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## Abstract

Human KC cell monolayer inoculated with concentrated Mason-Pfizer monkey virus (MPMV) showed syncytia formation within an hour. The cell fusion was blocked by the treatment of the MPMV with neutralizing antiserum. Treatment of the MPMV with beta-propiolactone resulted in the loss of infectivity although KC cell fusion ability of the virus still remained. KC cells inoculated with unconcentrated MPMV showed no cell fusion even after several transfers, although a chronic MPMV infection was established. The virus-producing KC cells were refractory to fusion by MPMV. Human embryonic lung cells (HEL) were infected by serially diluted MPMV harvested from virus-producing culture, transferred twice, then cultivated together with KC cells for syncytia formation to examine the end point dilution titer of the virus. HEL infected by  $10^{-4}$ -diluted MPMV still induced syncytia formation by cocultivation with KC cells.

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## STUDIES ON HUMAN KC CELL SYNCYTIA FORMATION INDUCED BY MASON-PFIZER MONKEY VIRUS

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*Abstract.* Human KC cell monolayer inoculated with concentrated Mason-Pfizer monkey virus (MPMV) showed syncytia formation within an hour. The cell fusion was blocked by the treatment of the MPMV with neutralizing antiserum. Treatment of the MPMV with  $\beta$ -propiolactone resulted in the loss of infectivity although KC cell fusion ability of the virus still remained. KC cells inoculated with unconcentrated MPMV showed no cell fusion even after several transfers, although a chronic MPMV infection was established. The virus-producing KC cells were refractory to fusion by MPMV. Human embryonic lung cells (HEL) were infected by serially diluted MPMV harvested from virus-producing culture, transferred twice, then cultivated together with KC cells for syncytia formation to examine the end point dilution titer of the virus. HEL infected by  $10^{-4}$ -diluted MPMV still induced syncytia formation by cocultivation with KC cells.

Mason-Pfizer monkey virus (MPMV), a new type of RNA tumor virus, was isolated from mammary cancer of monkey (1) and classified as D-type (Neither B- nor C-type) reverse transcriptase virus (2). Recently, it has been shown that MPMV induced cell fusion in KC cells (3, 4), a human glioma cell line infected with an avian sarcoma virus. Several RNA tumor viruses of various origins have been reported as inducing cell fusion in rat XC and/or human KC cells (5). The mechanism of cell fusion which results in syncytia formation is still unknown, although in rat XC cell fusion by murine leukemia virus (MuLV), the fusion was only from without, viral spikes playing an important role in the initiation of cell fusion (6).

In this report we studied the characteristics of KC cell syncytia formation by MPMV and tried to titrate the infectivity of MPMV using the KC cell fusion phenomenon.

### MATERIALS AND METHODS

*Cells.* The monkey mammary tumor cell line (CMMT, #3328-281; Pfizer Inc., obtained from Dr. Gelderblom, Berlin) was derived from mammary tumor cells of a rhesus monkey which had been cocultivated with normal rhesus

embryo cells. The culture produces MPMV. The human KC cell line originally named 118 MG-EM (7) was supplied by Dr. J. Pontén, Uppsala, Sweden. Human embryonic lung cells (HEL) were prepared by primary culture from human fetal lung. All cells were maintained in Eagle's minimal essential medium (Dulbecco's modification) supplemented with 10% heat-inactivated calf serum.

*Antiserum.* Goat antiserum against MPMV (# 2S-761) from Pfizer Inc., obtained from Dr. H. Bauer, Giessen, Germany, was previously shown to block cell fusion by cocultivation of KC cells with MPMV-producing cells (8). Non-immune goat serum was the control.

