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ERRATA

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16–17	Photo legend	Florescent-antibody	Fluorescent-antibody
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57	11	evolutinary	evolutionary
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97	4	sharaply	sharply
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Virology

possibility that the persisting virus genomes may interact with latent virus genomes suspected in the cells employed, regardless of what their derivation in tissues or species. If these interactions could be proven as an activation of latent virus or a formation of hybrid virus between latent and persistently infected virus, the existence of endogeneous latent virus in some cells might be shown also from this type of experiment. Some evidence for these possibilities have been presented in the persistent infection with Rubella virus of hamster cells, in addition to inducibility of latent virus by drug treatment or irradiation effect. Furthermore, we cannot overlook the possibility that persistent virus genomes may have effect on normal differentiation of embryonal cells, resulting in the occurrence of anomalies *in vivo*.

From these aspects, we have selected our subjects (I-III) as the most interesting projects described later in detail (Abstract 9–14). Unique problems concerning CMV latent or persistent infection in human cells and its possible activation by the other virus infection such as Coxsackie virus is also one of interesting targets related to our Abstract 11 and 12. They were derived from clinical observations in which a close correlation was found between occurrence of anencephalic fetus and high rate of double infections with CMV and Coxsackie B-5 virus in mothers. These problems are now being studied by Dr. Y. Yabuki's group, Ishikawa Prefectural Central Hospital. Several characteristics observed in the persistent infection may be also analyzed using the temperature-sensitive mutants of viruses (Abstract 9–12).

As a good model for viral carcinogenesis predicted in human, the infection of cultured cells *in vitro* with EBV is now believed to have an important meaning. Much direct or indirect evidence to suggest its etiological role in development of Burkitt's lymphoma and NPC has already been presented up to date. However, infection with EBV and subsequent transformation of human epithelial cells have not yet been confirmed completely, except in a few cases of non lymphatic human cells. Therefore, we have designed experiments to show an establishment of EBV infection in nasopharyngeal epithelial cells and a malignant transformation of infected cells *in vitro* under coworks with Dr. R. Umeda's group (Dept. of Otorhinolary., Kanazawa Univ.) (Sub. IV and Abstract 15). In these experiments, we have also tried a chromosomal analysis of cells transformed by EBV infection, including effects of other virus infections possibly occurring naturally in nasopharyngeal tissues. For these analyses, a procedure employing sister chromatid exchanges (SCE) was introduced as the finest technique in the field of chromosomal analysis. One more attempt in this case exists in the possible demonstration that a true SCE may occur in mild infection capable of establishment of persistent infection in human cells and may result in viral carcinogenesis, even though no evidence on this point has yet been shown (Sub. IV and Abstract 17).

In cooperation with Dr. N. Hattori's group (Dept. of 1st Int. Med., Kanazawa Univ.), in which the clinical significance of Interferon produced by infection with Hepatitis B virus was the first target of investigation, antiviral substances(AVS) have been found in the sera from liver diseases (hepatitis, hepatoma etc.). Starting from an establishment of reproducible assay systems for these AVS, characterization of their inhibitory activities aginst VSV(Vesicular Stomatits Virus) and several other viruses is now in progress. At present, it is clear that these active proteins in the sera completely differ from Interferon, because of their distinguishable action mechanisms. They also show a molecule distinct from IgM or IgG fraction, suggesting a special entity apart from usual viral antibodies which may be contained in some amounts. However, it is not denied at present that a complement system activated by unknown factors in the liver diseases may participate in these inactivations of several viruses (VSV, HVJ, Coxsackie, Pox etc.) under our experimental conditions. Our interests in this AVS originated from clinical evidence which showed a clear correlation between periodical changes of AVS activity and fatal prognosis of liver diseases, especially primary hepatoma. However, their inhibitory mechanisms on several viruses are attractive enough to induce further basic virological studies, as shown in Sub. V and Abstract 17.

