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SHORT COMMUNICATION

LACK OF EXPRESSIONS OF ENDOGENOUS (gs, CHICK HELPER FACTOR) AND EXOGENOUS AVIAN RNA TUMOR VIRUSES IN THE MOB-1 AND MSB-1 LINES DERIVED FROM MAREK'S DISEASE LYMPHOMAS

KAZUYOSHI IKUTA and SHIRO KATO

Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

TADASHI ANDO and TAKEO KONOBE[1]

Department of Tumor Viruses, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

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Herpesviruses have been established to be the etiologic agents of some lymphomas (Churchill and Biggs, 1968; Witter et al., 1969; Melendez et al., 1969) and to be closely associated with some human tumors (Epstein et al., 1964; zur Hausen, 1970). Burkitt's lymphoma has been closely linked by seroepidemiological studies (Wright, 1967; Henle et al., 1969; Gunven et al., 1970), electron microscopic examination (Epstein et al., 1964) and molecular hybridization (zur Hausen et al., 1970) to the Epstein-Barr herpesvirus. Moreover, Burkitt's tumors have been reported to contain RNA related to the RNA of murine leukemia virus (Kufe et al., 1973), and particles that form a band in sucrose gradients at 1.16–1.19 g/cm^3 and have both 60–70S RNA and reverse transcriptase (Kufe et al., 1973). Peters et al. (1973) and Frankel et al. (1974) reported that development of gross Marek's disease (MD) tumors and death of MD virus (MDV) infected LSI–SPF chickens only occured on concurrent infection with avian leukosis virus (ALV) such as Rous-associated virus (RAV)-2. These data suggest the possible implication of RNA tumor virus in the oncogenesis of these oncogenic herpesviruses. On the other hand Witter et al. (1975) claimed that chickens free of exogenous ALV infection, replicating endogenous ALV (RAV–O), gs antigen and/or chick helper factors (chf) were fully susceptible to induction of MD by MDV alone. Thus, exogenous ALV infection at least does not appear to be a requisite for MDV-induced oncogenesis in vivo. Two MD lymphoma cell lines have been established and designated MOB-1 and MSB-1, respectively (Akiyama et al., 1973; Akiyama and Kato, 1974). These line cells were proved to be closely associated with MDV. However, no evidence was found for association of ALV with these line cells in complement-fixation for ALV (COFAL) and resist-

1 Present address: Kanonji Institute, The Research Foundation for Microbial Diseases of Osaka University, Kanonji, Kagawa.

ance-inducing factor (RIF) tests or by electron microscopic examination for C particles in thin sections of samples of the line cells, although the gs test on MOB-1 cells was ±. A third MD lymphoma cell line, the MOB-2 line, has recently been established and found to be dually infected with MDV and exogenous ALV (Ikuta et al., 1976). The dual infection of this cell line prompted us to reinvestigate the possibility of association of ALV with the MOB-1 and MSB-1 cell lines.

The present work confirmed the lack of evidence for the expressions of endogenous (gs, chf) and exogenous avian RNA tumor viruses, in MOB-1 and MSB-1 line cells.

MOB-2 line cells were used as a positive controls. All three line cells were cultured as described previously (Akiyama and Kato, 1974; Ikuta et al., 1976).

Complement fixation assay of gs antigen of avian leukosis-sarcoma viruses, was performed on samples of homogenized cells. Anti-gs serum was obtained from pigeons in which wing web tumors induced by the Schmidt-Ruppin strain of RSV, subgroup D, had regressed. The MOB-2 line gave a positive result in this test but the MOB-1 and MSB-1 lines both gave negative results even though previously (Akiyama and Kato, 1974) the MOB-1 line had given a ± result.

The chf assay was performed as descrived by Weiss et al. (1973) with a minor modification. The MD cell lines to be tested were seeded at concentrations of 4×10^5 cells per 60-mm Petri dish in PRMI-1640 supplemented with 10% fetal calf serum and 1 μg/ml of Fungizone or Medium 199 supplemented with 10% tryptose phosphate broth, 5% calf serum, 2% bicarbonate, 1 μg/ml of Fungizone and 1% chick serum. Immediately after seeding, the cells were infected with 1×10^5 focus-forming units of Bryan high titer strain (BH) of RSV (RAV-7) both in the presence and absence of 2 μg/ml of polybrene. BH-RSV (RAV-7) was propagated on gs (−), chf (−), C/BE cells once from the original stock kindly given by Dr. P. K. Vogt. The culture fluids, harvested on day 5, were centrifuged at 3,000

