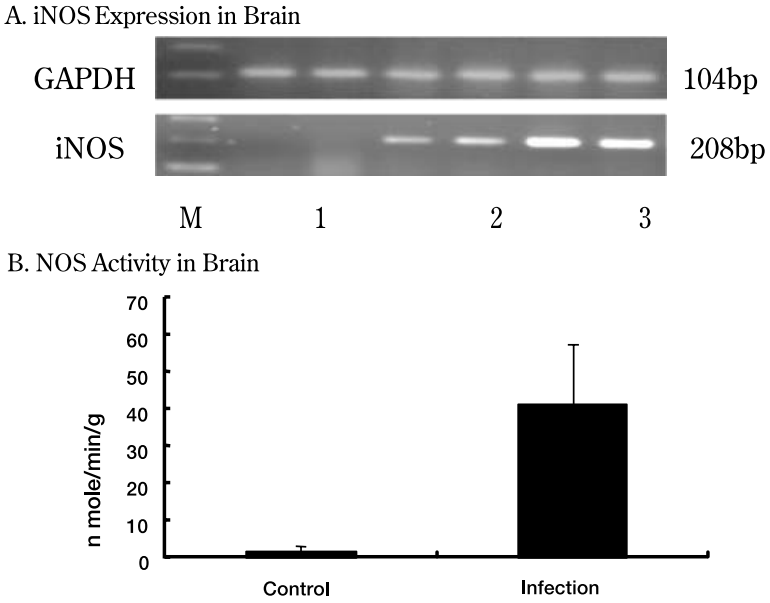


Fig. 3. Expression of TNF-α in the brain of newborn mice after IAV infection.
The levels of TNF-α in the brains of IAV-infected newborn WT mice were analyzed by enzyme-linked immunosorbent assay (EIA) on days 3 and 5. A significant increase in TNF-α concentrations was measured on day 5 in IAV-infected mice (P <0.05). Treatment with diclofenac further increased the concentrations of TNF-α on days 3 (P <0.05) and 5 (P <0.01).

ous infectious diseases, including influenza (28). IAV infection promotes the release of reactive oxygen (ROS) and nitrogen (RNS) species, such as NO, into the extracellular space. ROS and RNS induce tissue injury, and treatment with exogenous antioxidants attenuates tissue damage and lowers mortality in IAV-infected mice. NO, a product of iNOS, is produced by activated macrophages, neutrophils, type II pneumocytes, and airway epithelial cells after virus infection (29). In the brain of newborn WT mice, the concentrations of iNOS-mRNA increased over time after virus inoculation (Fig. 4A), and significant differences in these concentrations (P <0.05) were measured on day 5 between the infected and non-infected brains (Fig. 4B).

NO contributes to the antimicrobial host defense. However, these oxygen and nitrogen reactive intermediates cannot discriminate between exogenous invading pathogens and the host itself and function as mediators of nonspecific innate defense against various microbes. Reactive NO can affect bacteria rather selectively, while the surrounding normal tissue remains intact (Fig. 4C). In contrast, in viral infections, free radical mediators cause nonspecific oxidative injury as well as oxidative stress to the virus-infected tissues.



