Cytomegalovirus Cell Tropism

C. Sinzger(✉), M. Digel, G. Jahn

Contents

Abstract The human cytomegalovirus (HCMV) can infect a remarkably broad cell range within its host, including parenchymal cells and connective tissue cells of virtually any organ and various hematopoietic cell types. Epithelial cells, endothelial cells, fibroblasts and smooth muscle cells are the predominant targets for virus replication. The pathogenesis of acute HCMV infections is greatly influenced by this broad target cell range. Infection of epithelial cells presumably contributes to inter-host transmission. Infection of endothelial cells and hematopoietic cells facilitates systemic spread within the host. Infection of ubiquitous cell types such as fibroblasts and smooth muscle cells provides the platform for efficient proliferation of the virus.

C. Sinzger
Institute of Medical Virology, University of Tübingen, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany
christian.sinzger@med.uni-tuebingen.de

T.E. Shenk and M.F. Stinski (eds.), *Human Cytomegalovirus.*
Current Topics in Microbiology and Immunology 325.

The tropism for endothelial cells, macrophages and dendritic cells varies greatly among different HCMV strains, mostly dependent on alterations within the UL128-131 gene locus. In line with the classification of the respective proteins as structural components of the viral envelope, interstrain differences concerning the infectivity in endothelial cells and macrophages are regulated on the level of viral entry.

Target Cells of HCMV Infection

The question of which cell types in which tissues are targets of HCMV infection derives its relevance from the trivial fact that a virus can only live inside its host cell. The biology of a virus and, even more, its pathogenic effects within the infected organism are therefore inevitably linked to the spectrum of susceptible cell types.

Target Cells of HCMV In Vivo

Generally speaking, HCMV is tightly restricted to humans on the host level, but within the human host it can spread to virtually any tissue due to an exceptionally broad range of target cell types. In fact, it is easier to list the cell types that do not support HCMV replication: despite early reports about some degree of IE gene expression in lymphocytes in cell culture (Rice et al. 1984), we have not found IE antigens in cells of lymphoid origin during extensive immunohistochemical analyses of various organ tissues from acutely infected patients (Sinzger et al. 1995). A similar block of viral replication occurs in polymorphonuclear leukocytes. While these cells can take up virus particles and express IE antigens to some extent, transcripts and proteins of the early and late phase of viral replication are not found (Grefte et al. 1994; Sinzger et al. 1996). These two exceptions are faced by a long list of susceptible cell types, including various cells of ectodermal, mesodermal and endodermal origin. Most prominent examples are epithelial cells of glands and mucosal tissues, connective tissue cells in various organs, smooth muscle cells predominantly in the gastrointestinal tract and vascular endothelial cells (Sinzger et al. 1993; Ng Bautista and Sedmak 1995; Sinzger et al. 1995). Due to the strict host specificity, HCMV infection cannot be studied experimentally in animals, and in vivo data are hence only available from the analysis of diagnostic patient samples or autopsy materials. Dynamic aspects of viral replication and spread have therefore only been addressable within the blood compartment (Emery et al. 1999). Nevertheless, multiple circumstantial evidence strongly suggests successful viral replication in the above-mentioned cell types: Numerous capsids in the nucleus of infected cells as detected by electron microscopy unequivocally represent late-stage infection (Donnellan et al. 1966; Martin and Kurtz 1966; Kasnic et al. 1982; Balazs 1984; Francis

et al. 1989; Schwartz et al. 1990; Grefte et al. 1993). Likewise, detection of late structural viral proteins in infected cells argues in the same direction, and the combination of the latter approach with the additional detection of cell marker proteins allowed for a reliable identification of the respective cell types (Sinzger et al. 1993, 1996, 1999a; Digel and Sinzger 2006). Together with the often focal distribution of clusters of infected cells this provided strong evidence that mucosal epithelial cells, connective tissue cells, smooth muscle cells and endothelial cells can produce and transmit viral progeny to their environment (Fig. 1a). HCMV replication can be detected in almost every organ during acute infection under certain conditions, e.g., severe cases of intrauterine infection (Bissinger et al. 2002). Liver, gastrointestinal tract, lung, retina and brain are predominant sites of clinical manifestations of HCMV infections in immunocompromised hosts (Plachter et al. 1996). Within these organs, highly specialized parenchymal cells are frequent targets of HCMV infection, including hepatocytes in the liver (Sinzger et al. 1999a), alveolar epithelial cells in the lung (Ng Bautista and Sedmak 1995; Sinzger et al. 1995), and neuronal cells in retina and brain (Wiley and Nelson 1988; Schmidbauer et al. 1989; Rummelt et al. 1994). In principle, HCMV can thus cause extensive lesions because of its cytolytic nature, which is, however, in most cases limited by a marked cellular immune response (Sinzger and Jahn 1996).

