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Review

Congenital Infection and Disorders of Brain Development: With Special Reference to Congenital Cytomegalovirus Infection

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ABSTRACT Intrauterine infections of various microorganisms have the potential to induce brain abnormalities in the fetus and newborn infant. Among the infectious pathogens, cytomegalovirus (CMV) is the most significant infectious cause of brain abnormalities, with variations from fatal cytomegalic inclusion disease to functional brain disorders, such as mental retardation or epilepsy. Here, we present three autopsy cases of congenital CMV infection, and we review the features of the brain abnormalities of congenital CMV infection. A ventriculofugal spread of infection seems to be characteristics of congenital CMV-infected brains and is suggested to be the cause of neuronal migration disorders, resulting in brain malformations, such as microcephaly, lissencephaly and polymicrogyria. We also present an experimental animal model for congenital CMV infection. With the model we showed that murine cytomegalovirus (MCMV) infection in the developing mouse brain caused a disturbance of neuronal migration and neuronal cell loss, detected with morphometric measurements by labeling the neuronal precursor cells with BrdU. As shown by immunohistochemical double staining of BrdU and viral-antigen, infected neuronal cells constituted only a part of the disordered neuronal cells, suggesting that an indirect effect of vital infection on neuronal cells also contributes the migration disorders presumably mediated by cytokines or the induction of apoptosis. Neuronal migration disorders caused by MCMV infection are also discussed with reference to those caused by X-irradiation.

Key words: congenital infection, cytomegalovirus, brain development

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Effects of congenital infection on the developing brain

Intrauterine infections of microorganisms including cytomegalovirus (CMV), rubella virus, herpes simplex virus (HSV), Toxoplasma gondii, Group B streptococcus, Escherichia coli and others result in resorption of the embryo, abortion, stillbirth, intrauterine growth retardation, prematurity and congenital malformation (Klein et al., 1983). Although the consequences depend on the virulence of the agent and the susceptibility of the fetus, the route through which the organism gains access to the fetus and the time of gestation when the infection occurs also influence the outcome (Zeichner and Plotkin, 1988).

TORCH (Toxoplasma, Other agents, Rubella virus, Cytomegalovirus and Herpes simplex virus) agents, especially CMV, rubella virus and toxoplasma, are significant in that they cause brain abnormalities in congenital infection. However, the abnormalities are different, depending on cellular tropism of the organisms and modes of the effect on the cells (Zeichner and Plotkin, 1988). Following the initiation of mass vaccine actions for it in 1969, the incidence rate of congenital rubella syndrome decreased markedly (South and Sever, 1985). It was reported that the incidence of clinical severe congenital toxoplasmosis was one in 4,000 pregnancies (Larsen, Jr., 1977), and the incidence seems not to have changed.

In the case of CMV, congenital anomalies of the central nervous system (CNS) are induced by intrauterine infection with an average incidence of about 1% of all live births (Waller, 1971; Stagno et al., 1986; Ho, 1991). It is estimated that 5% to 10% of infected infants have generalized cytomegalic inclusion disease at birth (Ho, 1991). Another 10% or so of such infants have subclinical congenital infection and will subsequently have various degrees of conceptual, auditory, and visual complications during their preschool years (Pass et al., 1980; Conboy et al., 1986). Therefore, congenital CMV infection is the most serious cause of brain abnormalities among the congenital infections (Yow and Demmler, 1992). In this review, we first describe the brain findings of autopsy cases with congenital CMV infections, and then discuss the mechanisms of disorders of brain development caused by infectious agents, compared with the findings from mouse experimental models of congenital CMV infection.

Brain abnormalities caused by human congenital CMV infection

Autopsy cases

Three autopsy cases of congenital CMV infection were analyzed.

One male case from Toyohashi City Hospital was born at 40 weeks of gestation (weight 2,460 g), with symptoms of microcephaly, petechia, jaundice and hepatosplenomegaly; he died 5 days after birth. At autopsy, the brain weight (Br. Wt.) was only 48 g (14.5% of the average of normal Br. Wt. for the age). The brain showed dilatation of the ventricle and calcification of the ventricular walls (Fig. 1*B*). The cerebral surface was almost lack of the gyrus, like lissencephalic brain (Fig. 1*A*). The brain stem and the cerebellum were markedly hypoplastic (Fig. 1).

The second male case from Aichi Prefectural Colony was born at 35 weeks of gestation, weighed 1,560 g and died 12 days after birth. At autopsy, the brain weight was 166 g (43% of the average of normal Br. Wt. for the age). Moderate dilatation of the lateral ventricle (Fig. 2*A*) was observed with spotted calcification in the ventricular and subventricular zones, and uneven thickness of the ventricular zone with reactive inflammatory proliferation (Fig. 2*B*). Although the cerebral gyrus was formed, it looked rather polymicrogyric at the restricted region of the frontal lobe (not shown), while some regions in the temporal and occipital lobes seemed to be pachygyric (Fig. 2*A*). Immunohistochemical staining using antibodies specific to the immediate-early antigens of human CMV (HCMV) revealed that viral antigen-positive cells with swollen cytoplasm

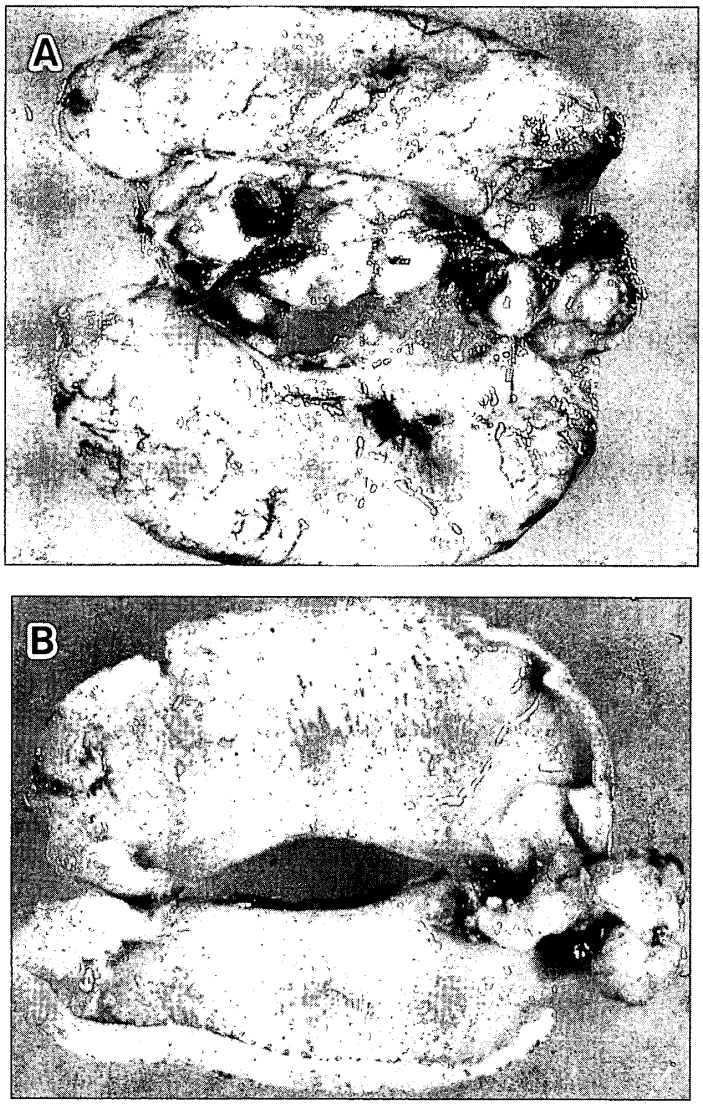

Fig. 1 Congenital CMV-infected brain (case 1) with lissencephalic cerebral surface (**A**) and calcification of the ventricular wall (**B**) (from Dr. Y. Nishimura, Toyohashi City Hospital).