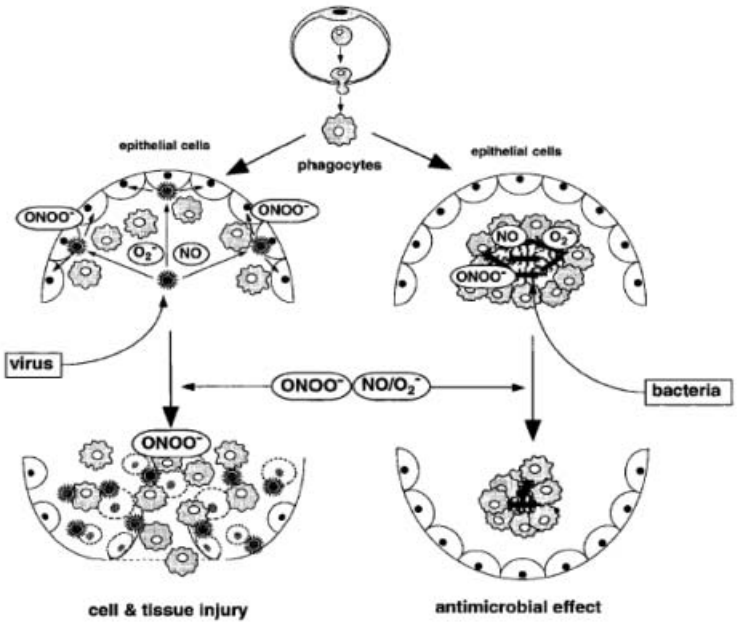


Fig. 4. Increase in the expression of iNOS mRNA (A) and NOS activities (B) in the brain of newborn mice after IAV infection. Role of NO in virus and bacterial infection (C).
(A) Expression of iNOS mRNA in the brain was analyzed by RT-PCR on days 1, 3 and 5 after IAV infection. Lines 1 & 2 correspond to day 1, lines 3 & 4 correspond to day 3, and line 5 & 6 correspond to day 5 after IAV infection. No positive band is present on day 1, and the expression of iNOS mRNA increased over time after infection.
(B) Total NOS activity in the brain on day 5 after IAV infection and in non-infected controls. A significant difference in NOS activity (P <0.05) was observed between the infected and non-infected animals (n=5 in each group).
(C) Schematic representation of the different biological effects of free radicals, such as O_2^- and NO, and their product, $ONCO^-$, in viral and bacterial infections (28).

ENDOTHELIN ISOPEPTIDES AS INFLAMMATORY MEDIATORS

The endothelins [ETs : ET-1, ET-2 and ET-3 (1-21)], a family of 21-residue peptide were first isolated from the culture medium of porcine endothelial cells and shown to be vasoconstrictors (30). Recent studies indicated that ETs are distributed in various tissues and cells and serve a variety of physiological and pathological functions, not only as smooth-muscle constrictors, but also as inflammatory mediators. Furthermore, the bioactive ET peptide family has expanded. New, smooth-muscle-constricting, 31-amino acid endothelins [ETs(1-31)], have been discovered by our group (31, 32). Attention has been attracted to the new functions of these ET isopeptides, such as chemokine function for eosinophils, monocytes and neutrophils (33, 34), and mediators of brain edema formation (35). In the brain, ETs and their receptors localize in neurons, glial cells and smooth muscle cells, and microvessel endothelial cells play pathophysiologic roles by modulating neuronal functions and cerebral blood flow. We found that, after 5 days of IAV inoculation, the concentrations of ET-1 (1-21) and ET-1 (1-31) in the brain of WT newborn mice were significantly higher than in non-infected brains. In the lungs, changes in the concentrations of ET-1 (1-21) were insignificant, whereas the concentrations of ET-1 (1-31) were significantly increased. Treatment with diclofenac had no effect on cerebral ET-1 concentrations. Putting these observations together, an increase in mRNA concentrations of ET-1 (Fig. 2) and in cerebral protein concentrations of ET-1 (1-21) and ET-1 (1-31) (Fig. 5) cause the formation of edema in the brain after IAV infection.

CONCLUSIONS

The pathological mechanisms of influenza encephalitis associated with an increase in the expressions of inflammatory mediators and accumulation of mini-plasmin in the brain capillaries have been presented in the light of current information. The proinflammatory cytokine TNF-α and IAV proteins cause mitochondrial injury, which, in turn, may stimulate the gene expression of adhesion molecules, such as V-CAM-1 and E-selectin, in the brain capillaries, causing the accumulation of mini-plasmin on the surface of cerebral capillaries. Mini-plasmin is a processing enzyme of HA of IAV, which induces the viral envelope fusion activity and allows the viral genome to enter the cells. This accumulation may potentiate the multiplication of IAV in the brain capillaries. In addition, increases in the mRNA expressions of iNOS and ET-1, which cause brain injury and an increase in the BBB permeability, lead to severe brain edema in IAV encephalitis or encephalopathy. A greater understanding of the changes in the expression of inflammatory cytokines and of the molecular mechanism of mini-plasmin accumulation

