Studies of early stage of mouse polyomavirus infection

ABSTRACT

The long-term research project of our laboratory is concentrated on the studies of the life cycle of the mouse polyomavirus. One of the many fields of our interest is targeted on entry and transport of the mouse polyomavirus into cells. Endocytic pathways are not completely understood but in general, it is known that the polyomavirus does not use only one endocytic pathway. After its binding to a receptor containing sialic acid, the virus is internalised into smooth monopinocytic vesicles. Monopinocytic vesicles carrying virions fuse often with early endosomes. Acidic pH of endosomes is necessary for successful virus infection. The role of caveolin in the above-mentioned process still remains unclear. Three hours post infection, a signal of the virus can be found in endoplasmatic reticulum and also in recycling endosomes. Regadless multiplicity of infection, only few virions sucessfuly deliver their genomes into the cell nuclus for their expression. Where virus uncoating occures and how virus genomes enter the nucleus remains unexplained. As well, the contribution of the minor structural proteins VP2 and VP3 is unclear. It is only partial information about processes of defence mechanisms of cell. To contribute to elucidation of some of these processes, we chose, besides virons, VLPs (pseudocapsids) , which do not contain viral genomes and are not able to induce productive infection but consist of capsid structures to the virons.

We isolated mouse polyomavirus with infection titre 2.10^{11} from infected whole mouse embryo cells and VP1 and VP1/3 pseudokapsids from insect cells infected by recombinant baculoviruses. With these isolates we studied whether pseudocapsids (composed of VP1 only) or (containig one of the minor proteins) can be competitive inhibitors of polyomavirus infection when added to virus inoculum prior infection. We found that VP1/3 have ability to compete infection 60% (in ratio 100 pseudocapsids per 1 viron) and VP1 pseudocapsids 43% (under the same conditons). We further studied colocalisation of entering virions and pseudocapsids by confocal microscopy. Only the minority of entering virons (and pseudocapsids) colocalised with caveolin-1 20 minutes post infection. Substantialy higher level of colocalisation of these structures was observed 3 hours post infection, in particular in perinuclear areas. Surprisingly, the most abundant colocalisation of VP1 and caveolin 1 could be seen throughout the cytoplasm 12 hours post infection. This sugests, that caveolin rich vesicles may be involved in discharging of disassembled virus particles from cells.

Key words: polyomavirus, VLP, inhibition of infection, virus entry, caveolin, VP1