were scattered in the subventricular zone (Fig. 2*C*, *arrows*), and a few positive cells in the cortex (Fig. 3) some of which looked like neurons with processes (Fig. 3*C*) were observed in the cortex of the temporal lobes. However, it was difficult to find viral antigen-positive cells along the ventricular and subventricular zones which showed inflammation and calcification.

The gestational age of the third female case from Aichi Prefectural Colony was not known. The infant weighed 1,650 g at birth, and died 2 days after birth. At autopsy, the brain weighed 220 g (66.0% of the average normal Br. Wt. for the age), had a well developed gyrus without irregularity and non-dilated ventricles (not shown). However, restricted parts of the ventricular zone of the lateral ventricle showed reticular dilatation with a proliferation of cells (Fig. 4).

In all three cases, cytomegalic inclusion cells were found in the alveolar macrophages of the lungs and the tubular epithelium of the kidneys (not shown).

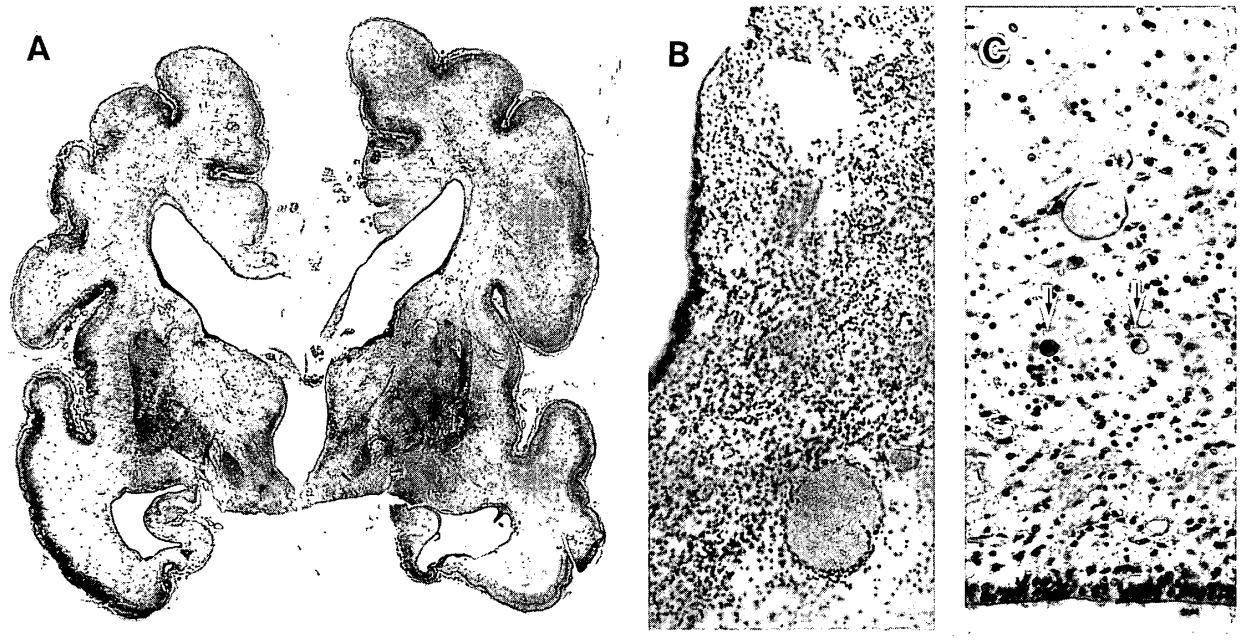

Fig. 2 Coronal brain section of congenital CMV infection (case 2).
A: A whole section through mammillary bodies.
B: Subventricular proliferation of cells. × 90.
C: Viral infected cells were scattered in the subventricular zone stained with antibody specific to the immediate-early antigen of HCMV (*arrows*). × 180.

Characteristics of brain abnormalities caused by CMV infection

Microcephaly has been reported to be the most prominent feature of symptomatic congenital CMV infection (Ho, 1992). There is great variation in the severity of CNS damage caused by CMV; however, the pathological findings of congenital CMV infection are often periependymitis, periventricular necrosis and subventricular calcification with scattered cytomegalic inclusion cells (Becroft, 1981; Periman and Argyle, 1992). It is possible that the cells of the ventricular wall are the primary target for CMV infection in the CNS. The ventricular zone in the developing brain is derived from the neuroepithelial cells which are precursors of neural cells, including neurons and glia. The brain vessels of the ventricular zone and the choroid plexus seem to be the most vulnerable in terms of viral infection in the stage when the blood-brain barrier is not completely formed. In the brains with congenital CMV infection, the ventriculofugal spread of viral infection seems to be characteristic, and the same manner of infection was reported in a MCMV-infected adult patient with AIDS (Willey et al., 1986). Periman and Argyle (1992) reported that among 15 premature infants with lethal congenital CMV infection examined, polymicrogyria (33%) and lissencephaly (6%) were observed in the autopsied brains in addition to microcephaly (77%). Lissencephaly is the failure of the cerebral cortex to form convolutions because of defective neuronal migration in early gestation, while polymicrogyria is a disorder of the late neuronal migration in addition to lesions of vascular and ischemic etiology (Sarnat, 1992). These findings suggested that a disturbance of neuronal migration may be induced in the developing brain by CMV infection (Periman and Argyle, 1992).

