BIKEN JOURNAL, Vol. 15, 105-109, 1972

SHORT COMMUNICATION

LACK OF VIRUS-INDUCED CELL SURFACE ANTIGEN IN METAPHASE-ARREST CELLS INFECTED WITH POXVIRUS

MASANOBU MANTANI and SHIRO KATO

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

(Received January 17, 1972)

Initiation of viral DNA synthesis of poxvirus in cells at any phase of the cell cycle, stops the cell cycle of these cells. Moreover poxvirus multiplication occurs in cells at any phase of the cell cycle except during mitosis (Mantani et al., 1968; Mantani and Kato, 1971). When mitotic cells obtained in the presence of colchicine or vinblastine sulfate were infected with cowpox virus or vaccinia virus, no synthesis of viral DNA or viral antigens or vaccinia-induced hemadsorption was observed (Mantani and Kato, 1971; Marcus and Robbins, 1963). In poxvirus-infected cells, virus-induced cell surface antigen (VICSA) was demonstrated by the unfixed fluorescent antibody technique and immune hemadsorption (Miyamoto and Kato, 1968, 1971). This paper reports that metaphase-arrest cells had no VICSA under similar conditions to those described previously (Mantani and Kato, 1971).

FL cells were grown as monolayers using Eagle's minimum essential medium supplemented with 10% calf serum, as growth medium (GM). Cells were arrested in metaphase by addition of 5×10^{-8} M colchicine (final concentration) and incubated for 17 hr with GM containing colchicine. Aliquots of 1 ml of a preparation of cowpox virus (LB red strain Av^- marker) were then inoculated into each bottle (50 ml prescription bottle) at a multiplicity (moi) of 0.5 plaque forming unit (PFU)/cell. At this time the mitotic index (MI) was 45. After adsorption for 1 hr, cells were re-incubated with GM containing colchicine.

Samples were taken 0 hr (soon after the adsorption period), and 6 and 11 hr after adsorption (PA). The cells in the bottles were separated into two phases by shaking. Metaphase-arrest cells were mainly recovered in the fluid phase (M) while interphase cells mainly remained attached to the surface of the glass as a monolayer (I). The latter were treated with EDTA and collected in test tubes. Cells in both phase (I) and phase (M) were washed three times with Hanks' solution by centrifugation and incubated for 1 hr at 37 C with 1.5 ml of anti-CPV-rabbit γ-globulin conjugated with fluorescein-isothiocyanate.

Then cells were rewashed three times with Hanks' solution by centrifugation and a little of the precipitate from each test tube was examined under a dark-field fluorescence-microscope. The rest of the cells were smeared on a slide glass, fixed with methanol and stained with Giemsa solution, to determine the MI and percentage of cells bearing " B " type inclusions (" B " cells). As shown in Fig. 1, the MI in the (M) phase remained between 70 and 85%

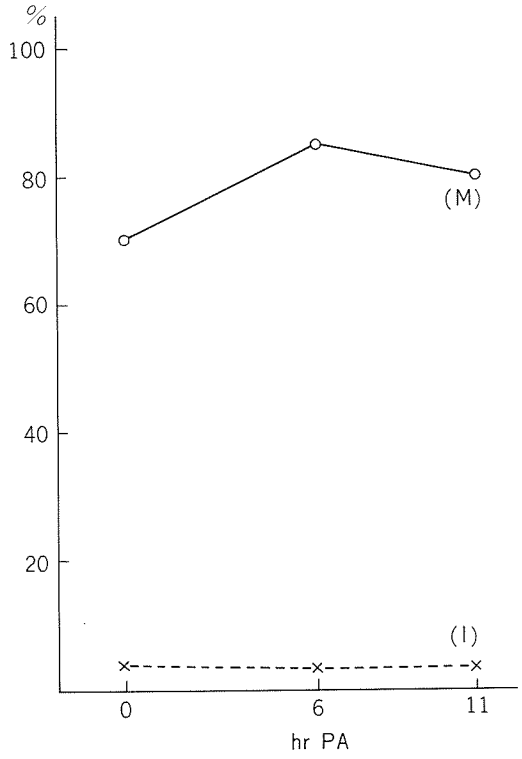


FIGURE 1. *Mitotic index of infected cells in phase (M) and phase (I) obtained by shaking bottles.*
○———○: *phase (M)*, ×-----×: *phase (I)*.

