SCIENTIFIC REPORTS

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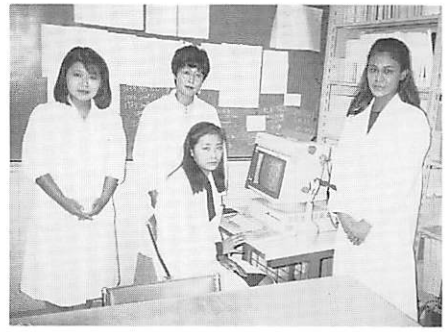
Virology



DEPARTMENT OF VIROLOGY

GENERAL SUMMARY

Recent advances through studies on viral oncogenesis and its related mechanisms in human tumor are far more rapid than anyone might have supposed 5-6 years ago. Those viruses strongly associated genetically or seroepidemiologically with human tumor have been proven at present to be Herpesviridae, Papovaviridae, Hepadnaviridae and Retroviridae. Besides the direct induction of human tumor through these viral infections in target cells or organs, considerable evidence concerning the reactivation or mutation of cellular oncogene (c-onc) related or unrelated to viral oncogene (v-onc) of Retroviridae and subsequent oncogenesis is also presented.

Accordingly, analysis of etiological c-onc in many human tumors seems to be the most interesting results, not only for viral oncogenesis, but also for general cell biological events in life science. It is now becoming the generally approved concept that the action of biologically active substances produced in the cells by activation or mutation of the above c-onc may play an important role in oncogenesis of animals including human, possibly overcoming the immunological surveillance systematized in vivo. In a recent trend in virological research, most experiments are being carried out intensively to make clear how limited amounts of genetic information (one or a few virus genes) can so profoundly alter the growth or characteristic behavior of cells, resulting in the final occurrence of a neoplastic state or cytopathic effect. Those studies on viruses, regardless of whether they are oncogenic or nononcogenic, are now done preferably by in vitro cultured cells, because of their easy analysis at cellular and subcellular levels. Animal infection experiments are usually limited to a particular purpose.

Under these circumstances, our interests are directed toward how to examine the type of virus-cell interactions, and how to use the information to develop possible applications to the problems of human tumor viruses. Among many viruses, even nononcogenic viruses, it is well known that the viral persistent or chronically latent infections are widely spread in humans, sometimes with or usually without any signs of apparent clinical symptoms. Cell oncogenesis may be another type of example to show such a persistent or chronically latent existence and to exhibit a subsequent expression of the respective oncogene. Thereafter, it may often be recognized as an occurrence of some phenotypic alterations of the cells. The importance of this type of viral infection has recently increased in close correlation with many lethal or serious diseases as shown in slow virus infection (Abstract No. 2).

In the case of in vitro transformation of cells as the first step of viral oncogenesis, one of the essential criteria may be a virus-cell interaction with the virus gene's integration into the host cell chromosome or its persistent existence in other types. In addition to the above problems

concerning the viral gene's existence, cellular functions which may sometime control the viral gene's expression or on occasion are kept under control by it appear to be worthy of energetic examination as our most exciting thema. Therefore, our stimulating projects have been directed towards the following subjects for more than 15 years.

1) Cellular and viral factor(s) important for the decision concerning permissive multiplication or persistent and latent existence of Paramyxovirus (HVJ: Sendai Virus and MV: Measles Virus) in tumor cells of neural origin (Abst., No. 1, 2).
2) Characteristic persistent and latent infection of human cells with Herpesviridae, HCMV (human cytomegalovirus) in correlation with possible oncogenesis (Abst., No. 3-6).
3) Unique properties of Herpesviridae, EBV (Epstein-Barr Virus) and its DNA derived from NPC (nasopharyngeal carcinoma)(Abst., No., 7, 8).