Target Cells of HCMV in Cell Culture

An increasing number of cell culture models almost perfectly reflect the in vivo situation concerning susceptibility of the various cell types. Again, lymphocytes and granulocytes are among the few cell types that were not found to support replication of HCMV in vitro, although they may still act as a passive vehicle for HCMV transmission. On the contrary, the list of susceptible primary cell cultures is long, including skin or lung fibroblasts, vascular smooth muscle cells (Tumilowicz et al. 1985), retina pigment epithelial cells (Tugizov et al. 1996), placental trophoblast cells (Halwachs-Baumann et al. 1998), hepatocytes (Sinzger et al. 1999a), neuronal and glial brain cells (Poland et al. 1990), kidney epithelial cells (Heieren et al. 1988), monocyte-derived macrophages (Ibanez et al. 1991; Lathey and Spector 1991), monocyte-derived dendritic cells (Riegler et al. 2000), and vascular endothelial cells (Ho et al. 1984; Waldman et al. 1989). All of these primary cell types support the complete viral replication cycle, acquire a uniform cytomegalic appearance during the late replication phase and are finally lysed (Fig. 1b). In addition, limited replication can be achieved in a number of immortalized cell lines such as glioblastoma cells, teratocarcinoma cell lines or monocytic cell lines. However, some kind of differentiation is often necessary to render such cell lines supportive of a complete replication cycle (Shelbourn et al. 1989; Ibanez et al. 1991; Lathey and Spector 1991; Spiller et al. 1997; Sinclair and Sissons