Among many usual acute virus diseases, the frequent occurrence of viral respiratory disorders looks most familiar to every person, and may also be important subjects in medical virology apart from possible problems of viral carcinogenesis in human. There is some statistic evidence that the frequency of suffering from "colds" including "flu" in every person, regardless of age and sex, may be at least four or more times a year. The etiological agents responsible for these diseases are now believed to be several virus groups consisting of Myxo-, Paramyxo-, Corona-, Entero-, Adenovirus group etc., together with other microbiological agents such as Mycoplasma, pathogenic Cocci etc.. Moreover, it is also reasonably concluded that incidence of respiratory diseases caused by the above virus infections may be three times higher than those from bacterial or mycoplasma infection. This background concerning viral etiology of respiratory diseases makes us undertake the basic studies connected with problems in public health. In our coworks with Dr. N. Kimura's group, Ishikawa Hygiene and Public Health Institute, isolation of responsible viruses and characterization or identification of isolated viruses using cultured cells are now going on as shown in Sub. VI and Abstract 18.

These studies especially concerned Sub. I and II were supported by a Grant-in-Aid for Cancer Research and a Grant-in-Aid for Scientific Research, from the Ministry of Education, Science and Culture, Japan.

ABSTRACT

(9) Antigenic conversion of tumor cell membrane xenogenized by persistent infection with nononcogenic viruses (HVJ=Sendai virus or Rubella virus).

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

It is well known that tumor cells usually show a poor antigenicity *in vivo* or *in vitro*. Therefore, various trials to enhance tumor specific antigencity have been described to date and they consist of viral infection, treatment with chemicals, cell-fusion techniques etc.. Since our first report on the lowered transplantability of tumor cells persistently infected with HVJ (Hemagglutinating Virus of Japan), we have been engaged in the xenogenization of tumor cell membrane by persitent infection with nononcogenic viruses. In the case of HVJ persistently infected cells (HVJ carrier cells), their decreased transplantability was clearly shown to be due to the enhanced cellular immune response in hamster transplanted with the cells. However, the carrier cells lacked the viral CPE and showed a non -retarded growth in culture. These enhanced cellular immune responses which were proven by the migration inhibition test and cell-mediated cytotoxicity test were significantly higher and more durable than those by the inoculation of non-carrier tumor cells. In this analysis, the HVJts (temperature-sensitive mutant) genomes carried in the cells played an important role in the antigenic conversion of cell membrane, enhancing cellular immune response *in vivo*.

Since all these experiments were done under an allogeneic system, the ones in a syngeneic system seemed to be essential for further analysis of immune response. Therefore, we have recently established syngeneic tumor cells (GEN10 and GM2) from inbred hamsters, GN strain. In a test to see the virus infection effect on their transplantabilities, these cells showed similar phenomena to those in the allogeneic system (THEL). Moreover, the persistent infection with Rubella virus also revealed the lowered effect (Table). We are now looking forward to carrying out further analysis of immune response in the new system and to obtaining an applicable approach through these procedures to human cancer in the future.

Table Tumorigenicity of HVJts- or Rubella-carrier cells.

cells		tumor incidence at 8 w	latent period (days)	mean size ± s. d. (mm) at 8 w
THEL	10^4	14/14	10–18	43.5 ± 10.2
THEL-HVJts	per n. i.	6/14	28–42	8.6 ± 4.3
THEL-Rub	GH	2/11	23–28	5.8
GEN10	10^6	3/3	7	11.2 ± 0.6
GEN10-HVJts	per i.	2/4	7	1.5
GEN10-Rub	GNH	1/8	21	3.8
GM2	10^6	4/4	7	49.8 ± 5.6
GM2-HVJts	per i.	0/4	/	/
GM2-Rub	GNH	1/3	7	21.0

n. i. GH : non-inbred golden hamster, i. GNH : inbred golden hamster, GN strain.

(10) Enhancing Effect of persistent infection with Paramyxovirus (HVJ) as one expression of persisting HVJts genomes on the formation of CPV (Cow pox virus) specific cell surface antigen.