Starting from our first examination of the effect of virus persistent infection on tumorigenicity of hamster tumor cells in 1972, the phenomena were analyzed through the induction of strong augmentation of cellular immunity in transplanted syngeneic animals with virus-carrier tumor cells. They were further extended to the problems of both viruses and tumor cells employed. Nononcogenic and low pathogenic mutant viruses such as HVJ, Rubella virus and HCMV, which have exhibited capability of easy establishment of persistent infection, were shown to be available. Not only animal tumor cells, but also human tumor cells were converted to non-tumorigenic ones in nude mice after the persistent infection with these viruses.

During these experiments, however, the most important and difficult problems to overcome were how to more effectively and preferably establish a persistent infection in tumor cells in vivo by an inoculation of mutant virus. For these purposes it seems to be essential to analyze viral or cellular factors required for virus permissive multiplication as shown in the present Abstracts of HVJ, MV and HCMV persistent or latent infection. The expression of persisting virus genes in cells, as well as gene mutation suitable for easy establishment of the persistent infection, should first be examined. From such examinations it may be possible to isolate a unique mutant virus with a preferable tropism to human tumor cells. Genetical analysis of such tropism will be a further interesting topic in the next step. One of those trials is partly presented in the case of EBV persistent infection as shown in Abst., No. 7 and 8.

On the other hand, through long term persistence of the virus gene in human cells some other type of possibility leading to the occurrence of oncogenesis in latently infected cells with some virus such as HCMV also appears to be worthy of examination, because of its extremely high frequency of latent infection in humans. Such an experiment is now going on as one model in human cell oncogenesis by direct infection with potentially oncogenic but usually nononcogenic virus.

These studies (Abst. No. 3-6) were partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

(1) Growth characteristics of Paramyxovirus: HVJ (Sendai Virus) wild type in neural tumor cells—Host-dependent temperature-sensitivity and release inhibition by papaverine, an inhibitor of cAMP phosphodiesterase[1), 2)].

H. Ogura, H. Sato and M. Hatano

The synthesis of M protein of HVJ wild type in rat glioma cells (C6) was selectively reduced at nonpermissive temperature (39°C), in contrast to the other viral proteins such as L, P, HN, F_0 and NP, as already shown in our previous report (J. gen. Virol., 65, 639, 1984). Here, we could add further evidence concerning these phenomena.

First, viral nucleocapside (NC), which consists of L, P and NP proteins, was confirmed to be formed within the infected cells at both permissive (32°C) and nonpermissive (39°C) temperatures. Hemagglutinin and neuraminidase activities expressed by the migration of HN proteins to the cell surface were also detected at 39°C. However, the L, P and NP proteins which are usually associated with the plasma membrane were absent in the membrane of cells incubated at 39°C. These results suggest that NC and glycoproteins synthesized at 39°C could not assemble effectively at the plasma membrane because of a lack of M protein. Moreover, these evidences were not observed in other HVJ susceptible cells. Thus, the host-dependent temperature-sensitive (ts) lesion of HVJ in rat glioma cells was considered to be mainly in M protein synthesis.

Since it is well known that cAMP is present in relatively high levels in cells of neural origin such as C6 cells and is playing an important role in cell differentiation, growth etc., we have examined the effect of cAMP-related agents on HVJ replication in rat glioma C6 cells. In our present case, papaverine, theophyline and caffeine which enhance endogeneous cAMP showed a dose-dependent inhibitory effect, but no significant effect was found in cAMP- or dibutyryl-cAMP-treated C6 cells. Moreover, simultaneous addition of cGMP did not block the effect of papaverine. Since the differentiation of C6 cells is proved to be affected by cAMP or dibutyryl cAMP, the inhibitory effect of papaverine may not be a consequence of increased endogeneous cAMP, but of its direct or other actions. In examinations of cellular events in HVJ-infected C6 cells, both viral genome replication and transcription were suppressed slightly by treatment of the cells with papaverine. In the cells cultured in the presence of papapverine, the synthesis of viral proteins and their phosphorylation occurred at normal rates. Membrane immunofluorescence and cell surface immunoprecipitation showed that the viral glycoproteins HN and F_0 were expressed on the cell surface of the papaverine-treated cells. Moreover, all the viral structural proteins were associated with plasma membrane isolated from the treated cells. These results indicate that papaverine treatment suppresses some part of virus budding at the plasma membrane.