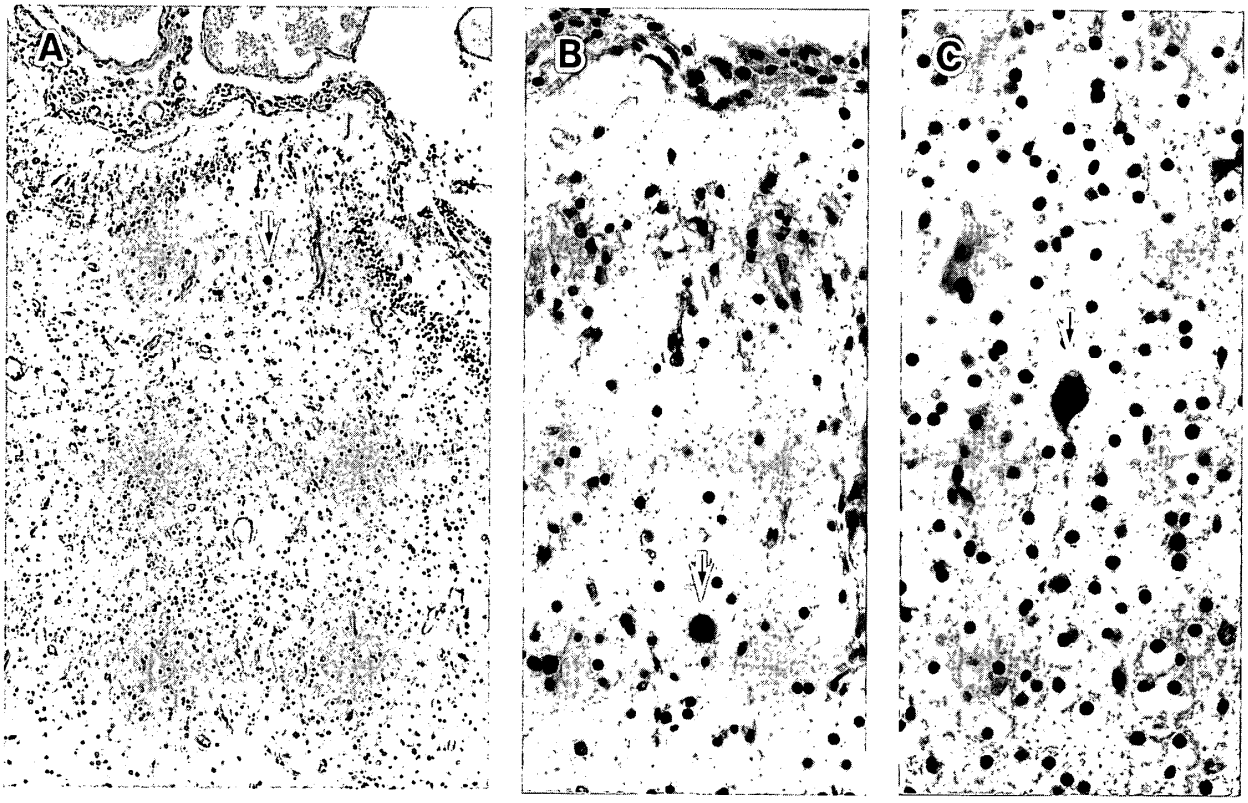

Fig. 3 Immunohistochemical staining of the cerebral cortex of the CMV-infected brain (the same case as Fig. 2).
A: Temporal lobe. × 90. **B:** × 360.
C: Parietal lobe. × 360.

Brain disorders in mouse models of congenital CMV infection

Brain abnormalities

Although there have been significant advances in the knowledge of the molecular biology of human CMV (HCMV) *in vitro*, the investigation of the *in vivo* pathogenesis and immunology has been limited by the strict species specificity of the virus. To overcome this limitation, the CMV of other species, in particular murine cytomegalovirus (MCMV), has been used as a model of human infection (Staczek, 1990; Ho, 1992; Bruggeman, 1993; Tsutsui, 1995).

We reported that mouse embryos in the early gestational stage did not show susceptibility to MCMV (Kashiwai et al., 1992) and that when isolated embryos on day 7.5 of gestation were adsorbed with a high titer of MCMV and cultured, they were susceptible in that MCMV-infected cells were observed in the various regions of the embryos, although the MCMV infection had little effect on their survival and development during 3 days of culture (Tsutsui and Naruse, 1987). Brain abnormalities, such as microophthalmia and cerebral hypoplasia were induced in mice by injecting MCMV into the conceptus on day 8.5 of gestation (Kashiwai et al., 1992; Tsutsui et al., 1993). The conceptus of ICR mice on day 8.5 of gestation were injected with MCMV through the uterine wall, and the pregnancies were allowed to continue. On day 15.5 of gestation, microphthalmia was observed in 19% of the MCMV-infected embryos. Since the survival rate decreased when the pregnancies were allowed to continue further, the incidence of microphthalmia decreased, whereas cerebral hypoplasia was observed in 17% of the surviving mouse fetuses on day 18.5 of gestation. Viral

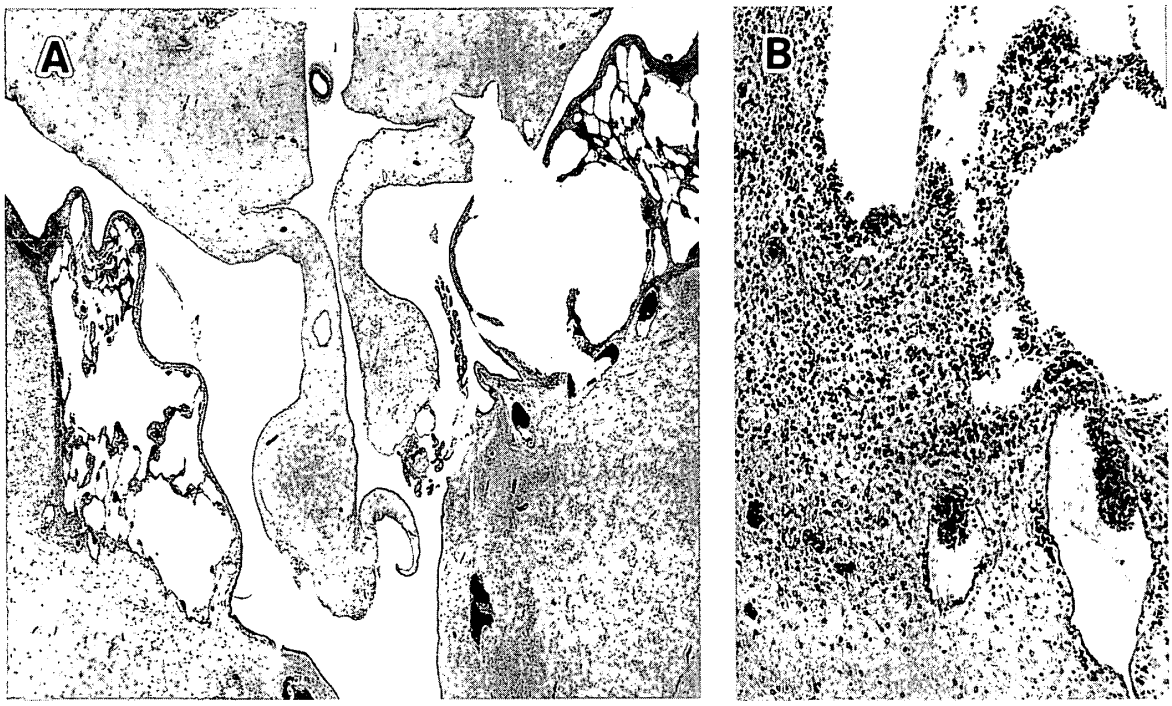

Fig. 4 The lateral ventricular zone of the brain of the congenital CMV infection (case 3).
A: Reticular dilatation of the ventricular zone. × 4. **B:** × 90.

antigen-positive cells were widely distributed in the mesenchyme around the eyes and the brain, especially in the endothelial cells of the vessels and perivascular mesodermal cells (Tsutsui et al., 1993). The primary target of CMV infection in the fetal stage was thus suggested to be the mesenchymal cells.