J. Tanaka, H. Ogura and M. Hatano

In an attempt to apply the cell surface antigen (S-ag) produced by CPV as the xenogenized tumor cell membrane antigen, this project was introduced. However, the CPV S-ag formation did not occur to any great extent in some tumor cells. This trouble was resolved by either an introduction of trypsin treatment of the cells or an establishment of HVJts persistent infection in the cells. In these studies, we have been interested in the mechanisms governing the above phenomenon especially in the case of carrier cells. At first, it became clear that this effect absolutely requires an expression of HVJts genomes which were clearly proven by experiments in which the culture temperature of the carrier cells was shifted up and down (39°C and 32°C, non-permissive and permissive temperature for HVJts). In comparison with intracellular growth of CPV, eclipse phase and one step growth in the carrier cells occurred within much shorter period, while the CPV adsorption showed no difference from those on parent cells. Further analysis proved that some protease activity significantly increases in the carrier cells, suggesting a probable responsible factor for acceleration of the CPV uncoating process. Therefore, ^{3}H-labeled CPV infected carrier and parent cells were fractionated by sucrose gradient centrifugation, employing ^{14}C-CPV as a marker intact virus. Fig. shows that ^{3}H-CPV infected into carrier cells appeared in the lighter fraction early within 2–3 hours after the infection, while it presented a delayed profile in parent cells. These results seemed to indicate an earlier occurrence of lighter CPV particles which were partially and more rapidly uncoated in the carrier cells. Similar analysis of ^{3}H-CPV reacted with crude cell extracts from carrier cells *in vitro* also manifested more rapid degradation of CPV. From these results, we have now concluded that HVJts-carrier cells acquired an accelerated uncoating activity, which may be greatly due to an increased protease activity, resulting in the enhanced formation of CPV S-ag in the carrier cells.

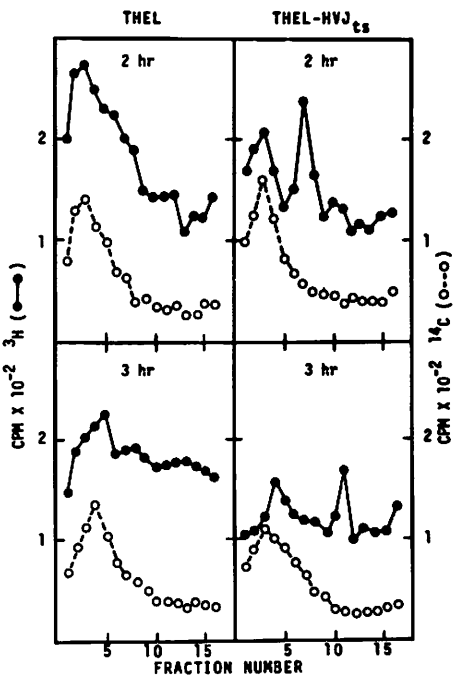

Fig. Sucrose gradient centrifugation of CPV infected to THEL and its HVJts-carrier cells.

(11) Effect of lytic or persistent infection with HVJ or Rubella virus on a possible activation or production of latent endogeneous C type RNA virus.

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

Following recent progress in the studies of latent endogeneous C type RNA virus, its etiological role and significance have grown as topics in human cancer. In view of this present status, our interests are directed toward a possible activation of latent virus by other nononcogenic virus infection. Some papers have described an activation of murine latent C type RNA virus by infection with irradiated Herpes virus[1], or by lytic or persistent infection with Rubella virus[2]. It is also well known that these latent viruses are inducible by the treatment of cells with drug (IUDR, chemical carcinogen etc.) or by irradiation of the cells. In the case of activation or induction by other virus infection, it may be possible to propose some kind of hybrid virus formation between latent and infected viruses, if there exists some homology between the nucleic acid of these two viruses. This possible formation of hybrid viruses seems to be of a completely different character and to have more attractive meanings than those produced by drugs or irradiation effects. In our preliminary experiments connected with the decreased transplantability of HVJ_{ts}- or Rubella-carrier tumor cells, HVJ_{ts}-carrier hamster cells were shown to be negative in a clear activation of latent C type RNA virus assayed by a measurement of reverse transcriptase(RT). Studies on the effect of Rubella virus infection are now in progress. On the other hand, we have now started an examination of optimal conditions for production of endogeneous viruses from spontaneously producing mouse tumor cells(MC) and human cells(A204 cells persitently infected with baboon endogenous virus). The effect of HVJ_{ts} or Rubella virus infection are also programmed in our next trials. In the case of MC cells, the oncogenicity of these tumor cells persistently infected with the above two viruses will be tested in a syngeneic system. One more possibility that hydrid virus formation between Rubella and endogeneous viruses in human cells including A204 cells may occur is also worthy of investigation. If this type hybrid virus could be obtained, it would also be promising to apply it to our projects in Abstract 12 and it may contribute greatly to the prospective problems of human endogeneous latent virus.

