Entry mechanisms of the human cytomegalovirus (HCMV) into GM- and M-monocyte derived macrophages

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Human cytomegalovirus (HCMV) is a species-specific member of the Herpesvirus family, widespread in humans, a leading cause of birth defects and a major cause of morbidity and mortality for immunocompromised individuals. During natural infection, macrophages play central roles as targets of HCMV by supporting viral replication and secreting a plethora of soluble factors. Highly pure subsets of pro-inflammatory (M1) and anti-inflammatory (M2) macrophages were generated by stimulating *ex vivo* monocytes with the lineage-determining cytokines GM-CSF and M-CSF, respectively [1]. In a previous series of experiment we had found that the efficiency of HCMV infection was opposite in the two types of macrophages: very high in M2 and very low in M1 macrophages. Since it has been suggested that the way of viral entry into a target cell, namely membrane fusion or endocytosis, can determine the infection efficiency [2], we investigated how HCMV enters into the two types of macrophages.

The intracellular distribution of the viral particles was visualized by indirect immunofluorescence (**Fig. 1**). While in M1 macrophages the viral particles were retained in the cellular periphery, in M2 macrophages the viral particles accumulated around the nucleus. For electron microscopy, the two types of macrophages were incubated for 90 min with HCMV, immobilized by high pressure freezing and cryo-substituted using a protocol optimised for membrane visibility [3]. Electron microscopic investigations of viral entry are much more difficult than studies of viral egress [4], because the few viral particles that enter a cell are hard to find. So far we could not directly observe viral particles at the very moment of fusion with the plasma membrane (schematic drawing in **Fig. 2**), most likely because this event is short-lived and unlikely to be trapped by our fast freezing methods. We assumed that while fusion should lead to the presence of naked capsids in the cytosol, endocytosis leads to the formation of cytoplasmatic vesicles containing virions. In fact, we observed both situations in both types of macrophages (**Fig. 3**). In quantitative analyses (**Fig. 4**) we found that M1 macrophages contain much more virions in vesicles than naked capsids in the cytosol, indicating that endocytosis is the main entry mechanism in M1 macrophages. M2 macrophages, however, show as many naked capsids as vesicles containing complete virions, indicating that both, endocytosis and fusion, are taking place. (In order to discern vesicles from invaginations of the plasma membrane, colloidal gold particles were added to the medium and washed away prior to high pressure freezing. So, invaginations of the plasma membrane are free of gold particles, whereas endocytotic vesicles do contain gold particles (**Fig. 3b**). In conclusion, we propose that the difference in the efficiency of HCMV infection of M1 and M2 macrophages may be due to the differences in viral entry.

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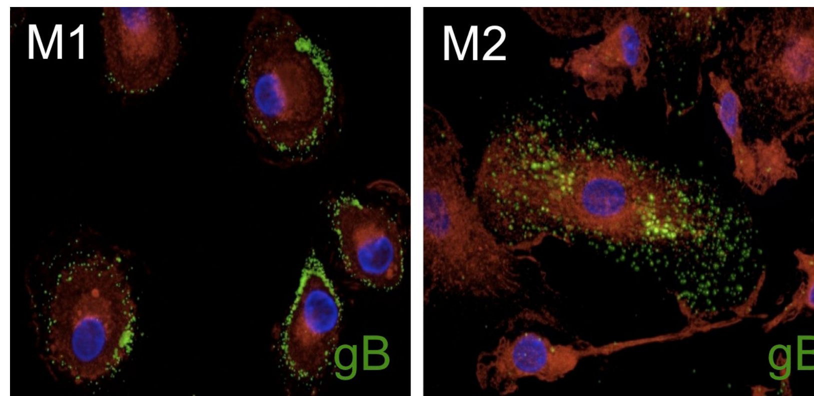

Figure 1. Localisation of HCMV viral particles in M1 and M2 macrophages. The primary cells were infected with strain TB40E at a MOI 10 for 90 min. gB, a glycoprotein of the viral envelope, was detected by indirect immuno-fluorescence (Alexa-488, green signal). The cells were counterstained with evans blue (cytoplasm, red signal) and with DAPI (nuclei, blue signal). In M1 macrophages the viral particles are retained in the periphery of the cells, whereas in M2 macrophages the viral particles spread over the whole cells.

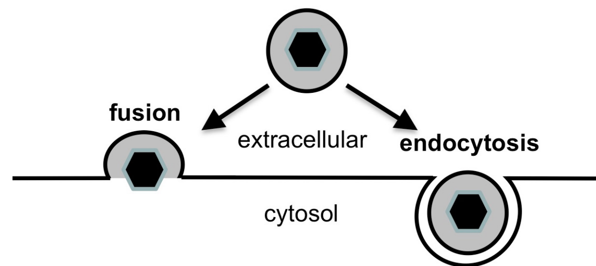

Figure 2. According to Sinzger (2008) [2] HCMV viral particles can enter a macrophage by membrane fusion or by endocytosis.

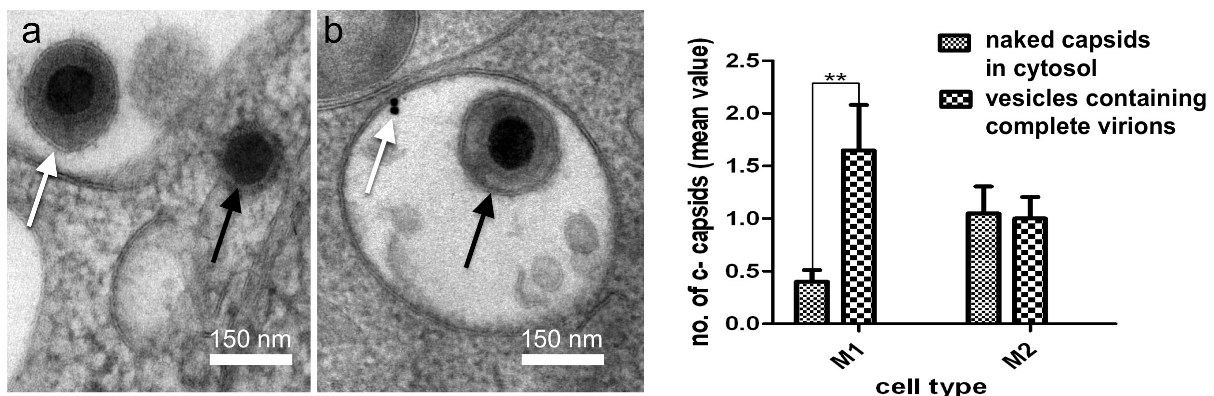

Figure 3. (left) The result of a viral particle (white arrow in Fig 3a) that fuses with the cell membrane of a macrophage is a naked capsid in the cytosol (black arrow in Fig. 3a). The first result of an endocytosis is a vesicle containing a complete virion (black arrow Fig 3b). (White arrow points to endocytosed gold particles.) Samples were high-pressure frozen. **(right)** Statistical analysis of the entry mechanism. **M1 macrophages** contain much more virions in vesicles than naked capsids in the cytosol, indicating that **endocytosis is the main entry mechanism**. M2 macrophages, however, show as many naked capsids as vesicles containing complete virions, indicating that **endocytosis and fusion are taking place**.

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