To analyze the direct effect of MCMV infection on the developing mouse brain, we developed a model system for brain abnormalities induced by MCMV injection into the cerebral ventricles of mouse embryos at the late stage of gestation (Naruse and Tsutsui, 1989; Tsutsui et a1., 1989). The pathological findings of the brains of the offspring from 7 to 14 days after birth, infected with a relatively high viral titer, sometimes showed acute lesions with cerebral necrosis and inflammation. In the cortical region where the laminal architecture was maintained, viral antigen-positive cells detected with a monoclonal antibody specific to the early nuclear antigen of MCMV (D5) were diffusely distributed in a laminar array in accord with neuronal lamination (Fig. 5*A*). In the hippocampus, viral antigen-positive cells were also distributed in accord with the pyramidal neurons (Fig. 5*B*).

Neurotropism of MCMV

To determine which types of brain cells are susceptible to MCMV, we performed immunohistochemical double staining using antibodies specific to neuron-specific enolase (NSE) or glial fibrillary acidic protein (GFAP) and the antibodies to the early nuclear antigen (D5) or the immediate early antigen (N2). The viral antigen-positive cells detected by the D5 antibody, which were localized in the cortex and hippocampus, were double-stained with the NSE antibody (Tsutsui et al., 1989). This finding directly demonstrated that MCMV infects neuronal cells. The MCMV-infected neuronal cells were observed for a prolonged time after birth when mouse embryos were infected (Tsutsui et al., 1995). In contrast, the infected cells stained with the N2

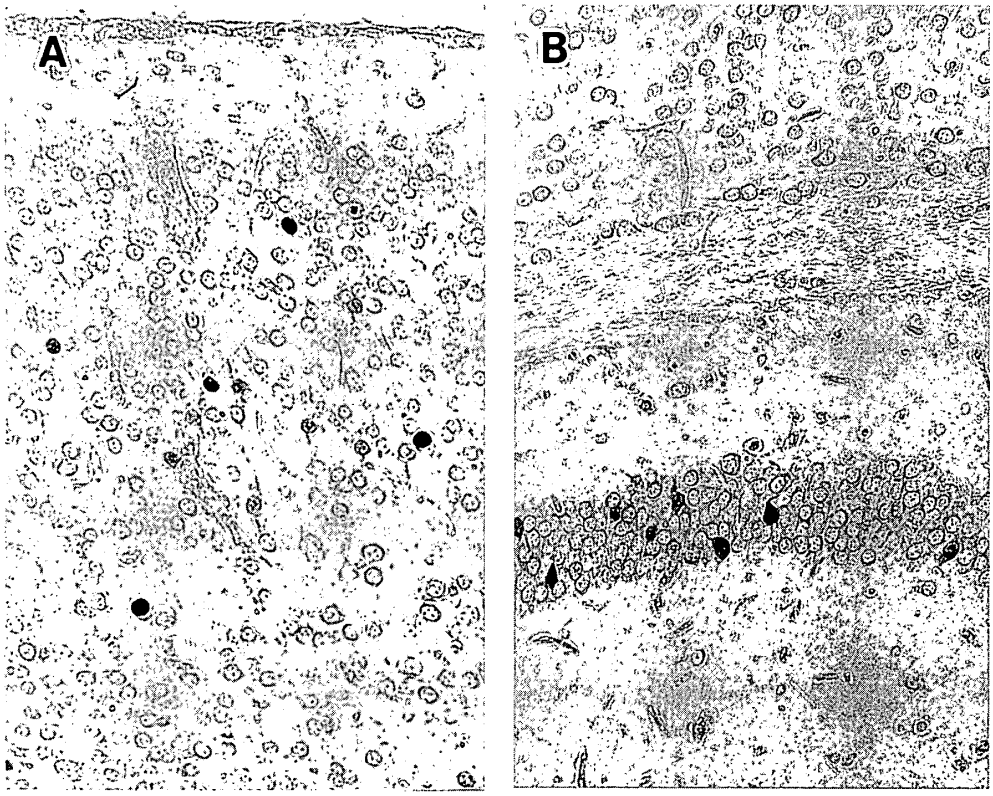

Fig. 5 Immunohistochemical staining of viral antigen-positive cells in 14-day-old mice that were intraperitoneally injected with MCMV on day 15 of gestation.
A: Cerebral cortex.
B: Hippocampus. × 180.

antibody, which were localized mainly in the ventricular wall and subventricular zone, were double-stained with the anti-GFAP antibody (unpublished results). These findings suggest that the dynamics of MCMV infection are different between neuronal cells and glial cells. In humans, inclusion-bearing cells have been identified in neurons, glia, ependyma, choroid plexus, meninges and vascular endothelium (Becroft, 1981). An *in vitro* model of the normal human brain has been developed in which fetal human brain cells form three dimensional aggregates consisting of astrocytes, neurons and oligodendrocytes (Pulliam et al., 1988; McCarthy et al., 1991). These cultures have been shown to be susceptible to HCMV infection. Poland et al. (1994) reported that a human primary nontransformed neuronal cell line was permissive to HCMV infection, and that the virus replication was extensively increased when the cells were induced to differentiate by nerve growth factor (NGF), dibutyryl cAMP (dbcAMP), or 1-isobutyl-3-methyl xanthine (IBMX). HCMV also replicated in human retinal tissues which were grafted into the anterior chambers of immune-deficient mice (DiLoreto et al., 1994). These findings indicate that human neuronal cells as well as glial cells and microglia are susceptible to HCMV as shown in MCMV, although neurotropism seems to be more prominent in MCMV than in HCMV.

Disorders of neuronal migration in developing mouse brains infected with CMV

Brain abnormalities like microcephaly, lissencephaly and polymicrogyria have been reported to be some-

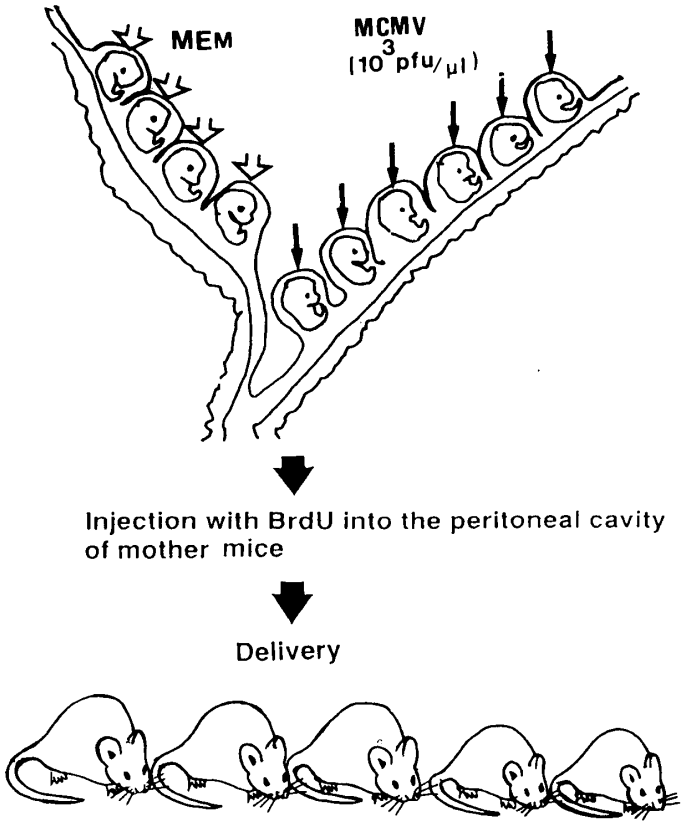

Fig. 6 Schematic illustration of the experimental procedures for comparison of neuronal migration of MCMV-infected brains with MEM-injected brains. MCMV-infected mice usually gains less body weights than uninfected mice.