1) Hamper, B., Aaronson, S. A., Derge, J. G., Chakrabarty, M., Showalter, S. D. & Dunn, C. Y., Proc. Natl. Acad. Sci. U.S., 73, 646–650, (1976).
2) Sato, M., Yamada, T., Yamamoto, K. & Yamamoto, N., Virology, 69, 691–699, (1976).

(12) Epidemiologic studies on teratogenic effect of Rubella virus infection in pregnant women by measurement of Rubella variant virus antibodies.

K. Sugiura, A. Asamoto, Y. Yabuki, H. Onishi and M. Hatano

It is well known that Rubella virus infection within an early gestation period results in the occurrence of serious anomalies in the fetus. Sato and Yamamoto (1976) described the isolation of hybrid Rubella virus (mpl virus) between Rubella virus and latent virus from the hamster cells (mpl cells) persistently infected with Rubella virus. This mpl virus shows antigenicity of both Rubella and latent oncorna viruses, and also exhibits reverse transcriptase activity. With this evidence, we have examined the relationship between the incidence of anomalie fetus and mpl (variant virus=V) antibodies (ab) in mothers during recent Rubella epidemic in 1975–1977 in Japan. Table shows the significance of the main results obtained. It is surprising that there is a clearer correlation between V-ab titers and occurrence of anomalies than that examined by usual measurements of W(wild virus)-ab. Anomalies found in our cases mainly consisted of omphalocele, anophthalmia, hydrocephalia, heart diseases, intrauterine death of fetus etc.. Starting from these observations, the distribution of V- and W-ab in asymptomatic (inapparent or uninfected) or apparent Rubella infection groups, both of which consisted of pregnant women, nonpregnant adult women, adult men and children was exanimed. Among these, the pregnant women's group apparently infected with Rubella virus showed significantly high population rate (ca. 30%) exhibiting V-ab higher than 1 : 128, while low in children's group even after the apparent infection (ca. 5%). In usual healthy adults, the population rates showing the above high V-ab titers is within 10%. In searches for other diseases with high V-ab, regardless of Rubella virus infection, breast cancer, lymphatic leukemia, liver diseases (decompensated cirrhosis) etc. were found. This evidence appears to indicate the usefulness of concomitant V-ab measurements in Rubella virus infection, especially in pregnant women, with the usual W-ab ones, and in some human diseases. However, it still remains to be made clear at present why mpl variant virus antigen can react with human antibodies or what role this V-virus plays in the induction of anomalie fetus or in the above diseases.

Table Measurements of wild(W) and variant(V) Rubllea virus antibody in relation to occurrence of abnormal fetus.

1) Rubella virus infected group during pregnancy.
(63 cases=W-ab higher than 1 : 128)

	normal birth	abnormal incidence
cases	43(68.3%)	20(31.7%)
V-ab≧1 : 128	2(3.1%)	10(15.7%)
	(2/43 : 4.6%)	(10/20 : 50%)

2) total cases= 119 examined both W- and V-ab
cases with V-ab alone higher than 1 : 128 --- 19/119(16.0%)
(normal birth : 5/19 (26.3%))
(abnormal incidence : 14/19 (73.7%))
cases with both W- and V-ab higher than 1 : 128 --- 12/119 (10.1%)
(normal birth : 2/12 (16.6%))
(abnormal incidence : 10/12 (83.3%))

(13) Regional high incidence of anencephaly and its epidemiologic and etiological observation from double infections with CMV (Cytomegalovirus) and Coxsackie B virus.