TABLE 1. *Chf test on three MD cell lines*

Assayed on			Q/BC[a]		C/BE[b]
Inoculum size			1 ml	0.1 ml	0.1 ml
Cell line	Medium	Polybrene	Focus forming units/60 mm petri dish		
MOB-1	RPMI[c]	+	0	0	1.1×10^3
		−	0	0	1.3×10^2
	M. 199[d]	+	0	0	8.9×10^2
		−	0	0	1.5×10^2
MSB-1	RPMI[c]	+	0	0	2.4×10^2
		−	0	0	1.3×10^2
	M. 199[d]	+	0	0	5.5×10^2
		−	0	0	1.2×10^3
MOB-2	RPMI[c]	+	2.7×10^3	5.4×10^2	1.3×10^3
		−	2.3×10^3	7.5×10^2	2.4×10^3

[a] Quail cells resistant to subgroups B and C of avian leukosis-sarcoma virus.
[b] Chicken cells resistant to subgroups B and E of avian leukosis-sarcoma virus.
[c] RPMI-1640 supplemented with 10% fetal calf serum and 1 μg/ml of Fungizone.
[d] Medium 199 supplemented with 10% tryptose phosphate broth, 5% calf serum, 2% bicarbonate, 1 μg/ml of Fungizone and 1% chick serum.

rpm for 10 min and then the supernatants were assayed for focus-forming units on C/BE as well as on Q/BC. As shown in Table 1, the MOB-1 and MSB-1 lines both gave negative results in the chf test, while the MOB-2 line which released ALV, subgroup A, gave a positive reaction.

For reverse transcriptase assay, culture fluids were collected from suspension cultures of the MOB-1 and MSB-1 lines, and from quail embryo fibroblasts (QEF) and chick embryo fibroblasts (CEF) co-cultivated with MOB-1, MSB-1, or MOB-2 cells and transferred once or twice at 3- to 4-day intervals. The cultures were centrifuged at 2,000 rpm for 10 min, and then the culture fluids were centrifuged at 22,000 rpm for 120 min and the latter precipitates were resuspended in 1/100 volume of standard buffer [0.1 M NaCl, 0.001 M EDTA, 0.01 M tris (hydroxymethyl) amino methane-HCl, pH 7.4]. The activity of reverse transcriptase of these samples was measured with exogenous templates using the DNA polymerase assay system of Haase et al. (1974) with a slight modification (Owada et al., 1975). As shown in Table 2, the MOB-1 and MSB-1 lines did not show any increased activity of reverse transcriptase, while the MOB-2 line did.

TABLE 2. *Reverse transcriptase activity of culture fluids after boostered replication on QEF and CEF*[a]

Cell line	Co-cultivated with	Relative incorporation[b] (pmoles)
MOB-1	QEF	0.1
	CEF	0.3
MSB-1	QEF	0.1
	CEF	0.3
MOB-2	QEF	14
	CEF	20
None	QEF	0.1
	CEF	0.3

[a] Each sample was concentrated 100-fold before the enzyme assay.

[b] Poly(rA): oligo $d(pT)_{10}$ was added to the reaction mixture and the relative incorporation was measured as uptake of ^{3}H-thymidine.

An immunofluorescent test for avian reticuloendotheliosis virus on these three cell lines was negative.

All the results are summarized in Table 3. None of the results gave any evidence of the association of expression of endogenous and exogenous avian RNA tumor viruses with the two MD lymphoma cell lines, MOB-1 and MSB-1. This, however, does not exclude the possibility of participation of endogenous RNA tumor virus genes in transformation by MDV. The results also indicate that transformation by MDV does not necessarily cause any expression of endogenous RNA tumor virus, even if it exists in these cells.

TABLE 3. *Summary of evidence for association of avian leukosis virus with MD lymphoma cell lines*

	Cell lines		
	MOB-1	MSB-1	MOB-2
gs antigen	−	−	+
chf test	−	−	+
Reverse transcriptase	−	−	+
C particles	−[a]	−[a]	+[b]
COFAL test	−[a]	−[a]	+[b]
RIF test	−[a]	−[a]	+[b]
AREV[c]	−	−	−

[a] Data cited from the paper of Akiyama and Kato, 1974.

[b] Data cited from the paper of Ikuta et al., 1976.

[c] Avian reticuloendotheliosis virus.

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