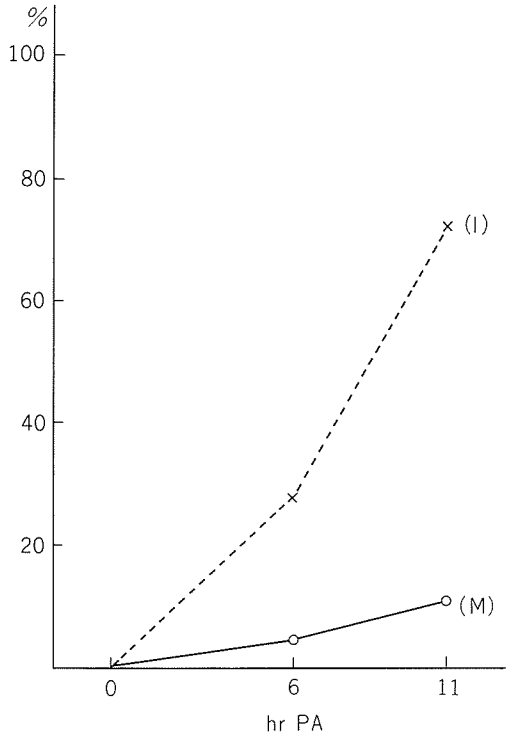


FIGURE 2. *Percentages of cells bearing inclusion bodies in phase (M) and phase (I).*
○———○: *phase (M)*, ×-----×: *phase (I)*.

throughout the experiment while that in the (I) phase was less than 3%. Neither the percentage of cells showing fluorescence around the cell-margin, as shown in Fig. 7, nor that of " B " cells in the (M) phase increased (Fig. 5), although those in cells in the (I) phase, (Fig. 6 and 8) increased to more than 60 percent 11 hr after viral adsorption, as shown in Fig. 2 and 3. The VICSA observed in the (M) phase 11 hr after viral adsorption seems to be that of interphase cells which constituted less than 30% of the cells in the (M) phase. The virus growth curves in FL cells in the presence and absence of 5×10^{-8} M colchicine were determined on BSCl cells using the agar overlay technique, as described previously (Mantani et al., 1970). As shown in Fig. 4, the virus growth when colchicine was added after infection (moi 0.18) was identical with that in the absence of colchicine. Cowpox virus grew as well in the presence of colchicine as in GM alone. However, growth of the virus was poor in a culture of FL cells, in which 43% of the cells were in metaphase-arrest at the time of infection (moi 0.22) 23 hr after treatment with colchicine. This also indicates that virus does not grow in cells during metaphase-arrest.

Summarizing these results, formation of cell surface antigens induced by cowpox virus does not occur in metaphase-arrest cells obtained with colchicine, but cowpox virus replication proceeds normally in the presence of 5×10^{-8} M colchicine.

Miyamoto and Kato (1968) observed surface antigens on FL cells induced by poxvirus in the presence of cytosine-arabinoside. Under these

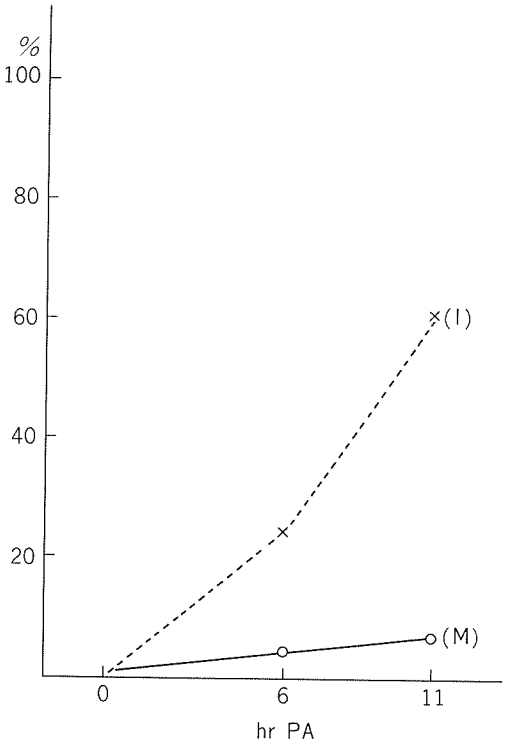


FIGURE 3. *Percentage of fluorescent cells among unfixed infected cells.*
○———○: *phase (M),* ×-----×: *phase (I).*