Y. Yabuki, R. Matsui, N. Kimura, J. Tanaka and M. Hatano

The frequency of latent infection with CMV in humans is high and possible activation of this occult virus is now believed to occur in some status such as pregnancy, delivery, transplantation of kidney, blood transfusion etc.. However, the mechanisms of how to activate this latent CMV in the human still remain unknown. Fortunately, we have encountered some clinical observations which may be used to explore the above possible activation in the human. First, for the past two years, 9 anencephalies were observed in Nanao General Public Hospital. This abnormal high incidence made us follow up epidemiological investigations of anencephaly in Nanao and in the surrounding area. In the total 78 cases of anencephaly in this area during an additonal five years (1971–1975), three times repeated anencephalic births and one concomitant anencephaly with twins may be noted. It is also remarkable evidence that the incidence ratio of anencephaly (ten per 2456 births, 0.4%) during the same five years in the Nanao area was clearly four or five times higher than usual ratio. Some suspected causative factors which we were able to follow up were as follows; 3 cases of marriage betweem cousins, 8 of genital bleeding, 9 of hyperemesis, 9 of common colds and 16 cases in which the individual received some kind of drug in the first trimester of pregnancy. In order to investigate the possibility that some virus infections may account for the occurrence of anencephaly, 29 kinds of virus antibodies have been examined in the anencephalic newborns, and their mothers's sera obtained. As shown in Table, the patients with anencephaly showed high incidence of double infection with both CMV and Coxsackie B type viruses, in contrast to those of normal delivery. However, other virus antibodies such as influenza, measles, rubella, herpes etc. were not significant, when compared with those of normal delivery. Therefore, we are now assuming that a possible activation of latent CMV may occur by other virus infections such as Coxsackie virus, and may bring about an occurrence of this abnormal fetus. With this in mind, we are also preparing to carry out experiments such as establishment of CMV persistent infection *in vitro* and induction of CMV by the other virus infection.

Table Distribution of complement-fixing(CF) antibodies against CMV and Coxsackie B virus in 13 patients with anencephaly compared with that in 40 womens with normal delivery.

virus ag	CF-ab positive* case (%)	
	normal (40 cases)	anencephaly (13 cases)
CMV	52.5 (21/40)	92.3 (12/13)
Cox-B 4	12.5 (5/40)	84.6 (11/13)
Cox-B 5	15.0 (6/40)	84.6 (11/13)
CMV + Cox-B 4 and 5	5.0 (2/40)	76.9 (10/13)

* higher than 1 : 4

(14) Growth factor released from virus-transformed or virus-persistently infected cells*.

J. Tanaka and N. Yamamoto

Serum for culturing cells is usually essential for the growth of diploid primary culture and non-transformed cells. Although a reduced serum requirement for the growth has been considered to be a property of the transformation state, it also shows the capability of the transformed cells to survive even in such a circumstance as limited serum. If we could culture transformed cells in a serum-free synthetic medium, several advantages in research for detection of a growth factor released from transformed cells, analysis of the nature of tumor-specific antigen, effect of chemicals including growth hormones without serum modification etc. would be much more promising. In order to demonstrate the production of a growth factor we first cultured 3T3 and SV3T3 (3T3 cells transformed by SV40) cells in a serum-free synthetic medium. The addition of conditioned medium (CM) collected from SV3T3 cells cultured in serum-free medium resulted in a stimulation of DNA synthesis and cell division of both 3T3 and SV3T3 cells (Table). For their maximun stimulation, 3T3 cells required an addition of more than 50% CM in fresh medium, whereas SV40-transformed 3T3 cells showed smaller amounts of CM (12.5%). Complete substitution with CM (100%) brought about an inhibitory effect on both DNA synthesis and cell divisions of SV3T3 cells. The growth of 3T3 cells in the above 100% CM was further accelerated by the addition of insulin which is known as one of the growth factors in serum. However, in the case of SV3T3 cells, some proteolytic enzyme inhibitors inhibited its growth. In the examination of the chemical nature of this growth factor, it was revealed to be a non-dialyzable and heat-labile macromolecule, probably a proteolytic enzyme. A similar growth factor was also detected in the conditioned medium from BHK-21 cells transformed by Rous sarcoma virus. We are now examining a possible growth factor released from cell cultures persistently infected with nononcogenic virus, HVJ or Rubella virus.

Table Effect of CM on DNA synthesis and cell division of 3T3 and SV3T3 cells.

