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POLYKARYOCYTOSIS INDUCED BY AMPHOTROPIC MURINE C-TYPE VIRUS (AP129) IN SAC-CELLS NONPRODUCTIVELY TRANSFORMED BY MOLONEY MURINE SARCOMA VIRUS (MO-MSV)

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Summary. — Nonproducer cells of STU mouse origin (Sac) transformed with Moloney murine sarcoma virus (Mo-MSV) and superinfected with the amphotropic murine C-type virus strain AP 129 unexpectedly formed polykaryocytes. The multinuclear giant cells appeared about 30 days after AP 129 infection as confirmed in three independently performed experiments. The majority of the Sac-cells became involved in syncytium formation. The polykaryocytes disappeared during continued culture transfers. Various cell lines obtained by limiting dilution showed the same reactivity as the parental cell line. Release of sarcoma-helper virus complex was observed before and during the appearance of the polykaryocytes as well as after their disappearance at about day 50 after infection. In normal STU mouse embryo fibroblasts, normal Balb/3T3 cells or another Mo-MSV transformed nonproducer cells (MSV85 C13; Balb origin) infected with AP 129 no polykaryocytes developed. The sarcoma-helper virus complex released from AP 129 infected Sac-cells led to transformation of cultured cells from different mammalian species (mink, goat, dog). However, when observed for at least 30 days these cultures did not show polykaryocyte formation.

Key words: polykaryocytosis; amphotropic murine C-type virus

Polykaryocytes (PK) can be induced by a variety of viruses in appropriate cells (Poste, 1970). Also some retroviruses — though generally considered to be noncytopathogenic — have the ability to induce PK in infected cell lines (for review see About and Huleihel, 1981; Saal *et al.*, 1983). Appropriate cell lines suitable for PK induction by retroviruses often are transformed cells (Klement *et al.*, 1969; Rand and Long, 1972; Nagy *et al.*, 1983; Saal *et al.*, 1983). The reason for this is not known. Perhaps surface alterations acquired during the process of transformation may be responsible for this property. Until now PK induction by murine amphotropic C-type virus has not been reported. We observed PK induction in a Moloney murine

sarcoma virus (Mo-MSV) nonproducer transformant of STU mouse origin (Sac; Weiland *et al.*, 1978) after infection with amphotropic murine C-type virus. Sac-cells differ from other MO-MSV transformants not suitable for PK induction (reported herein) with regard to expression of a serologically and biochemicaly detectable antigen (Hunsmann *et at.*, 1980). This is the first reported observation of PK induction by amphotropic murine C-type virus.

The Moloney murine sarcoma virus (Mo-MSV) transformed Sac-cells of STU-mouse origin were established from a secondary tumour that developed at the site where a primary Mo-MSV induced tumour had regressed. They contain sarcoma genome rescuable after superinfection with ecotropic murine C-type virus as described earlier (Weiland *et al.*, 1978). Further cell cultures used in this study were: primary murine embryo fibroblasts (MEF) of STU-mouse origin, normal Balb/3T3 cells (CCL 163), Mo-MSV nonproductively transformed MSV85 Ci 3 cells of Balb origin (Aaronson and Rowe, 1970), caprine dermal cells, canine foetal thymus cells (CFT, CRL 1430), mink lung cells (CCL 64) and Mo-MSV transformed S^+L^- mink cells ($MiCl_1$; CCL 64.1). Cells were grown in Eagle's minimum essential medium plus 10% inactivated foetal calf serum. Amphotropic murine C-type virus AP129 was provided by H. J. Thiel from this Institute. Virus stocks were prepared by infection of mink CCL 64 cells with the undiluted original probe in the presence of 2 μg/ml polybrene (Sigma, Chemical Co., St. Louis, U.S.A.). For rescue of sarcoma virus genome from Sac-cells by amphotropic murine C-type virus AP 129 the Sac-cells were plated at a density of 2×10^5 cells/Falcon-25 flask in the presence of 2 μg/ml polybrene. Eighteen hr after plating, the cells were inoculated with one ml of the virus stock containing 10^4 focus-inducing units in $MiCl_1$ cells. The cultures were transferred twice weekly at a density of 1×10^5 cells/ml. Titres of released sarcoma virus in terms of focus forming units (PFU) per 0.2 ml were determined by infection of mink CCL 64 cells and primary STU-MEF with serial dilutions and counting the foci 4—7 days later.

Transformation studies on different cell lines with Sac sarcoma virus released from Sac-cells after AP129 infection were performed applying the same procedure as for infection of Sac-cells.

About four weeks after infection the morphology of the culture gradually changed over a period of 1—3 weeks. No changes appeared in mockinfected or ecotropic murine C-type virus infected Sac-cells. After fixation and staining, polykaryocytic giant cells could be observed. As can be seen in Fig. 1-I, polykaryocytes contained large numbers of nuclei. During the peak of polykaryocytosis giant cell formation involved most cells of the monolayer. Plaques of fused cells were especially dominant in seven-day-old cultures in contrast to three-day-old ones suggesting that enlargement of the foci is a rather slow process. As seen on the large polykaryocyte in Fig. 1, vacuolic degeneration occurred in the centre of the syncytium. The nuclei of the giant cells had a large nucleolus. But this was also the case in non-affected cells (Fig. 1-II, see also Weiland *et al.*, 1979). After trypsinization and additional cultivation the amount of PK decreased gradually and finally the PK disappeared (about 7—8 weeks after AP129 infection). During the whole cultivation period — before and during appearance of the giant cells and after their disappearance — sarcoma-helper virus complex was released from the AP 129 infected Sac-cells. Infection experiments with AP129 were repeated using besides Sac-cells a further Mo-MSV nonproductively transformed cell line, MSV85 C13 of Balb origin, normal Balb 3T3 cells and normal primary STU-MEF. About four weeks later giant cells were evident only in AP129 infected Sac-cells, but did not appear in those addi-