*Concentration of MPMV.* MPMV from CMMT culture supernatant was centrifuged at 4,000 rpm for 20 min to remove cell debris and then the virus was centrifuged in an SW 27 rotor at 25,000 rpm for 45 min on to 0.5 ml of a 70% sucrose cushion (6) or pelleted without sucrose,

*KC cell fusion.* As DEAE-dextran (Pharmacia, Sweden) enhanced XC cell fusion by MuLV (6), this agent was also used for KC cell fusion by MPMV. DEAE-dextran was added to the medium of the KC cell monolayer to a final concentration of 25  $\mu\text{g}/\text{ml}$  for 30 min before they were subjected to the inoculation of concentrated MPMV. The virus concentrate either on a sucrose cushion or in culture medium after suspension of the pellet was added on to the monolayer KC cells for 10 min. Growth medium was added to the culture after removal of the virus solution. For mixed culture syncytia formation study, both trypsinized KC cells and trypsinized MPMV-producing cells were mixed (KC : MPMV-producing cells = 3 : 1). After overnight cocultivation, the cultures were fixed with 100% methanol, stained by Giemsa solution, and examined with the light microscope.

*Treatment of MPMV.* Overnight treatment of concentrated MPMV with a virus-inactivating drug,  $\beta$ -propiolactone (Sigma), known to inactivate the infectivity of Sendai virus (9) and MuLV (6) without affecting the fusion ability of these viruses, was performed at a final concentration of 0.02%. Treatment of MPMV with goat anti-MPMV serum or with non-immune goat serum was performed at a final serum dilution of 20% for 60 min at 37°C.

*Infectivity assay.* As HEL was susceptible to MPMV, HEL was chosen as the assay cells. Virus-containing culture supernatant of CMMT was serially diluted with growth medium and each 0.2 ml of them was added to DEAE-dextran treated HEL in 6 cm culture dishes. The cultures were incubated for 60 min, then 5 ml growth medium was added to the cultures which were then kept in a CO<sub>2</sub> incubator. They were transferred every 5 days. At every transfer, a part of the cells was cocultivated with KC cells. One day after cocultivation, the cultures were Giemsa stained and examined for the presence of KC syncytia.

## RESULTS

*KC cell syncytia formation by concentrated MPMV.* Forty-fold concentration of MPMV by centrifugation on to a sucrose cushion induced KC cell syncytia formation within an hour. The maximal syncytia formation was observed 4 hr

after virus inoculation. When MPMV was pelleted without a sucrose cushion on to the bottom of the centrifuge tube, then was added to KC monolayer cells, no KC cell syncytia formation was observed. Treatment of the concentrated MPMV with goat anti-MPMV serum blocked KC cell syncytia formation (Table 1 and Fig. 2) while non-immune goat serum had no such effect (Table 1 and Fig. 1). Overnight treatment of MPMV with  $\beta$ -propiolactone still induced KC cell

TABLE 1. KC CELL SYNCYTIA FORMATION BY CONCENTRATED MPMV

Treatment	Fusion activity	Infectivity
None	+	+
Nonimmune serum	+	+
$\alpha$ MPMV serum	-	-
$\beta$ -propiolactone	+	-
Dilution	-	+