% of CM in DME-TPB	DNA synthesis[a] (cpm/plate)		cell numbers[b] /plate ($\times 10^4$)	
	3T3	SV3T3	3T3	SV3T3
0	9,120	12,450	4.5	1.4
12.5	–	23,650	12.0	8.7
25.0	16,125	22,815	16.1	13.1
50.0	21,119	12,450	20.8	6.6
100	20,820	2,203	23.8	0.8

3.5×10^5 3T3 and 5×10^4 SV3T3 cells per Petri dish were plated in DME-TPB (Dulbecco's modified Eagle's medium plus tryptose phosphate broth). Twenty four hours later the medium was changed to DME-TPB containing various amounts of CM.

a. ^{3}H-dTR incorporation at 16 hrs after medium change.

b. at 48 hrs after medium change.

* This work was done at Fels Research Institute, Temple University, U.S.A. under the direction of Dr. N. Yamamoto.

(15) Experimental sutdies on viral etiology of Epstein-Barr virus (EBV) in nasopharygeal carcinoma (NPC): Establishment of EBV infection in human epithelial cells derived from nasopharyngeal tissues.

T. Takimoto, K. Morishita, M. Furukawa, R. Umeda and M. Hatano

First, during experimental reconfirmation of EBV genomes existing in NPC, we also found EBNA (EBV associated nuclear antigen) in 5–90% cells of 4/8 biopsy preparations examined. Nine tissues of 13 NPC biopsy specimens were cultured *in vitro* and among them 2/3 epithelial cells were shown to be EBNA-positive. In fibroblast-like cells from one case, floating cells were established spontaneously without EBV infection. These cells showed B lymphocyte markers and EBNA in more than 90% of the cells. EBNA-negative lymphocytes from cord-blood, adult peripheral blood and resected tonsilla were transformed *in vitro* by EBV (B-95-8 strain) infection, showing EBNA and lymphocyte markers. In contrast to lymphocytes, 7 human monolayered cells employed here did not show the above transformation. However, 3 floating cells (A1L, A2L and A3L) appeared about one month later in 6 nasopharyngeal fibroblast-like cells inoculated with EBV. These 3 established lines also showed EBNA and B lymphocyte markers. In nasopharyngeal epithelial cells (2-27-Ad) fused with EBNA -negative lymphocytes by UVed HVJ and then infected with EBV, EBNA was first observed only in the nucleus of polykaryons formed. From the second day after EBV infection, EBNA appeared in both nuclei of lymphocytes and 2-27 Ad cells of polykaryons, indicating an EBV infection in the epithelial cells. Moreover, the induction of early nuclear antigen (ENA) was possible by IUDR (70 ug/ml) treatment of the fused cells 3 days after EBV infection. This treatmemt also made the cells induce early antigen (EA) until the 4th day. Using 2-27 -Ad cells, the mutant cells (Ad-AH) defective in enzyme, HGPRT (hypoxanthine-guanine phosphorybosyl transferase), were selected after treatment of the cells with 8-aza-guanine. These Ad-AH cells were fused with EBNA- and HGPRT-positive A2L cells by UVed HVJ and then cultured with HAT (hypoxanthine-aminopterin-thymidine) medium. Ad-AH/A2L hybrid cells under these conditions could grow well, but other non-fused Ad-AH or floating A2L cells were eliminated. These somatic cell hybrids have been maintained for about 6 months with positive EBNA in more than 90% of the cells (Photo), showing a good model for the study of NPC *in vitro*.

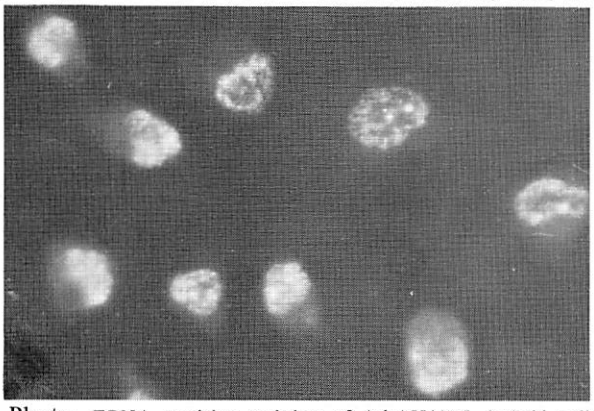

Photo. EBNA positive staining of Ad-AH/A2L hybrid cells maintained in the HAT medium for 6 months (X100, fluorescence-antibody staining).

