**Conclusions**

* FC are preferentially bound compared to EC.
* EC do not interefere with genome delivery to the nucleus.
* EC have lower affinity to sialic acid
* FC / EC Pufification from CsCl gradients
* Binding saturation curve (Moi 20) saturated
* Neuraminidase treatment kinetics

**5SG mutant shows prolonged cell viability upon infection.**

A9 cells (3 x 106) were productively infected with 5SG virions at a MOI of 5000 particles per cell.

Phospho-rich N-VP2 region mediates nuclear targeting and cytotoxicity of MVM.

In order to investigate a possible involvement of the distal serine phosphorylations within N-VP2 in MVM egress, we generated a mutant, referred to as 5SG, having the corresponding serine residues substituted by glycine. In Figure (ref..) several aspects of an infection with 5SG, such as cytotoxicity, nuclear targeting, and nuclear export are summarized.

* Fig S1

In a WT infection, the cells showed reorganization of the cytoskeleton as early as 20 hpi, resulting in a rounded phenotype of affected cells. Such changes in the cytoskeleton filaments are typical for MVM infections and have previously been described (Nüesch..). Increasing amounts of cells became rounded and partially detached showing apoptotic bodies 40 hpi eventually resulting in cell death and cytolysis (see Figure S1 ref.. 1st row). In contrast, 5SG virions were significantly less cytotoxic. Even as late as 40 hpi most of the infected cells still exhibited the typical fibroblastic phenotype and only few cells became rounded. No signs of apoptosis and cytolysis were observed (see Figure S1.. 2nd row). The delay in cell killing is at least in part explained by a less efficient initiation of the infection. 5SG was approximately 5x less efficient in generating viral DNA in the nucleus, indicating that less virions reached the nucleus, thus delivering less DNA templates in order to initiate viral replication. Indeed, similar quantities of progeny viral DNA were obtained when the cells were infected with 5x less WT virions (see Figure S1 B). However, nuclear export was not significantly affected. 5SG virions were efficiently exported from the nucleus even though showing a slight delay in cytoplasmic accumulation. This delay is obviously caused by defects in early steps of infection prior to the initiation of DNA replication, such as binding, endosomal escape, viral uptake, or nuclear targeting.

Similar observations have been reported by G. E. Tullis et al. (1992, ref) for a MVM mutation affecting the sequence but not the phosphorylations within N-VP2. A 7 amino acid deletion mutant lacking the trypsin-sensitive residues 17-23 within N-VP2 was slightly defective for binding and approximately 10-fold deficient compared to the WT in initiating a productive infection. These results suggest that this region is important for both binding and a subsequent step prior to the onset of DNA replication. Because this mutation affects both structural proteins VP1 and VP2, it is difficult to distinguish their relative contribution to the mutant phenotype. However, the binding defect is more likely a VP2 effect since virions lacking VP1 are not defective in binding to susceptible cells (Tullis,…).

In addition to its defect in cell binding, the mutant had an additional impairment at an early step in infection. Similar amounts of the 7aa deletion mutant produced approximately 10x less viral DNA in the nuclei of infected cells, suggesting that fewer mutant virions, on the average, reached the nucleus where they can initiate DNA replication. However, those that managed to reach the nucleus, replicated normally.

Equally, this mutant was delayed in escaping from the cells late in an asynchronous infection. However, mutant progeny virions were efficiently transported to the media early in the same infection, as well as in highly synchronized infections. Therefore, this effect might be a nonspecific defect in some aspect of cytolysis rather than a defect in an active egress mechanism.

Preliminary cytotoxicity experiments, based on the clonogenicity of infected cells on plastic dishes, suggest that VP(A2842-2863) kills host cells as efficiently as wild-type MVM, (L.B., unpublished observation). However, these experiments only measure the ability of the virus to kill cells and do not directly address the kinetics of cytolysis. VP(A2842-2863), for instance, might actually kill the cells earlier than would wild-type MVM, thereby shutting down export of virus to the media; alternatively, VP(A2842-2863) may kill cells with similar kinetics as wild-type MVM, but not lyse the plasma membrane sufficiently to release virions.

~~This deletion, however, did not disrupt all capsid functions; this domain was not required for viral assembly, encapsidation of DNA, or egress from the cells suggesting that the RVER region of VP1 and VP2 plays a role at specific steps in a MVM infection.~~

Additionally, an inhibition of plaque formation following DNA transfection was observed for this mutant even though DNA replication was normal. However, wildtype plaques were obtained following viral infection.

**Comparison of ECs and FCs in early virus infection.**

In addition to the previously characterized FC populations (FC-P1 and FC-P2), infected cells produce also a considerable amount of ECs. Due to the lack of DNA, EC band at lower density in a CsCl gradient compared to FC. While FC entered the gradient to a density of 1.46 g/cm3, EC already banded at 1.32 g/cm3, as determined by refractometry. A quantitative PCR analysis of the corresponding fractions confirmed that viral DNA containing particles were depleted from ECs to almost a thousand times. Approximately half of the overall viral progeny population represent ECs. Therefore, it is of interest to characterize their role during the course of infection. We studied their ability to bind to restrictive murine cells, their capacity to compete with FCs, and their potential to interfere with the progression of a natural infection.

