replication have a conserved target in the viral protein 3A similar to that of enviroxime

3. Guest Speaker's presentation

Professor Florence Morfin (Laboratory of Virology, Hospices Civils of Lyon, France, FRE 3011, CNRS / University of Lyon.

Antiviral drugs for herpes simplex virus and adenovirus: activity and resistance features

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シンポジウム発表演題の抄録

"Hematopoietic stem cell transplantation in Japan." —Special emphasis on cord blood transplantation—

Shunichi Kato
Tokai University School of Medicine

Hematopoietic stem cell transplantation (HSCT) has been increasingly applied to many patients with hematological disorders in many countries. However, stem cell source and donor selection differs between children and adults and varies from country to country. The Center for International Blood and Marrow Transplant Research (CIBMTR) reports worldwide use and outcome of HSCT annually, and 2009 report is as follows; Bone marrow is the primary graft source for transplantation in children, though the use of peripheral blood and umbilical cord blood grafts is increasing. During the period 2003 to 2007, peripheral blood grafts accounted for 28%, and cord blood accounted for 20% of allotransplants in patients younger than 20 years of age. Among adults older than 20 years, peripheral blood is the most common source of allogeneic grafts. The Japan Society for Hematopoietic Cell Transplantation (JSHCT) also reports national use and outcome of HSCT in Japan. BM is also the primary graft source in children and the use of CB is increasing while the use of PB is limited. During the period 2003 to 2007, BM, PB and CB accounted for 68%, 9% and 23% of allo-HSCT in patients younger than 20 years, respectively. Among adults older than 20 years, BM 56%, PB 23% and CB 21% in the same period. Thus, the proportion of cord blood is higher both in children and in adults in Japan than in other parts of the world.

Life-long observation of HSV-1 infections in a child with Wiskott-Aldrich syndrome: lessons learned from a patient

Masayuki Saijo. M.D., Ph. D.
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HSV-1 infections in a child with a congenital immunodeficiency, Wiskott-Aldrich syndrome, were observed over a 10-year period from the primary infection at the age of 3 to the fatal infections at the age of 13. The child was primarily infected with HSV-1 (TAP isolate) at the age of 3, and later suffered from recurrent and intractable mucocutaneous infections.

During the course of infections, ACV-resistant and forsarnet-resistant HSV-1 had been recovered. A human leukocyte antigen (HLA)-matched unrelated bone marrow transplantation (BMT) was performed at the age of 13, resulting in severe HSV-1 infections and progressive multifocal leukoencephalopathy. I have closely observed the clinical course of the HSV-1 infections in this patient and learned the important lessons: treatment, diagnosis, and pathophysiology of HSV-1 infections in an immunocompromised patient. In this presentation, I will show some important messages learned from this patient.

Pathology of Merkel cell polyomavirus in Japanese cases of Merkel cell carcinoma

Harutaka Katano
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Merkel cell carcinoma is a rare malignancy that sometimes occurs in the skin of elderly people. Recently, a new human polyomavirus, Merkel cell polyomavirus (MCV), was identified in Merkel cell carcinoma. MCV DNA was detected in Japanese cases of Merkel cell carcinoma, and we succeeded amplifying a full-length MCV genome from a patient with Kaposi's sarcoma. Immunohistochemistry using a newly developed rabbit polyclonal antibody against large T antigen showed nuclear expression of large T antigen in Merkel cell carcinoma. Nuclear localization may be important for functions of large T antigen. However, many mutations with stop codons have been detected frequently in the large T gene. We identified a nuclear localization signal of large T antigen using deletion mutants. I will show the association of the mutations with nuclear localization in the large T antigen, and the pathogenesis of MCV will be discussed.

Screening and characterization of chemical compounds that inhibit early phase of CMV and VZV infections.

Naoki Inoue, Ph. D., Department of Virology 1

To simplify the detection of infectious human cytomegalovirus (HCMV) and varicella-zoster virus, we generated cell lines that produced luciferase in a dose-dependent manner upon HCMV and VZV infection, respectively (Wang et al., 2006; Fukui et al., 2008). Using these cell lines, we screened a diverse library of 9,600 compounds and identified several anti-HCMV and anti-VZV compounds. We demonstrated that one of the compounds, 1-(3,5-dichloro-4-pyridyl)piperidine-4-carboxamide (DPPC), was effective against HCMV but not VZV, and that the compound targets the very early phase of CMV infection, probably by disrupting a pathway that is important after viral entry but before immediate-early gene expression. We also

analyzed another compound, named 133G4 that inhibited both HCMV and VZV infection. 133G4 inhibited the step of or prior to DNA replication of HCMV and VZV, but not expression of HCMV IE2 and VZV IE62. RT-PCR analyses indicated that expression of HCMV early and late genes was inhibited by 133G4. In transient transfection assay, 133G4 inhibited transactivation function of HCMV IE2 and VZV IE62. DPPC but not 133G4 inhibited growth of murine and guinea pig CMVs. To evaluate antiviral compounds against CMV easily in animal models, we developed an imaging-based evaluation method.