times associated with human congenital CMV infection. These abnormalities are thought to be caused mainly by disorders of neuronal migration (Sarnat, 1992; Mischel et al., 1995) and also by neuronal loss. In addition, ventriculofugal spread of viral infection seems to be characteristic in congenital CMV-infected brains. Although neurotropic features of infection are prominent in MCMV, neuronal infection in human CMV-infected brains has also been reported in addition to glial and microglial infections. These reports suggest that CMV infection may disturb neuronal migration in the brain development. However, there have been few reports showing direct evidence of the disturbance of neuronal migration by CMV infection.

Experimental model

We developed an experimental model for the assay of the effects of MCMV infection on neuronal migration in the developing mouse brains (Shinmura et al., 1997). Briefly, mouse embryos on day 15.5 of gestation were injected with MCMV or minimal essential medium (MEM) into the cerebral ventricles through the uterine wall. MCMV was injected into the embryos on one side of the uteri and MEM was injected into the embryos on the other side of the uteri. The pregnant mother mice were then injected intraperitoneally with 5-bromo-2-deoxy-uridine (BrdU) at various times after the MCMV/MEM injection. The embryos were allowed to develop and to be delivered, and were then fed by the mothers during the postnatal days (Fig. 6). The offspring were sacrificed, and their brains were removed, fixed and embedded in paraffin. Three coronal sections were made at the frontal, parietal and occipital lobes and stained with the D5 antibody. The brains

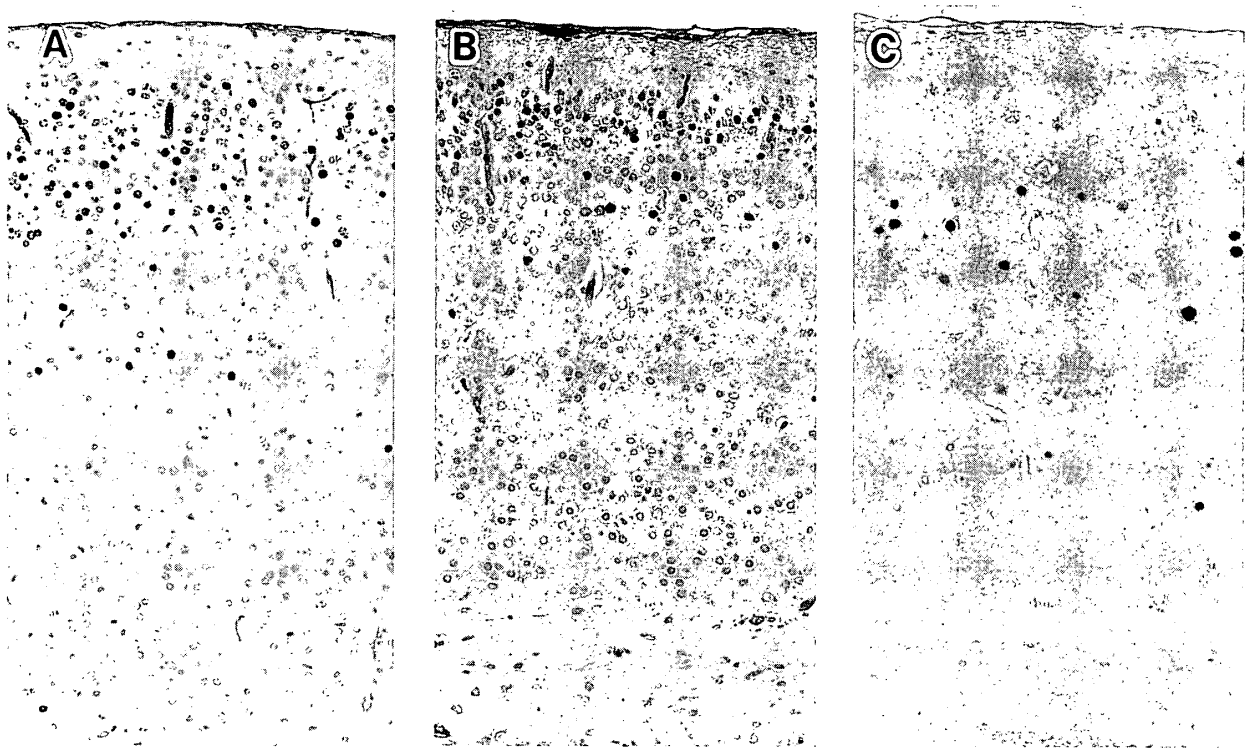

Fig. 7 Immunohistochemical staining of BrdU-labeled cells of the same parts of the parietal lobes of the 7-day-old offspring injected with MEM (**A**) and MCMV (**B** and **C**).
A, B: BrdU-staining.
C: Viral antigen-positive cells of the adjacent section of Fig. 7*B*.

with vital antigen-positive cells in any sections were regarded as the infected group, while the brains without positive cells were regarded as the uninfected group. By this definition, the MCMV-infected mice had significantly lower body weights and brain weight than did the uninfected mice. The adjacent sections of the parietal lobes were stained with anti-BrdU antibody. The BrdU-labeled cells were counted in each layer of the cerebral mantles. The percentages of BrdU-positive cells in each layer and the numbers of the labeled cells per unit area were calculated and compared between the infected and uninfected groups.

Neuronal migration disorder by MCMV infection

According to the time course of migration of BrdU-labeled cells in the cerebral cortex of the developing brains (Shinmura et al., 1997), more than 80% of the labeled cells were found in layer II–III of the brains of postnatal day 5 (P5), and then the number of labeled cells in layer IV–V increased due to the phenomenon known as upside-down migration (Rakic, 1988). In the MCMV-infected cortices of the P7 mice, for example, BrdU-labeled cells were localized mainly in layer II–III (Fig. 7*B*), while the numbers of BrdU-labeled cells in the deeper cortical layers of uninfected brains (Fig. 7*B*) were significantly higher than those in the MCMV-infected brains (Shinmura et al., 1997). The total numbers of BrdU-labeled cells in the infected brains were less than half of the uninfected brains (Fig. 8). These results suggested that the neuronal migration in the MCMV-infected brains was disturbed and that a loss of neuronal cells occurred during migration.