1) Ogura, H., et al., Arch. Virol., 94, 123-133, 1987.
2) Ogura, H., et al., J. gen. Virol., 68, 1143-1159, 1987.

(2) Restricted synthesis of membrane (M) protein and fusion (F) protein of Paramyxovirus: Measles Virus (MV) at elevated temperature (39°C) —Possible etiological role in the establishment of MV persistence in the acute infection[1), 2)].

H. Ogura, K. Baczko, E. K. Rima and V. ter Meulen

Subacute sclerosing panencephalitis (SSPE) provides one of the few examples of slow virus infection in which the etiological agent has been clearly identified as MV. The finding that many patients lack a localized central nervous system humoral immune response to the M protein of MV has focussed attention on it. In particular, the essential role of this protein in budding of the virus and alignment of nucleocapsides with external hemagglutinin (H) and F glycoproteins suggests that a defect in the synthesis or function of this protein could lead a lack of virus maturation and, hence, to persistence. In one SSPE case the accumulation of a readthrough transcript comprising the phospho (P) protein and the M protein gene provides an explanation for the restricted synthesis of the M protein.

Therefore, we have examined the effect of elevated temperature (39°C) usually observed in acute viral infection on the MV replication in persistently infected cells (C6/SSPE). This elevation of culture temperatures resulted in immediate selective inhibition of M protein synthesis which was confirmed by SDS-PAGE of total cytoplasmic lysate and immunoprecipitation with monoclonal antibody against the M protein in short-time labeling experiments. No specific disappearance of the mRNA coding for the M protein was observed when viral RNAs isolated from the infected cells were compared before and after a shift up by Northern blot analysis. Results of pulse-chase experiments did not show any significant difference in M protein stability between 35 and 39°C. This rapid block of M protein synthesis was observed, not only in clones isolated from C6/SSPE cells, but also in VERO, CVI and HeLa cells lytically infected with MV. Poly(A)$^+$ RNAs extracted from C6/SSPE cells before and after a shift to 39°C produced detectable P, nucleocapsid and M proteins in the cell-free translation system at 32°C. These data indicate that M protein synthesis is selectively suppressed at elevated temperature because of an inability of the translation apparatus to interact with the M protein-encoded mRNA.

In addition to the above evidence, elevated temperature was shown to cause a cessation of the F protein synthesis of MV in lytically infected VERO cells. The effect is caused by inhibition of translation of the corresponding mRNA rather than by a decrease in the synthesis or stability of the mRNA or by increased degradation of the F protein at 39°C. From the analysis of distribution of hemagglutinin and F mRNA in polysome the inhibition of F protein synthesis was suggested to be related to a cessation of elongation of the nascent polypetide chain rather than a defect in initiation of the translation of the mRNA at 39°C.

1) Ogura, H., et al., J. Virol., 61, 472-479, 1987.
2) Ogura, H., et al., J. gen. Virol., (in Press), 1987.

(3) Studies on cellular function(s) required for replication of Human Cytomegalovirus (HCMV)[1), 2)].

J. Tanaka, S. Kamiya, T. Ogura and M. Hatano

Replication of HCMV in vitro is supported efficiently only in human diploid fibroblast cells. Infectious progeny virus cannot be synthesized in cells of nonhuman origin, and generally limited amounts of virus are produced in human epithelial cell cultures. These results indicate that the replication of HCMV is dependent on a host cellular function(s). However, little is known about the cellular function(s) required for HCMV replication. Details concerning the nature of those function(s) are extremely important in our understanding of the viral disease, the molecular mechanisms involved in HCMV latency and reactivation, and transformation of cells by HCMV. In an attempt to clarify thier natures, we examined the effect of dimethyl sulfoxide (DMSO), which is known to cause a modulation of cellular functions, on the interaction of HCMV with host cells in a nonproductive state.