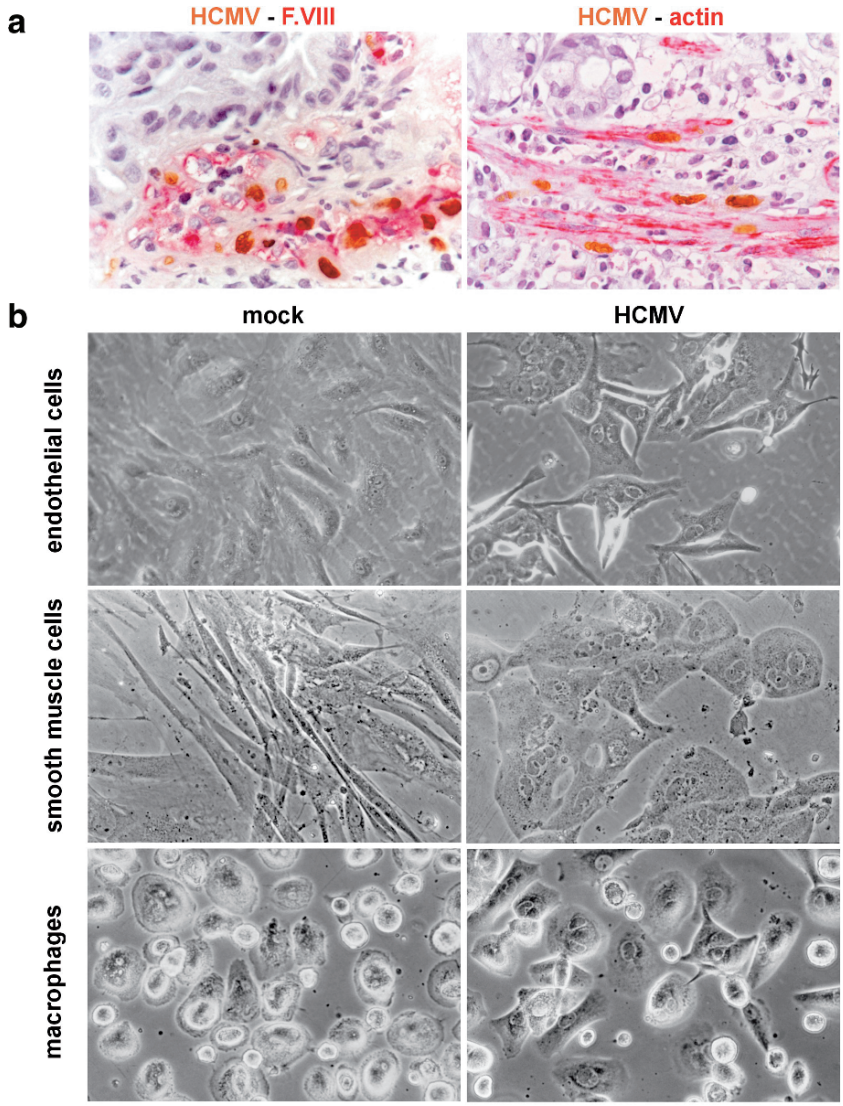

Fig. 1 **a** Immunohistochemical evidence of productive infection in endothelial cells and smooth muscle cells in vivo, as indicated by focus formation within the respective cell layers. *Brown nuclear signals*, detection of HCMV immediate early antigens by indirect immunoperoxidase labeling; *red cytoplasmic signals*, detection of F.VIII-related antigen (endothelial cells) and actin (smooth muscle cells) by indirect immunoalkaline phosphatase labeling; *blue nuclear signals*, counterstaining with hematoxilin. **b** Phase contrast micrographs of HCMV-infected cell cultures. Irrespective of the great morphological differences between cultured cells prior to infection, HCMV productive replication results in uniform morphological appearance with cytomegaly and nuclear inclusions

2006). While in vivo analyses were apt to descriptively identify the cell types infected by HCMV in its natural host, cell culture models made it possible to address quantitative aspects regarding susceptibility and productivity, thus revealing striking differences between cells of different origin: skin or lung fibroblast have always been the standard cell type for isolation and propagation of HCMV from patient samples and are still the most efficient producer cell line irrespective of the virus strain (Mocarski et al. 2006). For certain HCMV strains, vascular endothelial cells are also sufficiently susceptible and productive to allow long-term propagation of certain virus strains by passaging cell-free supernatants of infected cultures (Digel and Sinzger 2006). Other cell cultures, e.g., monocyte-derived macrophages, are low-level productive (Sinzger et al. 2006) and hardly release sufficient amounts of infectious progeny to maintain the virus during repeated passaging of cell-free supernatant on the respective cell type.

Cell Tropism

Along with the description of quantitative differences regarding susceptibility and productivity, different definitions of cell tropism may be applied to describe different aspects of HCMV-host cell interactions. In a broader sense, cell tropism refers to the simple fact that cells can be entered by the virus and will subsequently express viral genes. More functional definitions of cell tropism may take into account whether the infection is effective. From a biologist's point of view, cell tropism might define those target cells in which the virus can successfully reproduce. From a pathologists view, cell tropism might define those target cells that are damaged by the virus regarding cell survival and/or specific cellular functions.

Thus, even though HCMV infection of polymorphonuclear cells is abortive (Grefte et al. 1994), these cells can obviously contribute to hematogenous spread via the blood stream by carrying internalized virions (Gerna et al. 2000). Similarly, even though generation of viral progeny appears to be rather ineffective in monocytes/macrophages (Sinzger et al. 2006), this might suffice to start infection in an organ after transmigration through the vascular endothelium. Likewise, low-level production in placental trophoblast (Halwachs-Baumann et al. 1998), even though insufficient for long-term propagation of HCMV in trophoblast cultures, can contribute critically to intrauterine infection of the fetus (see also the chapter by L. Pereira and E. Maidji, this volume). Skin fibroblasts, lung fibroblasts and human umbilical vein endothelial cells are suitable for long-term propagation of clinical HCMV isolates, i.e., these cells have a reproductive index greater than 1. During the initial passages when isolates grow strictly cell-associated (Yamane et al. 1983), this would result in an increase of the fraction of infected cells within the culture. After adaptation to a cell-free infection mode, a reproductive index of greater than 1 would mean that progeny production exceeds virus input in the respective cell culture. The cell type used for