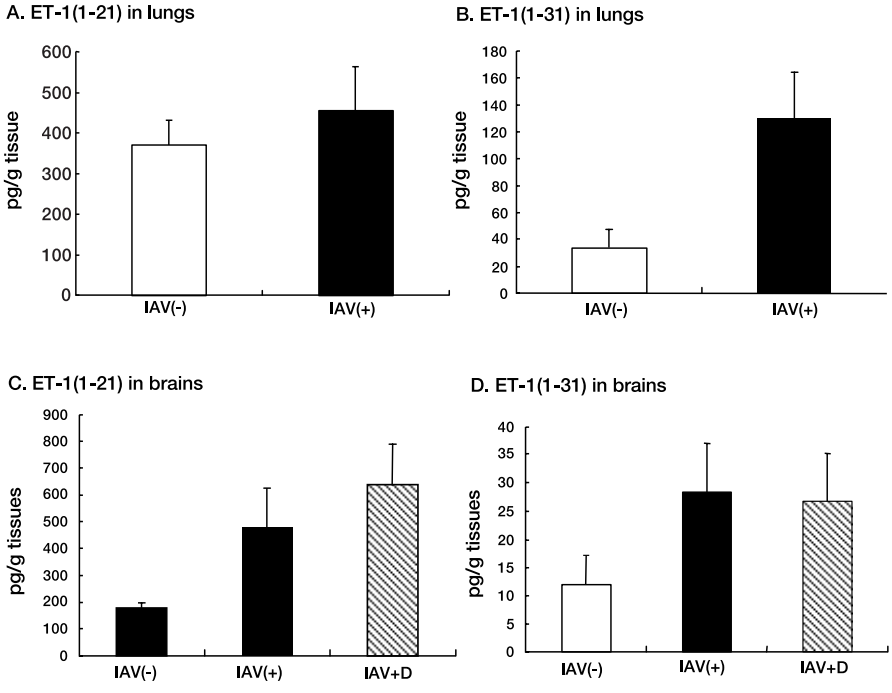


Fig. 5. ET-1(1-21) and ET-1(1-31) concentrations in the lungs and brains of newborn mice infected with IAV for 5 days.
Concentrations of ET-1(1-21) and ET-1 (1-31) were analyzed by EIA. Significant increases in ET-1(1-31) in the lungs and in ET-1(1-21) and ET-1(1-31) in the brains (P <0.05) were observed after IAV infection. Diclofenac (D) treatment had not effect on brain concentrations of ET-1(1-21) and ET-1(1-31).

in cerebral capillaries will provide profound insights into several components of influenza encephalitis.

ACKNOWLEDGMENT

This study was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture (13557014), and Research on Brain Science (H12-Brain-015) from the Ministry of Health Labor and Welfare of Japan.

REFFERENCES

1. Cox NJ, Subbarao K : Influenza. Lancet. 354 : 1277-1282, 1999
2. Ward AC : Neurovirulence of influenza A virus. J Neurovirol 2 : 139-151, 1996
3. Fujimoto S, Kobayashi M, Uemura O, Iwasa M, Ando T, Katoh T, Nakamura C, Maki N, Togari H, Wada Y : PCR on cerebrospinal fluid to show influenza- associated acute encephalopathy or encephalitis. Lancet 352 : 873-875, 1998
4. Sugaya N : Influenza-associated encephalopathy in Japan : Pathogenesis and treatment. Pediatrics Int 42 : 215, 2000
5. Okabe N, Yamashita K, Taniguchi K, Inouye S : Influenza surveillance system of Japan and acute encephalitis and encephalopathy in the influenza season. Pediatrics Int 42 : 187-191, 2000
6. Belay ED, Bresee JS, Holman RC, Khan AS, Shahriari A, Schonberger LB : Reye's syndrome in the United States from 1981 through 1997. N Engl J Med 340 : 1377-1382, 1999
7. Butterworth RF : Antipyretics and Reye's syndrome. Clin Invest Med 21 : 209-210, 1998
8. Lamb RA, Krug RM : Orthomyxoviridae : the viruses and their replication. In : Fields BN, Knipe DM, Howley PM, eds. Fields virology, 3rd ed. Lippincott-Raven Publishers. Philadelphia, Pa. 1996 pp. 1353-1395.
9. Takahashi M, Tango T : Estimation of excess mortality associated with influenza-epidemics by age and cause specific death in Japan, 1975-1999. Nippon Eiseigaku Zasshi (in Japanese) 57 : 571-584, 2002
10. Guo YJ : Influenza activity in China : 1998-1999. Vaccine 20 : S28-S35, 2000
11. Kido H, Yokogoshi Y, Sakai K, Tashiro M, Kishino Y, Fukutimi A, Katunuma N : Isolation and characterization of a novel trypsin-like protease found in rat bronchiolar epithelial Clara cells : A possible activator of the viral fusion glycoprotein. J Biol Chem 267 : 13573-13579, 1992
12. Towatari T, Ide M, Ohba K, Chiba Y, Murakami M, Shiota M, Kawachi M, Yamada H, Kido H : Identification of ectopic anionic trypsin I in rat lungs potentiating pneumotropic virus infectivity and increased enzyme level after virus infection. Eur J Biochem 269 : 1-9, 2002
13. Kido H, Murakami M, Oba K, Chen Y, Towatari T : Cellular proteinases trigger the infectivity of influenza A and Sendai viruses. Mo Cells 9 : 235-244, 1999
14. Murakami M, Towatari T, Ohuchi M, Shiota M, Akao M, Okumura Y, Parry MAA, Kido H : Mini-plasmin found in the epithelial cells of bronchioles triggers infection by broad-spectrum influenza A viruses and Sendai virus. Eur J Biochem 268 : 2847-2855, 2001
15. Crocker JFS, Digout SC, Lee SH, Rozee KR, Renton K, Field CA, Acott P, Murphy MG : Effect of antipyretics on mortality due to influenza B virus in a mouse model of Reye's syndrome. Clin Inves Med 21 : 192-202, 1998
16. Smith TCG : Reye's syndrome and the use of aspirin. Scott Med J 41 : 409, 1996
17. Mazo EL, Rusiaev VA, Fedorov AN, Iaroslavtseva NG, Kharitonenkov IG : Effect of the matrix protein on the influenza virus on oxidative phosphorylation in preparations of isolated liver mitochondria from white mice. Vopr Virusol 33 : 153-157, 1998
18. Chen W, Calvo PA, Malide D, Gibbs J, Schubert U, Bacik I, Basta S, O'Neill R, Schickli J, Palese P : A novel influenza A virus mitochondrial protein that induces cell death. Nature Med 7 : 1306-1312, 2001
19. Murphy MG, Crocker JFS, Her H : Abnormalities in hepatic fatty-acid metabolism in a surfactant/influenza B virus mouse model for acute encephalopathy. Biochim Biophys Acta 1315 : 208-216, 1996
20. Funato T, Kozawa K, Kaku M : Relationship of polymorphism in CYP2C9 to genetic susceptibility to diclofenac-induced influenza-virus-associated encephalopathy. Rinsho Byori (in Japanese) 50 : 140-145, 2002
21. Nishimura H, Itamura S, Iwasaki T, Kurata T, Tashiro M : Characterization of human influenza A (H5N1) virus infection in mice : neuro-, pneumo- and adipotropic infection. J Gen Virol