(16) Effect of DNA(Adeno and EB) or RNA(HVJ, Rubella and Coxsackie) virus infection on sister chromatid exchange (SCE) and chromosomal aberrations in lymphoblastoid cells from adenoid -tonsillar tissues.

M. Kase, M. Furukawa, R. Umeda and M. Hatano

In general virus infections in human, the first gate of virus invasion usually differs greatly. Among them, oral, nasopharyngeal or the upper respiratory route are often observed naturally in many cases such as Myxo-, Paramyxo-, Rubella, Entero-, Adeno viruses etc.. Probably, the first target cells for these viruses may be epithelial or endothelial cells of respective tissues. However, lymphatic tissues may also participate in the role of target cells, because they appear to be rich in these areas. On the other hand, many animal viruses have been shown capable of inducing chromosomal lesions in mammalian cells. Many procedures to examine chromosomal lesions have been presented to date, but the ones using observation of SCE after BUDR treatment are believed to have many advantages over the others. Therefore, this method is now often being introduced for the analysis of delicate chromosomal lesions induced by chemicals, irradiation etc.. In view of these recent development and a few reports on SCE occurrence by virus infection, either the lytic or the persistent one, we have examined the possible induction of SCE by viruses concerned with the nasopharyngeal infection route. Tonsillar lymphocytes established and transformed by EBV infection were first observed to be negligible alteration in SCE from those of primary normal ones uninfected. Using these cells(Ton-E), the resluts obtained by superinfection with Adeno type 5 virus are shown in Table. SCE/cell in this infection was not significantly altered from the control(uninfected with Adeno 5 virus), showing no effect of this virus infection. However, chromosomal aberrations mainly consisting of breaks in chromosomes occurred significantly and recovered to control those in the advance of infection periods. Similar results were also obtained in the case of infection with HVJ, Coxsackie etc.. The meaning of these results will be discussed after further analysis for comparison with the ones in persistent infection or other mutagens(chemicals and irradiations).

Table Frequencies of SCE and chromosonal aberrations in human tonsillar lymphocytes(Ton-E) transformed by EBV after superinfection with Adenovirus type 5. (moi: 1.0, BUDR 10μg/ml for 48 hrs before harvest)

hrs after infection	SCE/cell (mean ± s. d)	% cells with aberrations	breaks/cell
8 hrs	7.45 ± 0.28	43	1.21
24	7.93 ± 0.47	21	0.45
60	7.36 ± 0.46	13	0.17
108	7.06 ± 0.32	10	0.10
156	6.96 ± 0.22	7	0.07
control (uninfected)	6.88 ± 0.24	3	0.03

Each figure was determined on the basis of 30 metaphases.

(17) Studies on AVS (antiviral substances) found in the sera of liver diseases (hepatitis, cirrhosis, hepatoma etc.).