The confluent state of a human rhabdomyosarcoma cell line (A204 cells) showed a much lower susceptibility to HCMV when compared to that in subconfluent actively growing cells. Treatment of confluent cultures with DMSO (1-2%), however, converted many nonproductive cells to a productive state for virus replication. Infectious center assay revealed that approximately 100-fold more cells in the compound-treated cultures are able to produce infectious HCMV. The amount of infectious virus produced in DMSO-treated confluent cultures was enhanced by approximately 10,000-fold over production in untreated cultures and recovered to the level of that produced in subconfluent cultures (productive state for virus replication). The block of HCMV replication in confluent cultures seems to occur at the early stages in the HCMV replication cycle, since immediate early virus proteins and antigens were detectable at similar ratios in subconfluent and confluent cultures, whereas HCMV-specific DNA polymerase (an early virus function) was undetectable in confluent cultures. In contrast, in DMSO-treated confluent cultures appreciable amounts of HCMV DNA polymerase, viral DNA, and late virus antigens were synthsized. Pretreatment of confluent cultures with DMSO enabled the cells to support HCMV replication. Moreover, the most effective enhancement by DMSO was found in cultures that had been treated with it up to 5 hr after infection. These results indicate that this enhancing effect is primarily expressed through some host cellular function(s), and the early stages in the virus growth cycle are most likely to be under the control of DMSO action. These comparative studies described should provide useful experimental system for further studies on the cellular factor(s) that enables HCMV to replicate.

1) Tanaka, J., et al., Virology, 146, 165-176, 1985.
2) Tanaka, J., et al., Microbiol. Sciences, 2, 353-358, 1985.

(4) Interaction of Human Cytomegalovirus (HCMV) with cells of nonhuman origin[1), 2)].

S. Kamiya, J. Tanaka, T. Ogura, H. Sato and M. Hatano

Infection of nonhuman cells with HCMV usually results in an abortive infection. However, in these virus-infected non-human cells a number of changes in cell morphology and function are induced. The most attractive observation concerning the interaction between HCMV and nonpermissive animal cells is that hamster embryonic fibroblast cells are malignantly transformed by HCMV infection. Detailed studies on the virus functions expressed in nonpermissive animal cells are important for the understanding of virus replication and transformation by HCMV. In this study, we examined the interaction of HCMV with RK_{13} cells (an epithelial cell line derived from rabbit kidney).

RK_{13} cells exhibited a cytopathic effect characterized by cell rounding early after infection with HCMV. Two immediate early virus proteins with apparent mol. wt. of 72,000 (72K) and 76K were produced in both RK_{13} and human embryonic lung (HEL) cells, fully permissive for HCMV replication, within 3 hr after infection. Interestingly, synthesis of the 76K virus protein was greater than that of the 72K protein in RK_{13} cells, whereas the reverse usually occurs in permissive HEL cells. Moreover, of three early virus proteins which were expressed in productively infected HEL cells, two (88K and 80K) were not detected in abortively infected RK_{13} cells. HCMV-specific DNA polymerase, an another early virus protein, was also undetectable in RK_{13} cells. Neither late virus antigens and proteins nor infectious virus were produced in these animal cells. DNA analysis by isopycnic centrifugation in CsCl revealed that in HCMV-infected RK_{13} cells synthesis of host cellular DNA at 1 or 2 days after infection was enhanced 4- to 5-fold, compared to that in uninfected cells, although synthesis of virus DNA was not detectable. HCMV infection also caused a marked increase in the mitotic activity of RK_{13} cells. When subconfluent cells were infected, more than 20% of the cells demonstrated mitosis at 72 hr after infection. However, the rate of cell growth was considerably reduced when compared to that in uninfected cells. This reduction in the cell growth rate may be due to chromosomal damage caused by HCMV infection, since chromosomal changes such as fragmentation, aberrations, gap, break, deletion etc. were observed in virus-infected RK_{13} cells. The arrest in mitosis of the HCMV-infected cells and subsequent chromosomal changes are suggested to be associated with the altered expression of immediate early or early virus functions in these cells.