Phenotypic characterization method for HSV and VZV thymidine kinases

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We reported a novel phenotypic characterization method for HSV and VZV thymidine kinases (TK) to screen for acyclovir-resistant virus infection (Suzutani et al. J Clin Microbiol, 2000). This method determines the biochemical phenotype of TK polypeptide, which is synthesized in vitro from viral DNA using a procedure as follow. The TK gene of each sample virus strain is amplified and isolated under the control of a T7 promoter by PCR. The PCR products are transcribed with T7 RNA polymerase and translated in a rabbit reticulocyte lysate. Using this method, enzymatic characteristics and the size of the TK polypeptides encoding HSV and VZV DNA were defined in less than 2 days without virus isolation. The assay should be a powerful tool in monitoring drug-resistant viruses, especially in cases in which virus isolation is difficult.

Endotheliotropic elephant herpesvirus (EIHV-1) was described at 1999 as a novel virus causing fatal hemorrhagic disease of Asian and African elephants in zoo. The virus has not isolated, but partial nucleotide sequence was defined from DNA prepared from infected organs. The DNA sequence data showed the virus codes TK gene in its genome. We plan to study the TK using above methods as co-work with the National Zoo in Washington D.C.

Cellular kinase inhibitors that suppress enterovirus replication have a conserved target in the viral protein 3A similar to that of enviroxime

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Previously, we identified a kinase inhibitor, GW5074, that inhibits poliovirus (PV) and enterovirus 71 (EV71) replication, although its target has remained unknown. To

identify the target of GW5074, we searched for cellular kinase inhibitors that have anti-enterovirus activity similar or related to that of GW5074. With this aim, we performed screenings to identify cellular kinase inhibitors that could cooperatively with GW5074 or synthetically in the absence of GW5074. We identified MEK1/2 inhibitors (SL327 and U0126), an EGFR inhibitor (AG1478) and a phosphatidylinositol 3-kinase (PI3K) inhibitor (wortmannin) as compounds with a cooperative inhibitory effect with GW5074, and an Akt1/2 inhibitor (Akt Inhibitor VIII) as a compound with a synthetic inhibitory effect with MEK1/2 inhibitors and AG1478. Individual treatment with identified kinase inhibitors did not affect PV replication significantly, but combined treatment with MEK1/2 inhibitor, AG1478 and Akt1/2 inhibitor suppressed the replication synthetically. The effect of AG1478 in this synthetic inhibition was compensated with other receptor tyrosine kinase inhibitors (IGF-1R Inhibitor II and Flt3 Inhibitor II). We isolated mutants resistance to Flt3 Inhibitor II and GW5074 and found that these mutants had cross-resistance to each treatment. These mutants had a common mutation in viral protein 3A that results in an amino acid change at amino acid position 70 (Ala to Thr), a mutation that was previously identified in mutants resistant to a potent anti-enterovirus compound, enviroxime. These results suggest that some cellular kinase inhibitors and enviroxime have a conserved target in viral protein 3A to suppress enterovirus replication.

Antiviral drugs for herpes simplex virus, adenovirus and influenza virus: activity and resistance features

Pr Florence MORFIN, PharmD, PhD
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An overview will be presented about data on antiviral drugs used to treat herpes simplex virus (HSV), adenovirus (AdV) and influenza virus infections.
Antiviral drugs, and acyclovir as a first line, have been largely used to treat HSV infections. Resistance has been detected, mainly in immunocompromised patients. But no increase of resistance incidence could be detected in recent epidemiological studies. Genetic characterization of resistant viruses revealed numerous mutations in the thymidine kinase gene and some could be studied by site-directed mutagenesis.

Regarding AdV infections, they are often very serious especially among bone marrow transplant patients and a recent European survey described risk factors associated with this infection. Two antiviral drugs may be used to treat AdV infections: cidofovir and ribavirin. A large phenotypic study was conducted to evaluate *in vitro* susceptibility of reference strains and clinical isolates belonging to all AdV species. Cidofovir revealed to be active on all seroytpes whereas ribavirin was active on a limited number of species.

Influenza virus infections are more and more often treated by neuraminidase inhibitors, especially in the actual context of influenza pandemic. Resistance to neuraminidase inhibitors has been followed up for a few years, staying at a low level in most studies until 2007-2008 winter were oseltamivir-resistant H1N1 strains became predominant in most countries. A close survey of influenza susceptibility has now to be performed in the context of swine H1N1 pandemic.

Morfin教授の発表風景