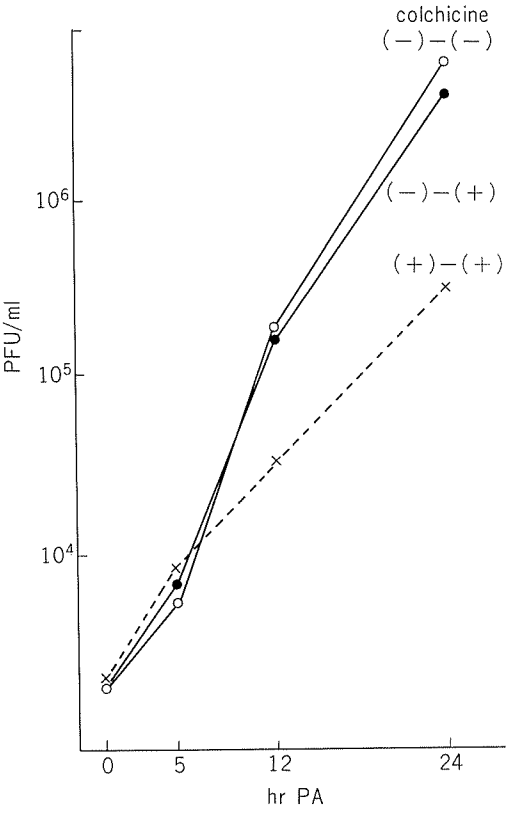


FIGURE 4. *Effect of colchicine upon cowpox virus multiplication.*
○———○: *in the absence of* 5×10^{-8} M *colchicine.*
●———●: *in the presence of* 5×10^{-8} M *colchicine after infection (moi 0.18).*
×-----×: *in the presence of* 5×10^{-8} M *colchicine 23 hr before infection and after infection (moi 0.22).*

conditions, viral and cellular DNA syntheses were both inhibited. In the case of Newcastle disease virus, virus attachment, penetration, and eclipse proceeded normally in metaphase-arrest cells, but viral development was suppressed in them (Marcus and Robbins, 1963). In the case of poxvirus infection, it is probable that the uncoating process or some process before uncoating may be suppressed in mitotic cells infected with poxvirus.

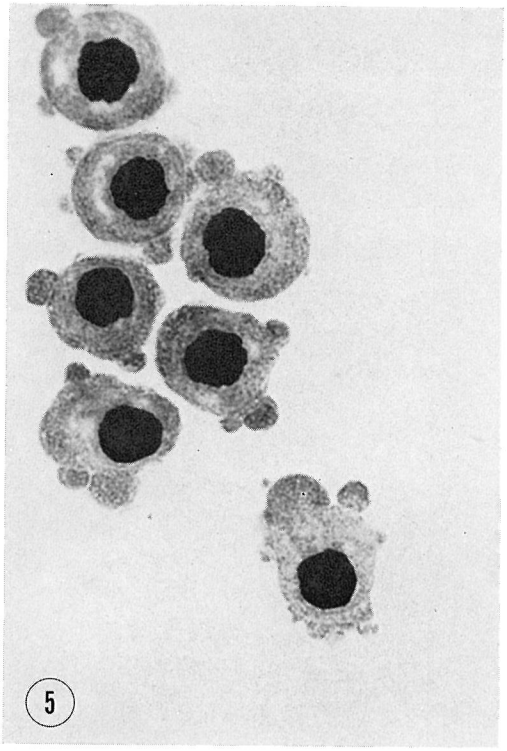


FIGURE 5. *Metaphase-arrest cells in phase (M), 11 hr after infection. No inclusions are seen.*

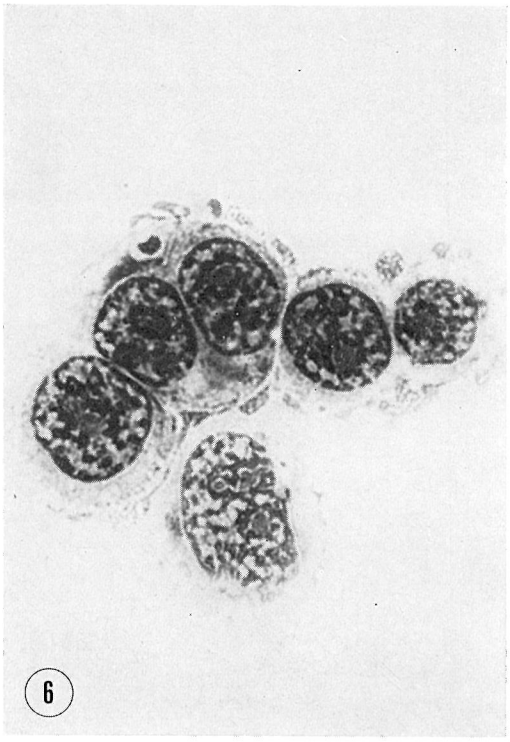


FIGURE 6. *Interphase cells in phase (I), 11 hr after infection. More than 70% of these cells had inclusion bodies in the cytoplasm.*

REFERENCES

Mantani, M., H. Miyamoto, and S. Kato. 1968. Effect of viral DNA synthesis of poxvirus upon nuclear DNA synthesis of synchronized FL cells. Biken J. 11: 71–90.