81 : 2503-2510, 2000
22. Julkunen I, Melen K, Nyqvist M, Pirhonen J, Sareneva T, Matikainen S : Inflammatory responses in influenza A virus infection. Vaccine 19 : S32-S37, 2001
23. Kaufmann A, Salentin R, Meyer RG, Bussfeld D, Pauligk C, Fesq H, Hofmann P, Nain M, Gemsa D, Sprenger H : Defense against influenza A virus infection : essential role of the chemokine system. Immunobiology 204 : 603-613, 2001
24. Aiba H, Mochizuki M, Kimura M, Hojo H : Predictive value of serum interleukin-6 level in influenza virus-associated encephalopathy. Neurology 57 : 295-299, 2001
25. Kaiser L, Fritz RS, Straus SE, Gubareva L, Hayden FG : Symptom pathogenesis during acute influenza : interleukin-6 and other cytokine responses. J Med Virol 64 : 262-268, 2001
26. Ito Y, Ichiyama T, Kimura H, Shibata M, Ishiwada N, Kuroki H, Furukawa S, Morishima T : Detection of influenza virus RNA by reverse transcription-PCR and proinflammatory cytokines in influenza-virus-associated encephalopathy. J Med Virol 58 : 420-425, 1999
27. Kallas EG, Reynolds K, Andrews J, Treanor JJ, Evans TG : Production of influenza-stimulated tumor necrosis factor-alpha by monocytes following acute influenza infection in humans. J Interferon Cytokine Res 19 : 751-755, 1999
28. Akaike T, Maeda H : Nitric oxide and virus infection. Immunology 101 : 300-308, 2000
29. Akaike T : Role of free radicals in viral pathogenesis and mutation. Rev Med Virol 11 : 87-101, 2001
30. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Yazaki Y, Goto K, Masaki T : A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332 : 411-415, 1988
31. Nakano A, Kishi F, Minami K, Wakabayashi H, Nakaya Y, Kido H : Selective conversion of big endothelins to tracheal smooth muscle-constricting 31-amino acid-length endothelins by chymase from human mast cells. J Immunol 159 : 1987-1992, 1997
32. Kido H, Nakano A, Okishima N, Wakabayashi H, Kishi F, Nakaya Y, Yoshizumi M, Tamaki T : Human chymase, an enzyme forming novel bioactive 31-amino acid length endothelins. Biol Chem 379 : 885-891, 1998
33. Sharmin S, Shiota M, Murata E, Cui P, Kitamura H, Yano M, Kido H : A novel bioactive 31-amino acid ET-1 peptide stimulates eosinophil recruitment and increases the levels of eotaxin and IL-5. Inflamm Res 51 : 195-200, 2002
34. Cui P, Tani K, Kitamura H, Okumura Y, Yano M, Inui D, Tamaki T, Sone S, Kido H : a novel bioactive 31-amino acid endothelin-1 is a potent chemotactic peptide for human neutrophils and monocytes. J Leukoc Biol 70 : 306-312, 2001
35. Sato M, Noble LJ : Involvement of the endothelin receptor subtype A in neuronal pathogenesis after traumatic brain injury. Brain Res 809 : 39-49, 1998