K. Tanaka, T. Nakagawa, T. Ikeda, Y. Kato, N. Hattori, H. Sato and M. Hatano

In the course of studies on Interferon (IF) in various liver diseases, AVS differing from IF activity was found in high level in hepatitis etc., while normal healthy persons showed lower levels. As one of the distinguishing characters of AVS, procedures such as pretreatment of cells which appear to be essential to IF activity were not needed for AVS determination. AVS also exhibited its activity even in non-human cells (mouse or monkey), showing non-species specificity which seems to be a different property from IF. Reproducible assay procedures have been established by both the INAS (inhibition of nucleic acid synthesis 50%) method which was developed for IF assay and has been modified by us, and the plaque reduction method employing VSV (vesicular stomatitis virus) and several other viruses (HVJ, Coxsackie B-5, Poxvirus etc.). Therefore, this AVS activity may be a non-specific one for the viruses employed, exhibiting some difference from specific virus antibodies in immunological meaning. The other characteristics found in this AVS were heat-lability (56°C, 30 min.), pH resistance (pH 2.0, 24 hrs) and distinct molecules from IgM or IgG in gel filtration. The titer of AVS against VSV in primary hepato-cellular carcinoma and gastric cancer with liver or lymphnode metastasis showed a result two or more times higher than that in healthy persons (Table). Concerning the serum proteins that resembled our AVS, a heat-labile serum factor (HLI) which has virus-neutralizing or -inhibiting activity has been reported with a wide range of viruses such as Myxo-, Paramyxo-, Poxviruses etc.. Though immunological significance of such facrors had been suggested, the true entity of their character still remains to be clarified. In the case of HLI, it was shown to be produced in healthy animals by usual virus immunization. Assuming protein synthesis in liver, production of proteins like AVS as a result of modification of proteins by disordered liver cells may be possible, showing no virus-specificity immunologically. Furthermore, in the case of more advanced stages of hepatoma etc., the above production or modification of AVS proteins seems to be repressed, because AVS in these stages of liver diseases was usually observed to be lowered. In addition to these considerations, a possible participation of complement system activated by unknown factors is not denied and should be determined in the near future.

Table Antiviral substance (AVS) in malignant tumors.
(mean units ± s. d. by INAS 50 against VSV)

normal adults (36)*	gastric cancer (41)*	lung cancer (15)*	hepatocellular carcinoma (13)*
243±112.4	567.2±439.4	437.1±352.7	395.5±261.1

* cases

(18) Isolation and characterization of etiological viruses in respeiratory diseases using cultured cells.

N. Kimura, T. Kaji, H. Onishi and M. Hatano

This project seems to be important not only from the point of view of public health but also from the standpoint of basic virology, because there is a prospect of suggestive evidence concerning viral mutation in the human. In order to isolate viruses responsible for respiratory diseases under conditions which resemble as closely as possible those in the human, the employment of tissue cultured cells(Vero=cell line derived from African green monkey kidney, AGMK=primary African green monkey kidney cells) has been recently introduced (Table). In the case of Vero cells, the supplement with diluted trypsin to the medium (VT system) is essentially required. This supplement is due to the evidence that trypsin makes some Paramyxovirus cleave its coat protein, resulting in complete growth of virus in the cells.

One more big problem may be influenza virus which usually becomes a topic every year, regardless of the scale of its epidemic. We have also tried to isolate this virus from this area's epidemics for the past 3 years (1975 −1977). In these experiments, MDCK(derived from canine kidney) cells supplemented with dilute trypsin(MT) have been introduced as a new system together with AGMK, VT and developing chick embryos(10dE). In a comparison of these systems, the MT system clearly showed several advantages such as higher isolation rate(ca. 30% from 373 specimens), earlier confirmation of isolation within 3 days, higher susceptibility to samples containing a few viruses etc.. The antigenicity of virus isolated in the MT system usually did not differ from that in the 10dE system which has been used as host cells for vaccine production. However, we have encountered an interesting virus strain(B/Ishikawa/1/76) by this MT system. This MT-isolated and -passaged variant virus showed a mosaic-like antigenicity which reacted differently with antibodies produced by 10dE-passaged variant virus(heterogenic reactivity) compared to those by homologous variant virus(MT-passaged variant virus). Moreover, the homologous antigenicity was rapidly lost and converted to a heterogenic one, when MT -passaged virus was replicated in 10dE or in the M(MDCK cells without supplement of trypsin) system. This means that some influenza virus from human has the ability to change its antigenicity easily depending on the host cells. It is also noted that it became possible to observe this evidence first only by the employment of susceptible cultured cells.

Table Isolation of responsible viruses from respiratory patients in VT- and AGMK -system during 1972–1976.

Period	No. of specimens	No. of isolated viruses in VT (AGMK)				
		Paramyxo	Influ.	Adeno.	Entero.	NI.*
1972	179	10(0)	-	9(1)	1(3)	5(3)
1973	412	10(6)	2(5)	26(2)	9(7)	0(6)
1974	498	21(8)	-	5(2)	5(26)	-
1975	487	31(14)	0(8)	19(4)	7(10)	0(9)
1976	344	4(0)	0(1)	15(5)	2(2)	6(17)
total	1920	66(28)	2(14)	74(18)	23(48)	11(17)

* not identified