To determine the correlation of BrdU-labeled cells and vital-infected cells, the immunohistochemical

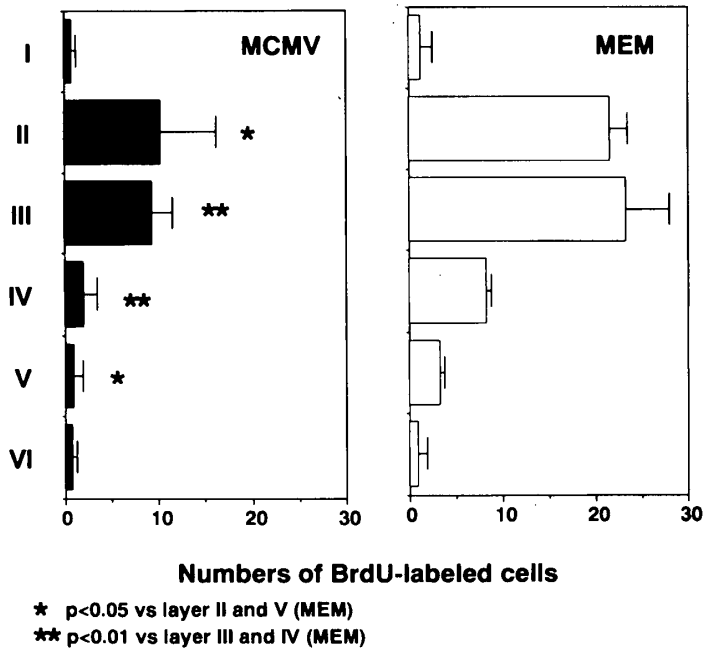

Fig. 8 Comparison of BrdU-labeled cells in each layers of the cerebral walls in the 7 day-old-mice which were injected with MCMV or MEM on day 15.5 of gestation and labeled with BrdU 6 h later.

double-staining was performed using anti-BrdU antibody, which stains BrdU-labeled nuclei blue by alkaline phosphatase (ALP) reaction, and using anti-MCMV antibody (Q3) which recognizes the early cytoplasmic viral antigen, stained red by 3-amino-9-ethylcarbazole (AEC) (Fig. 9). In the cerebral cortices of P7 brains which were infected with MCMV on day 15.5 of gestation and labeled with BrdU 6 h later, the BrdU-labeled cells were distributed mainly in layer II–III, while the viral antigen-positive cells were localized mostly in the deeper layers (IV–VI) (Fig. 9*A* and *B*). Although the double-stained cells constituted only 7.5% of the total number of BrdU-labeled cells, most of the double-stained cells were localized in the layers III–IV. A small number of double-stained cells were found in the deeper layers V–VI and in the ventricular walls. In the P1 MCMV-infected brains, double-labeled cells were found in the ventricular zone and intermediate zone (Fig. 9*C*) but not in the cortical plate (Shinmura et al., 1997). By the double staining, it was shown that the migration of most of the BrdU-labeled cells, mainly localized in layers II–III, tended to precede the viral antigen-positive cells, presumably because the progenitor cells were pulse-labeled by BrdU, while the infection of the progenitor cells was continuous. Although the double-stained cells were disturbed in migration, it is of interest that the MCMV-infected cells migrated to some degree in the developing brain, because neuronal migration is thought to be the active movement of the neuronal cell bodies along the radial glial fibers (Rakic, 1988).

Characteristics of MCMV-induced migration disorder

It has been known that ionizing radiation, when delivered at a critical gestational period, can profoundly affect the neuronal migration of the developing brain in mammals (Inouye et al., 1993; Jensh et al., 1995; Fushiki et al., 1996). In the irradiated brains, there is a threshold of the dose of ionizing irradiation, over which a disturbance of neuronal migration occurs (Fushiki et al., 1996), resulting in a prominent disturbance

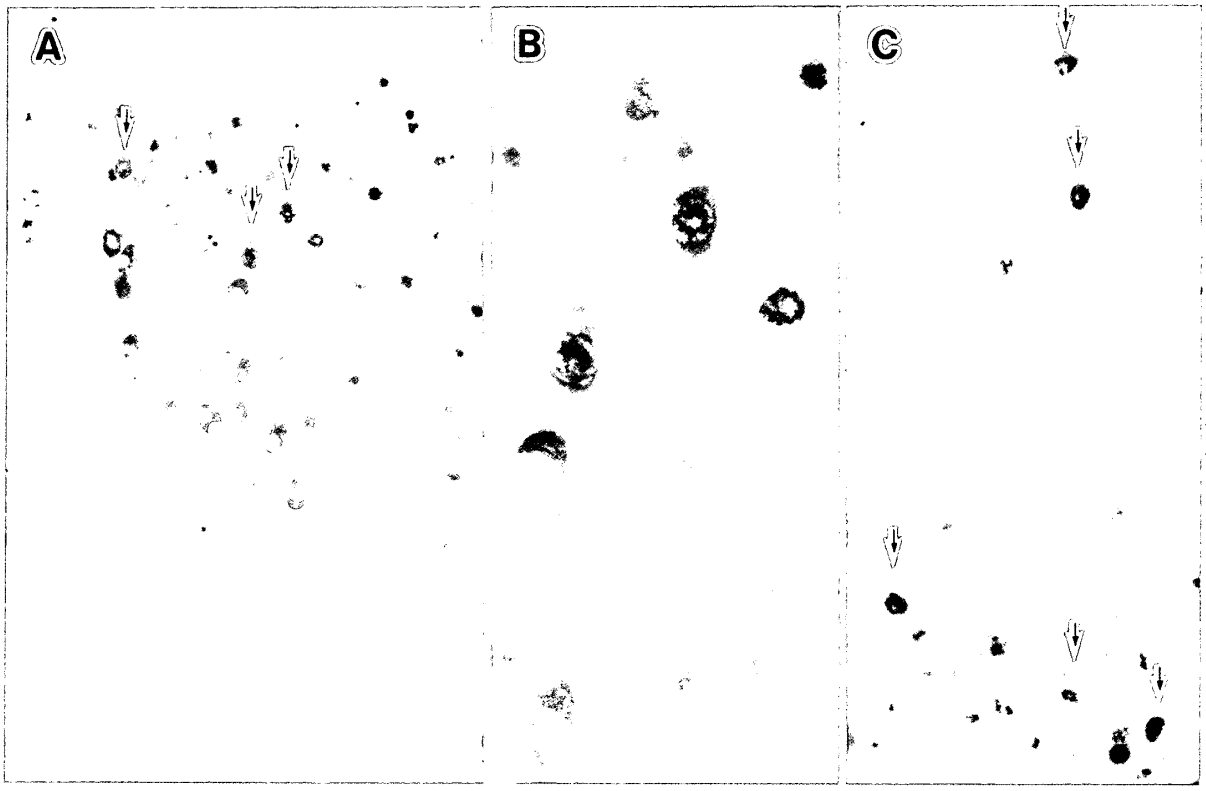

Fig. 9 Immunohistochemical double staining of BrdU- and viral antigen-positive cells in the cerebral walls of the offspring infected with MCMV on day 15.5 of gestation.
A: Labeled with BrdU 6 h after infection. × 90.
B: × 360. **C:** labeled with BrdU 72 h after infection.
A, B: Cerebral cortex.
C: Ventricular and subventricular zones.

in either the prenatal or postnatal period. In contrast, the disturbance of neuronal migration by infection with MCMV has not been to be uniform manner; there is a divergence of the migration disorder depending on types and intensity of infection. It is thus possible that radiation may affect the progenitor cells of the fetal brain evenly for a restricted duration, while MCMV may infect the progenitor cells in the ventricular zone unevenly and may need a certain time for the virus to proliferate. However, the progenitor cells seem to show susceptibility to both of these agents.