tionally investigated transformed and normal cells. Since not all of the adherent Sac-cells became involved in the giant cell formation it was of interest to compare the behaviour of cell clones gained after limiting dilution of Sac-cells with that of the parental cell line. Five clones infected with AP129 released sarcoma-helper virus complex and developed multinuclear syncytia about four weeks after infection. As also seen with the parental cells, giant cells did not involve all of the clone monolayer cells and disappeared constantly during continuation of culture after trypsinization.

Infection of normal mink lung cells, canine foetal thymus cells and goat fibroblasts with the sarcoma-helper virus complex released from AP129 superinfected Sac-cells led to transformation of the infected cells. During a cultivation period of at least 30 days after infection no PK could be observed.

The phenomenon described in Sac-cells at about four weeks after amphotropic C-type virus infection differs from the two known categories of cell fusion by viruses: fusion from without and fusion from within (Bratt and Gallaher, 1969) with regard to the time of its appearance. Fusion from without or "early" polykaryocytosis (Watkins, 1971; Guillemain *et al.*, 1981) arises within minutes or a few hours; it is a passive process which does not require any de novo virus synthesis but is dependent on the presence of virus proteins. Fusion from within or "late" polykaryocytosis becomes apparent within several hours or days after infection of sensitive cells and results from an active process depending on the integrity of the virus genome (Watkins, 1971; Guillemain *et al.*, 1981). The fusion process arising in AP129 infected Sac-cells differs from both categories of cell fusion described, as it was detected only several weeks after infection. Further investigation is needed to characterize the process leading to the very late Sac-cell fusion.

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References

Aaronson, S. A. and Rowe, W. P. (1970): Nonproducer clones of murine sarcoma transformed Balb/3T3 cells. *Virology* **42**, 9—19.

Aboud, M. and Huleihel, M. (1981): Rapid syncytium formation induced by Moloney murine sacroma virus in 3T3 NIH cells and its delay by mouse interferon. *Arch. Virol.* **70**, 103-114.

Bratt, M. A. and Gallaher, W. R. (1969): Preliminary analysis of the requirements for cell fusion from within and fusion from without by Newcastle disease virus. *Proc. natn. Acad. Sci. (U.S.A.)* **64**, 536—543.

Guillemain, B., Mamoun, R., Astier, T., and Duplan, J. F. (1981): Mechanism of early and late polykaryocytosis induced by the bovine leukaemia virus. *J. gen. Virol.* **57**, 227—231.

Hunsmann, G., Weiland, E., Mussgay, M. and Schneider, J. (1980): A non-virion surface antigen on Moloney sarcoma virus-transformed Non-producer and producer cells. *J. gen. Virol.* **49**, 375—384.

Klement, V. Rowe, V. P., Hartley, J. W., and Pugh, W. E. (1969): Mixed culture cytopathogenicity; A new test for growth of murine leukemia viruses in tissue culture. *Proc. natn. Acad. Sci. (U.S.A.)*, **63**, 753—758.

Nagy, K., Clapham, P., Cheingsong-Popov, R., and Weiss, R. A. (1983): Human T-cell leukemia virus type I: Induction of syncytia and inhibition by patients' sera. *Int. J. Cancer* **32**, 321—328.

Poste, G. (1970): Virus induced polykaryocytosis and the mechanism of cell fusion. *Adv. Virus Res.* **16**, 306—356.

Rand, K. H. and Long, C. (1972): Syncytial assay for putative human C-type virus RD-114 utilizing human cells transformed by Rous sarcoma virus. *Nature, New Biol.* **240**, 187—190.

Saal, F., Cavalieri, F., Sitbon, M. and Peries, J. (1983): Baboon endogeneous C type virus induced syncytium formation in Epstein-Barr Virus (EBV) carying lymphoblastoid human cell lines. *Arch. Virol.* **75**, 151—155.

Watkins, J. F. (1971): Cell fusion in virology. In M. Polard (Ed): *Perspectives in Virology*, Vol. VII, 159—178. Academic Press, New York.

Weiland, E., Mussgay, M. and Weiland, F. (1978): Nonproducer malignant tumor cells with rescuable sarcoma virus genome isolated from a recurrent Moloney sarcoma. *J. exp. Med.* **148**, 408—423.

Weiland, F., Weiland, E. and Mussgay, M. (1979): Growth pattern of tumours in mice induced by murine sarcoma virus — Moloney and by sarcoma virus transformed cells. *Brit. J. Cancer* **40**, 932—942.

Explanation of Micrographs (Plate XVII):

Fig. 1. Multinucleated giant cells in Mo-MSV transformed Sac-cells during 10th transfer after infection with amphotropic murine C-type virus AP 129. In I — polykaryocytes of different size are seen. The affected as well as the nonaffected Sac-cells exhibit a prominent nucleolus. The largest polykaryocyte reveals vacuolic degeneration. In II — mock-infected Sac-monolayer cells. Magn 225 ×.