propagation of an isolate has an effect on the cell tropism of the resulting HCMV strain. Apparently, long-term propagation in fibroblasts selects for HCMV strains with low endothelial cell tropism, whereas long-term propagation in endothelial cells maintains the broader cell tropism characteristic for recent clinical HCMV isolates (Waldman et al. 1991; Sinzger et al. 1999b). Whether propagation in other cell types such as smooth muscle cells or macrophages would also results in a restricted cell tropism has not been tested. Likewise, it is unknown whether an adaptation of HCMV to certain cell types also takes place during natural infection in vivo, i.e., whether tropism variants of HCMV exist within one patient. At present, the assumption of different cell tropism variants in different organ tissues is still speculative, but first hints in that direction come from reports on a strictly localized reactivation of HCMV, e.g., in the lactating breast (Hamprecht et al. 2003). Apart from viral determinants, a tissue-specific immune control might also contribute to differences in the apparent organ tropism of HCMV replication, similar to the apparent tropism of MCMV for the salivary gland (Jonjic et al. 1989). Experimental data from murine cytomegalovirus indicate that under the complex in vivo conditions the apparent cell tropism can be further modified by the microenvironment within a certain tissue. For example, proapoptotic stimuli from surrounding immune cells can limit infection in an otherwise susceptible cell type (Patrone et al. 2003), and this proapoptotic effect might even occur in a cell type-specific manner. For a refined understanding of HCMV's in vivo cell tropism, future work should therefore take into account how the complex organ-typical interactions might influence the susceptibility of target cells for HCMV infection, e.g., by analyzing complex organ tissue cultures (Reinhardt et al. 2003).

In conclusion, the strict host tropism of HCMV is contrasted by a remarkably broad cell tropism within its host, with epithelial cells, endothelial cells, fibroblasts and smooth muscle cells being the predominant targets for virus replication. The discrepancy between in vivo findings and cell culture data has diminished with the introduction of more recent HCMV strains and their application in various primary cell cultures. Since the basic cell culture tools reflecting the in vivo cell tropism of HCMV are now available, the analysis of compound cell culture systems representing the complex composition of organ tissues can be targeted in the future.

Pathogenetic Role of Selected Cell Types

The broad target cell range provides the basis for a highly complex interaction between HCMV and the human host, which can be adapted to many different situations during their lifelong relationship. It should always be kept in mind that HCMV can successfully enter its host, spread within the body, establish latency, reactivate frequently throughout life and be transmitted to other individuals mostly without ever causing clinically apparent disease (for aspects of latency, see the chapters by M. Reeves and J. Sinclair, this volume and M.J. Reddehase

et al., this volume). Many of these aspects of HCMV's silent life are still a matter of speculation. More robust information is available on the contribution of certain cell types to viral dissemination and organ infection under conditions when insufficient immune control allows virus replication to exceed the threshold of clinical manifestation (see also the chapter by W. Britt, this volume). Conclusions from analyses of severely ill patients on the behavior of HCMV in the normal host (Fig. 2) are therefore made, with the provision that an intact immune control may modify the apparent cell and organ tropism.