Although the fate of CMV-infected neuronal cells is not known, it is possible that MCMV-infected neurons are sustained in the brain for a rather prolonged time, based on the findings that vital antigen-positive neurons were retained in the cortex and hippocampus of mice when they were infected at a late stage of gestation with a low titer of virus (Tsutsui et al., 1995). Furthermore, Joly et al. (1991, 1992) reported that neuronal cells are deficient in the major histocompatibility class I molecules for the presentation of viral antigen to cytotoxic T lymphocytes, suggesting that viral infection would be persistent in these cells. It is worthwhile to mention that Oldstone proposed the concept that viral persistent infection can interfere with cell functions without disrupting the cell's vital functions (Oldstone, 1989, 1993). Modulations of neuronal migration have been reported in N-CAM mutation mice (Ono et al., 1994) and in slice cultures of the developing mouse cerebellum with blocked glutamate receptors (Komuro and Rakic, 1993). It is not known whether MCMV

infection in neuronal cells alters these functions.

Since we observed that the viral antigen-positive neurons were only a small fraction of the total number of BrdU-labeled neurons, it is possible that the migration of uninfected BrdU-labeled neurons was also disturbed in the MCMV-infected brains. Therefore, there may be two possibilities for the induction of a neuronal migration disorder in CMV-infected brains; one is by direct infection and the other is by indirect effects of the infection. It has been reported that viral infections induce various kinds of cytokines (Orange and Biron, 1996) and that cytokines regulate the cellular phenotype of developmental neuronal cells (Mehler et al., 1995). Viral infections induce apoptosis in infected and uninfected cells in the brains of HIV-infected AIDS patients (An et al., 1996). We have also obtained evidence that MCMV infection in the developing mouse brain induces apoptosis in uninfected brain cells (unpublished data). Neuronal cell loss in addition to disordered neuronal migration was observed in MCMV-infected brains (Shinmura et al., 1997), resulting in microcephaly.

CONCLUSIONS

Although intrauterine infections as environmental factors are thought to constitute only a few percents of causes of all congenital malformations (Kalter and Warkany, 1983), congenital CMV infection is the most common cause among the infectious agents (Stagno et al., 1986). The ventriculofugal spread of infection from the ventricular zone to the cortical plate of the fetal and neonatal brain seems to be characteristic of intrauterine infections, especially CMV infection. This infection pattern is also interesting from the aspect of brain development, because radial migration refers to the process by which neuronal cells from the ventricular and subventricular zones migrate along radial glial fibers to reach the cortex (Rakic, 1988). In cases of CMV infection, the ventricular zone seems to be the most vulnerable site of infection, reported as periependymitis with calcification in cytomegalic inclusion disease (Becroft, 1981). Brain malformations, such as microcephaly, lissencephaly and polymicrogyria, have been reported to be associated with congenital CMV infection (Becroft, 1981; Ho, 1991; Periman and Argyle, 1992). These neocortical malformations have usually been considered to be disorders of neuronal migration which have been classified with regard to morphology or putative events which occur in early, intermediate and late intrauterine life. (Mischel et al., 1995).

We developed an experimental model of congenital CMV infection. We observed neurotropism of MCMV and a ventriculofugal spread of infection, the same as has been observed in autopsy cases of congenital HCMV infection. We also obtained direct evidence of disordered neuronal migration caused by infection with MCMV in the developing mouse brains. In this model, we compared the neuronal migration of MCMV-infected neonatal mice with that of uninfected mice from the same litter; the comparison of the neuronal migration in the two groups was thus of brains under the same conditions. The relationship between BrdU-labeled neuronal cells and viral-infected cells during neuronal migration was analyzed by the method of double-staining. BrdU-labeled neuronal cells in the MCMV-infected mice were decreased in number and disturbed in migration compared with uninfected mice. However, only a small fraction of the MCMV-infected neuronal cells were detected by the double-staining method. Therefore, the disturbance of neuronal migration may be caused both by direct infection and by indirect effects of the infection mediated through cytokines or the induction of apoptosis.

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REFERENCES

An, S.F., Giometto, B., Scaravill, T., Tavolato, B., Gray, F. and Scaravilli, F. (1996) Programmed cell death in brains of HIV-1-positive AIDS and pre-AIDS patients. Acta Neuropathol. (Berl.), **91:** 169-173.

Becroft, D.M.O. (1981) Perinatal cytomegalovirus infection: Epidemiology, pathology, pathogenesis. Perspectives in Pediatric Pathology, (Rosenberg, H.S., Bernstein, J. ed.), Masson, New York, 203-241.

Bruggeman, C.A. (1993) Cytomegalovirus and latency: An overview. Virchows Arch. (B), **64:** 325-333.

Conboy, T.J., Pass, R.F., Stagno, S. Britt, W.J., Alford, C.A., McFarland, C.E. and Boll, T.J. (1986) Intellectual development in school-aged children with asymptomatic congenital cytomegalovirus infection. Pediatrics, **77:** 801-806.

DiLoreto, D., Epstein, L.G., Lazar. E.S., Britt, W.J. and del Cerro, M. (1994) Cytomegalovirus infection of human retinal tissue: An *in vitro* model. Lab. Invest., **71:** 141-148.

Dische, M.R. and Gooch, III, W.M. (1981) Congenital toxoplasmosis. Perspective in Pediatric Pathology. vol. 6: Infectious Diseases, (Rosenberg, H.S. and Bernstein, J. ed.), Masson Publ., New York, 83-113.

Fushiki S., Matsushita, K., Yoshida, H. and Schull, W.J. (1996) *In utero* exposure to low-doses of ionizing radiation decelerates neuronal migration in the developing rat brain. Int. J. Radiat. Biol., **70:** 53-60.

Ho, M. (1991) Congenital and perinatal human cytomegalovirus infections. Cytomegalovirus: Biology and Infection, 2nd ed., Plenum Press, New York, 205-227.

Inouye, M., Hayasaka, S., Sun, X.Z. and Yamamura, H. (1993) Disturbance of neuronal migration in mouse cerebral cortex by low-dose gamma-irradiation. J. Radiat. Res., **34:** 204-213.