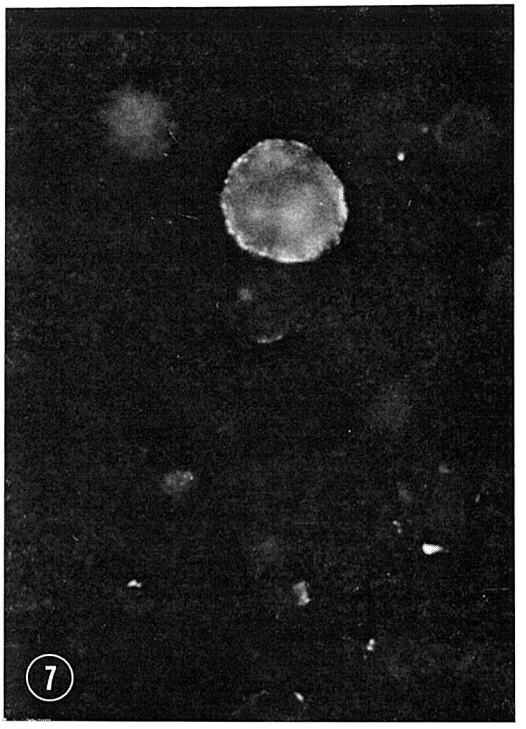
Mantani, M., Y. Sakaue, and S. Kato. 1970. Virus multiplication in culture cells synchronized by excess thymidine treatment. 1. Effect of excess thymidine upon growth of poxviruses and chikungunya virus. Biken J. 13: 353–364.

Mantani, M., and S. Kato. 1971. Virus multiplication in culture cells synchronized by excess thymi dine treatment. III. Effect of viral DNA synthesis of cowpox virus upon the mitotic phase of FL cells. Biken J. 14: 297–310.

Marcus, P. I., and E. Robbins. 1963. Viral inhibition in the metaphase-arrest cell. Proc. Nat. Acad. Sci. U.S.A. 50: 1156–1164.

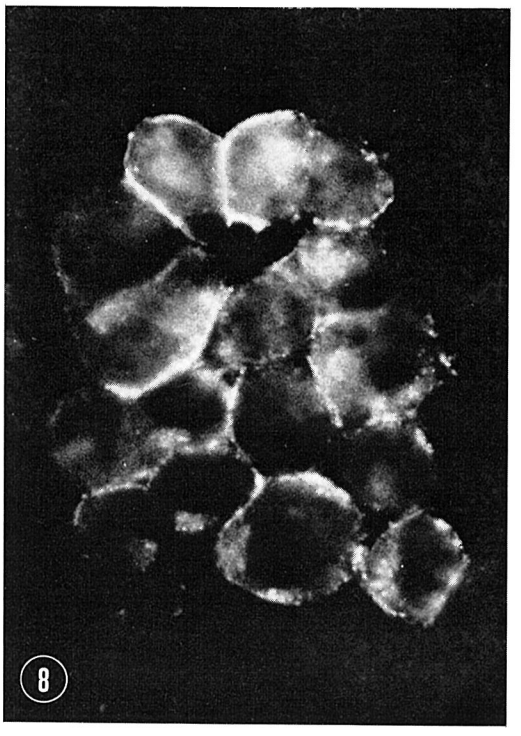
Miyamoto, H., and S. Kato. 1968. Immune hemadsorption by cells infected with poxviruses. Biken J. 11: 343–353.

Miyamoto, H., and S. Kato. 1971. Cell surface antigens induced by poxviruses. I. Effects of antimetabolites on cell surface antigens. Biken J. 14: 311–324.



◀

FIGURE 7. *Fluorescent cells in phase (M) 11 hr after infection. Less than 8% of unfixed infected cells in phase (M) had fluorescence around the cell margin.*



◀

FIGURE 8. *Fluorescent cells in phase (I) 11 hr after infection. More than 60% of these cells had fluorescence around the cell margin.*