1) Kamiya, S., et al., Arch. Virol., 86, 143-150, 1985.
2) Kamiya, S., et al., Arch. Virol., 89, 131-144, 1986.

(5) Establishment and characterization of in vitro Human Cytomegalovirus (HCMV) latency models[1), 2), 3)].

J. Tanaka, T. Ogura, S. Kamiya and M. Hatano

HCMV is capable of establishing both persistent and latent infections after a primary infection in vivo. The latent virus can be reactivated despite a significant level of antibody titer. Many of the diseases caused by HCMV are considered to be associated with reactivation of the virus from a latent state. Moreover, HCMV has been shown to possess the ability to transform human cells in culture after persistent infection. Since the virus has the capacity to establish a long-term interaction with the host, the possibility has been suggested that the latent HCMV may be associated with certain human cancers. Therefore, a better understanding of the various aspects concerning HCMV latency is important for control of virus infection and for examination of oncogenic potential of the latent HCMV. For study of the molecular mechanisms of HCMV latent infection in vivo, we established and characterized in vitro latency model systems using epithelial human cell lines.

When a human thyroid papillary carcinoma cell line (TPC-1) preheated at 40.5°C for 48 hr was infected with HCMV and incubated at a supraoptimal temperature (40.5°C), the cultures could be maintained for at least 65 days without detection of infectious virus. In contrast, when the infected cultures were incubated at 37°C, HCMV persistently infected cultures were established. HCMV was reactivated from the latently infected cultures by reducing the incubation temperature from 40.5°C to 37°C, and the cultures subsequently entered into virus persistent infection. Although HCMV-specific immediate early polypeptides and nuclear antigens were continuously detectable in the majority (more than 95%) of the cells during the latent period, a detectable level of virus-specified DNA polymerase (one of the early virus proteins) was not induced, suggesting that the blockage of HCMV replication in the latently infected cultures occurs at the early stages of the HCMV replication cycle. The latently infected cells were susceptible to superinfection with both homologous and heterologous strains of HCMV. In persistently infected cultures approximately 38% of the cells were lysed by reaction with HCMV immune serum and complement, whereas complement-mediated immune cytolysis could not be detected in the latently infected cultures.

A similar latency model system could also be established in a human ovarian teratocarcinoma cell line by incubation at an infraoptimal temperature (32°C). These in vitro models will provide the system to analyze the state of the HCMV genome and factors controlling its expression during maintenance of the latent state and during virus reactivation.

1) Tanaka, J., et al., Virology, 161, 62-72, 1987.
2) Tanaka, J., et al., Virology, (in press), 1987.
3) Kamiya, S., et al., Arch. Virol., 89, 131-144, 1986.

(6) Human Cytomegalovirus (HCMV) persistent infection in a human central nervous system cell line: production of a variant virus with different growth characteristics[1].

T. Ogura, J. Tanaka, S. Kamiya, H. Sato and M. Hatano

HCMV is the commonest agent causing foetal infection. Congenital HCMV infection causes a wide range of diseases including cytomegalic inclusion disease, congenital defects and interstitial pneumonia. Particularly, in infants whose brain is severely damaged by HCMV infection, symptoms such as microcephalus, epileptic encephalitis, cerebral palsy and optic atrophy are exhibited. Moreover, infants infected congenitally with HCMV asymptomatically have also been shown to exhibit mental retardation, sensorineural hearling loss, low intelligence and deafness. Studies on the response of murine nerve tissue cultures to murine cytomegalovirus infection indicated that all cell types of the nerve tissue showed cytopathic effects with typical cytomegalovirus inclusion and cell fusion. However, little information is available concerning the interaction between HCMV and human central nervous system (CNS) tissue cultures. Therefore, we have examined the susceptibility to HCMV of various cell lines derived from human CNS and the fate of HCMV-infected cultures.