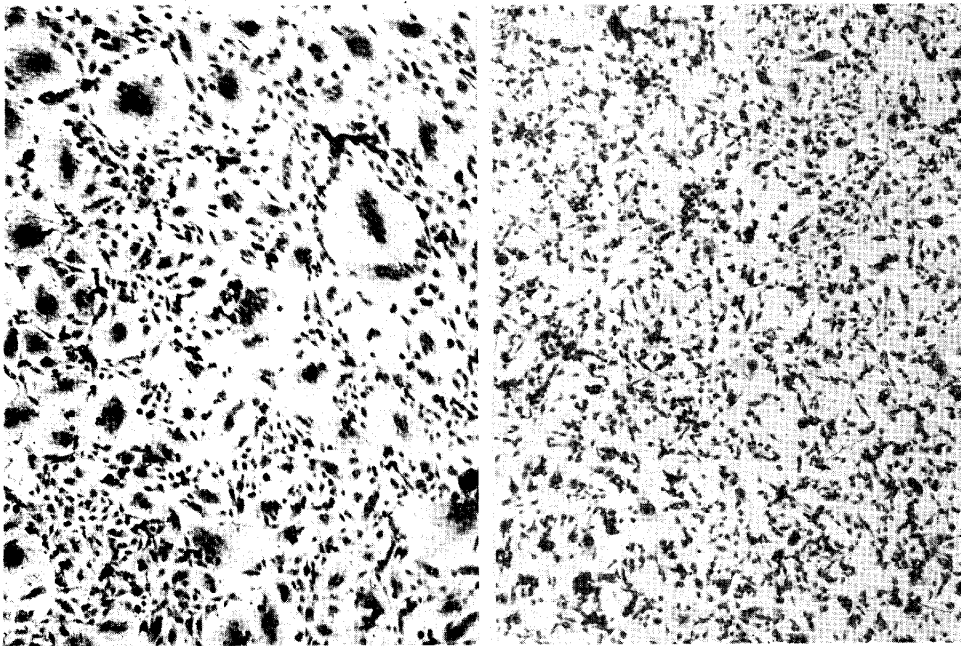


Fig. 1

Fig. 2

Fig. 1. KC cell syncytia formation by MPMV. Concentrated MPMV was incubated with nonimmune goat serum in a final dilution of 20% at 37°C for 60 min and then added on to KC cell monolayer. The culture was fixed 3 hr after virus infection with methanol and stained with Giemsa solution. Magnification  $\times 60$ .

Fig. 2. Syncytia formation block by neutralizing serum. Concentrated MPMV was incubated with goat anti-MPMV serum in a final dilution of 20% at 37°C for 60 min and then added on to KC cell monolayer. The culture was treated under the same conditions as described in the legend to Fig. 1. Magnification  $\times 60$ .

syncytia formation, although no infectivity was demonstrated when the treated virus was added to HEL (Table 1). When the concentrated MPMV was diluted ten-fold with growth medium or when unconcentrated MPMV was used for the inoculum, KC cell syncytia formation was no longer detected even after several transfers, although a chronic MPMV infection was established.

*Infective titer of MPMV assayed on HEL.* The end point dilution of the culture supernatant of CMMT which still contained infectious virus was  $10^{-4}$ . It was not until after the second transfer of the MPMV-infected HEL that they showed syncytia formation by cocultivation with KC cells. From the third transfer of the infected HEL, the number and the size of syncytia increased, although no syncytium was observed in mixed culture of HEL infected with  $10^{-5}$  dilution of MPMV and KC cells. This indicated that the infectivity titer of the MPMV was  $10^4$  syncytia forming units/0.2 ml.

*Resistance of syncytia formation of MPMV-infected KC cells.* KC cells showed extensive syncytia formation when they were inoculated with concentrated MPMV or cocultivated with MPMV-producing cells, however, MPMV-producing KC cells were refractory to syncytia formation under the same conditions.

#### DISCUSSION

Both 40-fold concentrated live MPMV and virus killed by  $\beta$ -propiolactone induced extensive KC cell syncytia formation within an hour. The infective titer of MPMV in the culture supernatant of CMMT was  $10^4$  syncytia forming units/0.2 ml. Therefore 40-fold concentrated virus solution contained about  $10^6$  syncytia forming units/ml. KC cells inoculated with diluted or unconcentrated MPMV induced no KC syncytia formation even after several transfers, although they produced progeny MPMV. This indicates that KC cell fusion is only fusion from without caused by inoculated virus and there is no fusion from within caused by progeny virus as with XC cell fusion by MuLV (6). As the anti-MPMV serum neutralized the syncytia forming ability of MPMV, it is most likely that viral spikes (glycoproteins) play an important role in syncytia formation as with paramyxoviruses (10, 11) and murine leukemia virus (6). The absence of syncytia formation when pelleted MPMV was used for the inoculum suggested that this procedure removed viral spikes. The susceptibility of the HEL used for assay cells for viral titration was too low for the development of clear plaque assay for MPMV as in the XC plaque test for MuLV (12). Though Rand *et al.* (4) described quantitative KC plaque assay for MPMV using RD and WI-38 cells as assay cells, it was necessary in our experiments to transfer the MPMV-infected HEL at least twice to obtain syncytia formation. Therefore, it was impossible to know the exact infective titer of MPMV, though the end point dilution titer could be determined.

It has been reported that MPMV-producing KC cells (3) and RD 114 feline virus-producing KC cells (13) resisted syncytia formation by respective viruses. Our results confirmed this finding. Similary MuLV-producing XC cells resisted cell fusion (6, 14) which was probably due to interference with inoculum virus by the MuLV from XC cells (6) which is known as the "virus interference phenomenon" in virology.

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