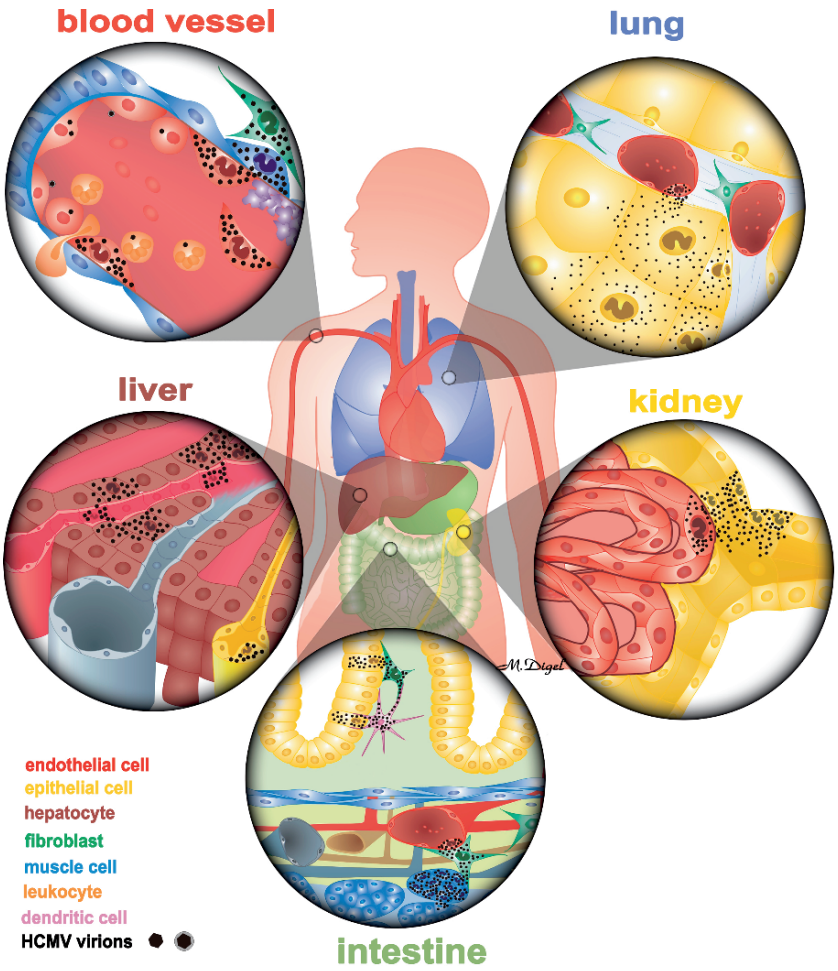

Fig. 2 Hypothetical contribution of various cell types to hematogenous dissemination and organ manifestation as deduced from immunohistochemical findings and cell culture data. *Black dots* represent virus

Epithelial Cells

Epithelial cells are a major target of HCMV infection (Sinzger et al. 1995) and can therefore be assumed to play an important role during host-to-host transmission as they line all external body surfaces. Most likely, HCMV enters a new host by infection of mucosal epithelium. For example, HCMV newborns and infants can be infected by breast milk of a seropositive mother, a highly efficient transmission route which accounts for the majority of HCMV transmissions during early childhood (Stagno and Cloud 1994). More than 95% of seropositive breastfeeding women reactivate HCMV locally, shed cell-free infectivity into the milk, and 30%-40% of them will transmit HCMV to their children (Hamprecht et al. 2001). The infants' mucosal surfaces throughout the gastrointestinal tract are exposed during feeding, and epithelial cells in all parts of the gastrointestinal tract are susceptible and obviously support productive infection. They are the most likely candidates for primary replication of incoming HCMV. However, as these first steps of infection are hardly ever recognized, there are no data available for a direct proof of these considerations. Alternatively, similar to HIV, dendritic cells could also contribute to entry via mucosal surfaces.

More direct data are available supporting a role of epithelial cells in shedding HCMV into body fluids. During acute productive infection, late-stage-infected epithelial cells have been detected in salivary glands, kidney and various parts of the gastrointestinal tract (Variend and Pearse 1986; Sinzger et al. 1995; Bissinger et al. 2002). Undoubtedly, these cells are a source of infectivity detected within saliva, urine and stool and may thus contribute to HCMV transmission via these excretions.

Dendritic Cells

Because of their complex biology, dendritic cells (DCs) may play various roles in the pathogenesis of HCMV infections, resulting in proviral as well as antiviral effects. Immature DCs are resident in virtually all mucosal and epidermal surfaces of the body, controlling for the invasion of foreign organisms. They are well equipped for highly efficient endocytic uptake of material from their environment, e.g., pathogens or remnants from apoptotic cells. Uptake of infectious HCMV into DCs can result in viral replication and release of viral progeny (Riegler et al. 2000). On the other hand, endocytic uptake of HCMV may lead to processing of viral proteins and presentation of viral epitopes by MHC class I and II molecules. This is counteracted to some extent by HCMV-induced downregulation of several immune-stimulatory surface molecules including MHC class I and II (Grigoleit et al. 2002; Moutaftsi et al. 2002; Hertel et al. 2003). As a consequence, HCMV-infection decreases the immune-stimulatory capacity of DCs (Grigoleit et al. 2002; Moutaftsi et al. 2002; Hertel et al. 2003). For immature DCs, antigen uptake is a