Significant amounts of infectious progeny virus were produced in 118MGC glioma and IMR-32 neuroblastoma, but not in KGC oligodendroglioma cells when the cultures were infected with wild-type virus (HCMV wt) at a multiplicity of 10 plaque-forming unit (PFU) per cell. Further passage of infected 118MGC cells resulted in the establishment of a long-term persistent infection, designated 118MGC/Towne cells. These cells continuously produced infectious virus (HCMVpi) with titers ranging from 10^2 to 10^5 PFU/10^6 cells up to 360 days after infection (corresponding to 50 subcultures). Since no temperature-sensitive mutants, defective interfering particles or interferon-like activity were found in the 118MGC/Towne cultures, maintenance of the persistent infection seemed to be due to a balance between the release of infectious virus and growth of uninfected cells. The HCMVpi produced in long-term persistently infected cultures was shown to be different from the HCMVwt originally used to infect by the following characteristics: (1) HCMVpi replicated slowly and yielded lower amounts of progeny virus than HCMVwt; (2) HCMVpi induced a 73,000 mol. wt. immediate early protein that was not synthesized in HCMVwt-infected cells; (3) HCMVpi had a different DNA structure from that of HCMVwt. These results suggest that HCMVpi is a slower growing variant of HCMVwt and probably plays an important role in the maintenance of the persistent infection. The persistent infection established in these studies (118MGC/Towne cells) will provide a useful experimental model for studies of the interaction of HCMV with the CNS in vivo.

1) Ogura, T., et al., J. gen. Virol., 67, 2605-2616, 1986.

(7) Heterogeneity and DNA analysis of Epstein-Barr Virus (EBV) derived from a nasopharyngeal carcinoma (NPC) epithelial hybrid cell line[1), 2), 3)].

H. Sato, T. Takimoto, H. Ogura, J. Tanaka and M. Hatano

In vitro EBV primarily infects B-lymphocytes, and virus replication has been studied mainly in human and marmoset lymphocytes. However, EBV has also been shown to infect epithelial cells (Glaser, R., 1984). Due to the lack of a permissive tissue culture system for propagation of the virus, only a limited number of isolates have been studied up to the present time. Two strains, the p3HR-1 and the B95-8 virus, which differ in their biological properties, were the most popular ones employed. Apart from the P3HR-1 virus, all other EBV isolates investigated, regardless of their origin, have been found to have transforming potential, but they cannot induce EBV-associated EA (early antigen) upon superinfection of Raji cells (a nonproducer cell line of Burkitt's lymphoma origin).

In NPC tissue, EBV DNA is detected in the malignant epithelial cells. NPC cells (epithelial) grown as explant cell culture can be induced to express viral antigens and produce mature infectious virus particles in some instances. However, biological analysis of the role of EBV in NPC is still hampered because the malignant epithelial cells usually cannot be cultured in vitro, and pathological tissue can be obtained only by biopsy.

Recently, we have established an EBV carrying epithelial NPC hybrid cell line (NPC-KT) by fusing primary NPC epithelial cells with an epithelial cell line derived from human adenoid tissue. This NPC-KT cells highly produce unique EBV (NPC-KT virus), and infection to cord blood lymphocytes (CBL) with low concentrations of NPC-KT virus induced the cells to form EBV-nuclear antigen positive continuous cell lines. However, the CBLs exposed to higher doses of virus showed stimulated DNA synthesis 2 days after infection, followed by cell lysis. EA-induction by NPC-KT virus was stimulated by dimethyl sulfoxide, but that by P3HR-1 virus was inhibited by the drug. EA polypeptides induced by superinfection of Raji cells with NPC-KT and P3HR-1 viruses were undistinguishable by SDS-PAGE. Viral structural proteins produced in NPC-KT and P3HR-1 cells were also undistinguishable by SDS-PAGE. Viral DNA was analyzed in comparison with DNA of B95-8 virus by use of the restriction endonuclease, EcoRI, Hind III and Sal I and separation of fragments in 0.4% agarose. The digestion fragments of these two viral DNAs were obviously similar, but could be distinguished from each other. The molecular weight of DNA of NPC-KT virus was about 120×10^6 daltons, which was about 8×10^6 daltons larger than that of B-95-8 virus DNA, indicating the homogeneity of NPC-KT virus DNA. These data obtained in cloned NPC-KT cells suggest the possibility that a single clone of virus has both transforming and EA-inducing activities.