**Both FC and EC bind specifically to SA residues on the cell surface**

In order to characterize the binding specificity of FC and EC, both capsid types were allowed to bind discretely to susceptible, restrictive murine fibroblasts at 4 °C. At such low temperature, active cell-mediated uptake through endocytosis is prohibited. Unbound viruses were removed by several washings of the adherent cells. For restrictive mouse fibroblasts, binding saturation is reached at MOIs higher than 10000 DNA-containing particles per cell, as determined by the quantification of bound FCs to adherent cells at 4 °C. Both capsid species restrictively bind to SA residues since they can be completely depleted from the cell surface by treatment with neuraminidase (see Table…), an enzyme that specifically hydrolyzes glycosidic linkages of neuraminic acids. Complete removal of attached viruses is even achieved under saturated conditions. In order to guarantee a complete removal of viral particles from the cellular surface, a minimal dose of 25 U/mL of the enzyme is required.

Binding sites: 500000 per A9 cell (P.LINSER, HELEN BRUNING, AND R.W. ARMENTROUT, 1977)

**EC and FC exhibit morphological differences**

The flexible, unordered N-terminus of the major structural protein, VP2, shows distinct conformation in either capsid population. N-VP2 is accessible to proteolytic digestion or specific antibodies only in FC, whereas it remains inaccessible in EC, indicating important structural differences between these capsid populations. The differences for N-VP2 accessibility can be used to distinguish FCs and ECs in IF experiments. Staining of FCs results in co-localization of a-Caps and a-N-VP2 antibodies whereas ECs are detected only by a-Caps antibodies.

**FC are preferentially bound to the SA residues on the cell surface**

*In silico* quantification of co-localization in representative IF pictures revealed that binding of FCs was not disturbed in the presence of ECs. When FCs and ECs were bound to cells at equal stoichiometry, FCs preferentially bound to the cell surface, indicating a higher binding affinity for FCs compared to ECs. Co-localization of both antibodies was higher than 95 % in the absence and in the presence of ECs, indicating that FCs bound preferentially to the cells. Even under non-saturated conditions, ECs were detected rarely when applied as mixed populations. Only when an equal amount of ECs was added prior to the FCs a slight increase in bound ECs was observed. Nevertheless, ECs did not represent 50 % of the bound population but only reduced co-localization marginally to approximately 75 %.

**EC do not compete with FC for cell surface receptors**

Quantitative competition experiments under saturated conditions confirmed that increasing amounts of ECs did not disturb the attachment of FCs to the cell surface. These results substantiate the preferential binding of FCs to susceptible cells previously observed in IF experiments. Even an unnatural 16-fold excess of ECs did not significantly disturb receptor binding of FCs.

Due to the differences in N-VP2 conformation among FCs and ECs, there is evidence that the N-VP2 termini may be involved in the stabilization of the binding

Binding sites: 500000 per A9 cell (P.LINSER, HELEN BRUNING, AND R.W. ARMENTROUT, 1977)

Linser et al. report a competition of FC (unlabelled) with FC labelled. However, an excess of more than 100x was required to observe competition!!

Because the amino terminus of VP2 is accessible to the viral surface, the simplest explanation is that this region facilitates adherence to the cell surface. However, this interpretation is not consistent with previous studies that showed that this domain can be physically removed from the viral particle by proteases without affecting the binding or infectivity of the virus (Clinton and Hayashi, 1976; Linser and Armentrout, 1978). ~~In addition, empty particles, in which the amino terminus of VP2 is resistant to~~

~~protease digestion, compete as efficiently as full particles for cellular binding sites (Linser and Armentrout, 1978).~~ Taken together these results suggest that this region exerts a secondary effect on the receptor-binding site rather than participating directly in receptor binding.

Additional Results Structure:

* EC also can be purified from SN of infected cultures
* Role of EC?
  + Display morphological differences
  + Bind specifically to SA residues on cell surface
  + Do NOT compete with FC in terms of binding and infection (do not disturb infection..)
* Role of N-VP2..? Binding? Stabilization? Internalization..?

Study of infectious virus populations by FPLC

* *In vivo*: AEX: Native, bound, internalized 🡪 Characterization of virus populations
  + B19V
    - Profile changes during course of infection (MonoQ)

FPLC

* Our lab: trafficking: Rearrangements (Mani, Nico,…)
* Nico 🡪 B19V in vivo aquires phosphorylations and undergoes structural rearrangements
* FPLC: chromatography surface features lead to separation
  + AEX: surface charges, Cl- gradient
  + Chromatofocusing: IP, pH-Gradient

**Add 5S/G differs in egress cos of early delay….!!**