maturation stimulus, and with maturation DCs downmodulate their endocytic activity and upregulate peptide processing and presentation. Upon maturation, they become mobile and are led by their homing receptors toward lymphatic tissues. Interestingly, immature Langerhans type DCs, which reside in epidermal and mucosal tissues, only become highly susceptible after maturation (Hertel et al. 2003; Reeves et al. 2005), whereas immature interstitial type DCs can be readily infected by HCMV (Riegler et al. 2000; Moutaftsi et al. 2002). It is tempting to speculate that HCMV is endocytosed by immature DCs in mucosal tissues, thus providing a maturation stimulus, which then leads to migration toward the draining lymph node and renders the cells permissive to HCMV replication. In the lymph node, productively infected mature DCs may spread the virus to other cells, whereas their immune-stimulatory capacity may be restricted. To further temper an antiviral immune response, infected mature DCs may directly and indirectly inhibit T cell functions (Raftery et al. 2001). However, despite such immunosuppressive effects, immunocompetent hosts regularly develop a strong T cell response protecting from clinical manifestations of the infection. Cross-presentation of viral antigens by DCs following uptake of apoptotic material from infected cells has been described as an explanation for the well-known robust immune response to HCMV in the normal host (Tabi et al. 2001).

Fibroblasts

Fibroblasts are not only the standard cell culture system for propagation of HCMV to high titers (Mocarski et al. 2006), but they are also among the major targets of HCMV in vivo (Sinzger et al. 1995). Efficient replication in such a ubiquitous cell type opens the possibility for HCMV to replicate in virtually every organ. Consequently, infected connective tissue cells are assumed to contribute to efficient spread of HCMV in organs as different as adrenal glands, bone marrow, heart, kidney, liver, lung, pancreas, placenta, small bowel and spleen (Bissinger et al. 2002). If the particular property of cultured fibroblast to generate and release high titers of viral progeny also applies for infected connective tissue cells in vivo, then they might contribute greatly to the highly dynamic proliferation of HCMV during acute infections (Emery et al. 1999).

Smooth Muscle Cells

Like fibroblasts, smooth muscle cells are also ubiquitously distributed throughout the body. Their basic function is generation of kinetic force by contraction of the actin-myosin skeleton, which may be controlled either by the autonomic nervous system, hormones or stimuli from neighboring cells. Given their spatial organization as multicellular layers in the wall of hollow

organs, they regulate the dynamic shape and intraluminal pressure of these organs and help in maintaining organ integrity. Smooth muscle cells are susceptible to productive HCMV infection (Tumilowicz et al. 1985), which may have important pathophysiological consequences. When the host's immune response is severely compromised, focal expansion with subsequent lytic replication in the gastrointestinal tract can result in ulceration (Sinzger et al. 1995) and perforation (Genta et al. 1993), with sometimes fatal outcome. In the immunocompetent host, infection of vascular smooth muscle cells may be pathogenetically important. In these cells, HCMV downregulates extracellular matrix proteins, which may contribute to the development of inflammatory vasculopathies (Reinhardt et al. 2006). In addition, lytic infection of vascular smooth muscle cells might provoke a response to injury reaction, and consequently HCMV is considered a possible pathogenetic (co)factor in the context of atherosclerosis (Stassen et al. 2006).

Endothelial Cells

Speculation on an association of HCMV with vascular damage is additionally supported by the marked endothelial cell tropism of HCMV in vivo. Again, the ubiquitous distribution of small vessels throughout the body is reflected by the detection of HCMV-infected microvascular endothelial cells in various organs, e.g., brain, lung, liver, kidney and the complete gastrointestinal tract (Myerson et al. 1984; Wiley and Nelson 1988; Roberts et al. 1989; Sinzger et al. 1995; Bissinger et al. 2004). They support productive lytic infection and can hence promote hematogenous dissemination HCMV from the circulating blood into organ tissues, often accompanied by a vasculitic response around infected vessel walls (Roberts et al. 1989; Sinzger et al. 1995). Macrovascular endothelial cells are also susceptible to productive lytic infection (Kahl et al. 2000) and combined damage of the endothelial layer and the underlying smooth muscle layer may initiate the cascade of defense reactions finally resulting in vascular lesions. While the contribution of HCMV to atherosclerosis in the general population is still a matter of debate (see also the chapter by D.N. Streblow, this volume), the association is very clear in patients after heart transplantation (Valantine 2004; Potena et al. 2006).