1) Takimoto, T. et al., JNCI, 74, 57-60, 1985.
2) Sato, H. et al., JNCI, 76, 1019-1024, 1986.
3) Sato, H. et al., Vriology, (in press), 1987.

(8) Establishment of superinfection in epithelial hybrid cells (D98/HR-1, NPC-KT and A2L/AH) with Epstein-Barr Virus (EBV) and its relationship to the C3d receptor as a proposed EBV receptor[1), 2)].

T. Takimoto, H. Sato, H. Ogura, T. Miyawaki and R. Glaser

Earlier studies have suggested that the EBV receptor was closely associated with the complement C3d receptor, a mr. wt. 145,000 membrane glycoprotein. However, D98/HR-1 cells, one of the human EBV genome positive epithelial cells could be superinfected with EBV derived from P3HR-1 (HR-1) cells (lytic nontransforming virus), and these cells were shown to have no detectable C3 receptor. Three different EBV genome positive epithelial/hybrid cell lines (D98/HR-1, NPC-KT and A2L/AH) were compared here in their susceptibilities to superinfection with EBV diffrently derived from the respective HR-1, NPC-KT and B95-8 cells, focussing on a possible detection of the C3 receptor. NPC-KT-EBV have shown both transforming and early antigen (EA)-inducing properties, while B95-8-EBV could only transform B lymphocytes. Demonstration of EBV antigen was observed to be positive after superinfection of D98/HR-1 cells with both HR-1-EBV and NPC-KT-EBV. The NPC-KT hybrid cells prepared by fusing human adenoid epithelial cells (AD-AH) and EBV genome positive NPC (nasophayrngeal carcinoma) explanted epithelial cells were susceptible to superinfection with HR-1-EBV but not with NPC-KT-EBV. The A2L/AH hybrid cells, which were prepared by fusion between AD-AH cells and lymphocytes transformed by B95-8-EBV could not be superinfected with any EBV employed here.

For further investigations, we examined cell surface markers on AD-AH, NPC-KT, A2L/AH and D98/HR-1 cells using monoclonal antibodies as well as by rosette formation. Thus, these three cell lines showed to express the OKB2 antigen, and the common acute lymphoblastic leukemia antigen is observed on the A2L/AH cells. We also found that NPC-KT parental cells and a clone of NPC-KT cells express erythrocyte antibody complement b and d, as determined by rosette formation, but were negative for C3b and C3d when monoclonal antibodies against these two markers were used. The D98/HR-1 cells were also confirmed to be negative for C3b and C3d. The data suggest that the C3d receptor may be a part of the EBV receptor but the C3d receptor, by itself, is not the only receptor to which EBV can bind.

In addition, A2L/AH cells among these three lines should be noted in their unique properties in which synthesis of EA, VCA (viral capsid antigen) and virus DNA or even formation of EBV particles occur by the treatment of the cells with iododeoxyuridine (IudR) as a rescuer. However, their parental A2L (lymphoid cells transformed with B-95-8-EBV) cells were nonproducer cells and were shown to be negative after similar treatment with IudR. The above mentioned virus rescued from the A2L/AH hybrid cells could transform human cord blood lymphocytes but could not induce EA in superinfected Raji cells.

1) Takimoto, T. et al., Cancer Res., 46, 2085-2087, 1986.
2) Takimoto, T. et al., Cancer Res., 46, 2541-2544, 1986.