Jensh, R.P., Eisenman, L.M. and Brent, R.L. (1995) Postnatal neurophysiologic effects of prenatal x-irradiation. Int. J. Radiat. Biol., **67:** 217-227.

Joly, E., Mucke, L. and Oldstone, M.B.A. (1991) Viral persistence in neurons explained by lack of major histocompatibility class I expression. Science, **253:** 1283-1285.

Joly, E., and Oldstone, M.B.A. (1992) Neuronal cells are deficient in loading peptides onto MHC class I molecules. Neuron, **8:** 1185-1190.

Kalter, H. and Warkany, J. (1983) Congenital malformations: Etiologic factors and their role in prevention. N. Engl. J. Med., **308:** 424-431.

Kashiwai, A., Kawamura, C., Kadota, C. and Tsutsui, Y. (1992) Susceptibility of mouse embryo to murine cytomegalovirus infection in early and mid-gestation stages. Arch. Virol., **127:** 37-48.

Klein, J.O., Remington, J.S. and Marcy, S.M. (1983) Current concepts of infections of fetus and newborn infant. Infectious Diseases of the Fetus and Newborn Infant, (Remington, J.S. and Klein, J.O. ed.), W.B. Saunders Co., Philadelphia, 1-26.

Komuro, H. and Rakic, P. (1993) Modulation of neuronal migration by NMDA receptors. Science, **260:** 95-97.

Larsen, J.W.Jr. (1977) Congenital toxoplasmosis. Teratology, **15:** 213-217.

McCarthy, M., Pesnick, L., Taub, F. Stewart, R.V. and Dix, R.D. (1991) Infection of human neuronal cell aggregate culture with a clinical isolate of cytomegalovirus. J. Neuropathol. Exp. Neurol., **50:** 441-450.

Mehler, M.F., Marmur, R., Gross, R., Mabies, P.C., Zang, Z., Papavasilliou, A. and Kessler, J.A. (1995) Cytokines regulate the cellular phenotype of developing neuronal lineage species. Int. J. Dev. Neurosci., **13:** 213-240.

Mischel, P.S., Nguyen, L.P. and Vinters, H.V. (1995) Cerebral cortical dysplasia associated with pediatric epilepsy. Review of neuropathologic features and proposal for a grading system. J. Neuropathol. Exp. Neurol., **54:** 137-153.

Naruse, I. and Tsutsui, Y. (1989) Brain abnormalities induced by murine cytomegalovirus injected into the

cerebral ventricles of mouse embryos *exo utero*. Teratology, **40:** 181-189.

Oldstone, M.B.A. (1989) Viral persistence. Cell, **56:** 517-520.

Oldstone, M.B.A. (1993) Viruses and diseases of twenty-first century. Am. J. Pathol., **143:** 1241-1249.

Ono, K., Tomasiewicz, H., Magnuson, T. and Rutishauser, U. (1994) N-CAM mutation inhibits transitional neuronal migration and its phenocopies by enzymic removal of polysialic acid. Neuron, **13:** 595-609.

Orange, J.S. and Biron, C.A. (1996) Characterization of early IL-12, IFN-αβ, and TNF effects on antiviral state and NK cell responses during murine cytomegalovirus. J. Immunol., **156:** 4746-4756.

Perlman, J.M. and Argyle, C. (1992) Lethal cytomegalovirus infection in preterm infants: Clinical, radiological, and neuropathological findings. Ann. Neurol., **31:** 64-68.

Pass, R.F., Stagno, S., Myers, G.L. and Alford, C.A. (1980) Outcome of symptomatic congenital cytomegalovirus infection; Results of long-term longitudinal follow-up. Pediatrics, **66:** 758-762.

Poland, S.D., Bambrick, L.L., Dekaban, G.A. and Rice, P.A. (1994) The extent of human cytomegalovirus replication in primary neurons is dependent on host cell differentiation. J. Infect. Dis., **170:** 1267-1271.

Pullian, L., Berens, M.E. and Rosenblum, M.L. (1988) A normal human brain cell aggregate model for neurobiological studies. J. Neurosci. Res., **21:** 521-530.

Rakic, P. (1988) Defects of neuronal migration and the pathogenesis of cortical malformation. Prog. Brain Res., **73:** 15-37.

Sarnat, H.B. (1992) Disorders of neuroblasts migration. Cerebral Dysgenesis. Oxford Univ. Press, 245-274.

Shinmura, Y., Kosugi, I., Masago-Aiba, S., Baba, S., Yong, L-R. and Tsutsui, Y. (1977) Disordered migration and loss of viral-infected neuronal cells in developing mouse brains infected with murine cytomegalovirus. Acta Neuropathol. (Berl.) (in press).

South, M.A. and Sever, J.L. (1985) Teratogen update: The congenital rubella syndrome. Teratology, **31:** 297-307.

Staczek, J. (1990) Animal cytomegaloviruses. Microbiol. Rev., **54:** 247-265.

Stagno, S., Pass, R.F., Cloud, G. Britt, W.J., Henderson, R.E., Walton, P.D., Veren, D.A., Page, F. and Alford, C.A. (1986) Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA, **256:** 1904-1908.

Tsutsui, Y. and Naruse, I. (1987) Murine cytomegalovirus infection of cultured mouse embryos. Am. J. Pathol. **127:** 262-270.

Tsutsui, Y., Kashiwai, A., Kawamura, N., Nagahama, M., Mizutani, A. and Naruse, I. (1989) Susceptibility of brain cells to murine cytomegalovirus infection in the developing mouse brain. Acta Neuropathol. (Berl.), **79:** 262-270.

Tsutsui, Y., Kashiwai, A., Kawamura, N. and Kadota, C. (1993) Microphthalmia and cerebral atrophy induced in mouse embryos by infection with murine cytomegalovirus in midgestation. Am. J. Pathol., **143:** 804-812.

Tsutsui Y., Kashiwai, A., Kawamura, N., Aiba-Masago, S. and Kosugi, I. (1995) Prolonged infection of mouse brain neurons with murine cytomegalovirus after pre- and perinatal infections. Arch. Virol., **140:** 1725-1736.

Tsutsui, Y. (1995) Developmental disorders of the mouse brain induced by murine cytomegalovirus: Animal models for congenital cytomegalovirus infection. Pathol. Intern., **45:** 91-102.

Waller, T.H. (1971) The cytomegalovirus: Ubiquitous agents with protean clinical manifestation. N. Engl. J. Med., **285:** 203-214.

Willey, C.A., Schrier, R.D., Denaro, F.J., Nelson, J.A. and Lampert, P.W. (1986) Localization of cytomegalovirus products and genome during fulminant central nervous system infection in AIDS patient. J. Neuropath. Exp. Neurol., **45:** 127-139.

Yow, M.D. and Demmler, G.J. (1992) Congenital cytomegalovirus disease-20 years is long enough. N. Engl. J. Med., **326:** 702-703.

Zeichner, S.L. and Plotkin, S.A. (1988) Mechanisms and pathways of congenital infections. Clin. Perinatol., **12:** 163-188.