Leukocytes

The disposition of HCMV to systemic dissemination and multiorgan involvement has already been mentioned. Leukocytes are assumed to be a central player with regard to hematogenous spread of the virus, whether by being a target of permissive

infection or by passively transporting infectious particles as a vehicle. The latter obviously applies for polymorphonuclear cells, which can take up virus particles and express viral immediate early proteins but do not support the full replicative cycle (Grefte et al. 1994). Even though these cells can not produce viral progeny, they are still capable of transmitting the infection to other cell types, as evidenced by frequent isolation of HCMV from polymorphonuclear cells of immunocompromised patients (Gerna et al. 1992), and this is most likely due to attachment and partial localized fusion of cell membranes with subsequent transfer of engulfed (sub)viral particles, as shown in the opposite direction for the transfer of HCMV from endothelial cells to polymorphonuclear cells (Gerna et al. 2000). In line with these hypotheses, (a) infectivity is predominantly found in the polymorphonuclear fraction of whole blood (Schafer et al. 2000), (b) detection of the viral structural antigen pp65 (pUL83) in polymorphonuclear cells can be clinically used as a marker of acute HCMV infection (The et al. 1990; Gerna et al. 1991) and (c) removal of white blood cells from whole blood prior to transfusion almost completely reduces the risk of HCMV transmission (Gilbert et al. 1989). Monocytes, although a minor target cell with regard to frequency, might also contribute to hematogenous spread of HCMV, particularly as monocyte-derived macrophages support the full replicative cycle (Ibanez et al. 1991; Lathey and Spector 1991). It is tempting to assume a scenario where monocytes rolling along the vascular endothelium take up infectious virus from productively infected endothelial cells at one site of the body, differentiate upon transmigration through an activated endothelial layer at a different site of the body (Waldman et al. 1995), and release virus progeny into the corresponding organ after maturation into tissue macrophages (Sinzger et al. 1996).

Apart from their role in acute HCMV infections, all susceptible cell types may in principle also be sites of viral latency, although experimental data point to a particular role of hematopoietic cells in that context (Sinclair and Sissons 2006 see also the chapters by M. Reeves and J. Sinclair, this volume). Taken together, the pathogenesis of acute HCMV infections is greatly influenced by the broad target cell range of this virus, with hematopoietic cells facilitating systemic spread, ubiquitous cell types like fibroblasts and smooth muscle cells providing the platform for efficient proliferation of the virus and epithelial cells contributing to interhost transmission.

Cell Biological Basis of HCMV Cell Tropism

The longstanding paradox between broad cell tropism of HCMV in vivo and a restricted target cell range of the available virus strains in cell culture has been resolved by the introduction of endothelial-propagated virus strains with a well-preserved natural cell tropism. This enabled recent progress toward the definition of viral genes governing interstrain differences in cell tropism and the first insights into the underlying virus-host interactions.

Interstrain Differences in Cell Tropism

The idea that HCMV strains may differ regarding their reproductive potential in certain cell cultures was already reported in 1980 (Albrecht and Weller 1980). The finding that extended propagation in fibroblasts regularly results in loss of endothelial cell tropism, whereas propagation in endothelial cells maintains a broad cell tropism of the respective strain (Waldman et al. 1991), made the issue of cell tropism accessible to further experimental analyses. Indeed, the phenotypic differences are very pronounced with a 100- to 1,000-fold reduction in endothelial cell tropism of fibroblast-adapted strains irrespective of the origin of endothelial cells (Kahl et al. 2000; Sinzger et al. 2000). Nevertheless, even severely fibroblast-adapted strains, such as AD169 or Towne, can infect endothelial cells to some extent, which may also depend on the origin of the endothelial cell culture. In consideration of this, such HCMV strains are more precisely classified as poorly endotheliotropic rather than nonendotheliotropic.

Interstrain differences in HCMV cell tropism occur as a cell culture artifact, but significant variation has also been described between recent clinical isolates from different patients (Sinzger et al. 1999b). Together with the finding that multiple isolates from the same patient behaved identically with regard to endothelial cell tropism, this suggests a natural interhost variability of HCMV cell tropism. This may contribute to the highly variable clinical course of HCMV infections in various patients (Sinzger et al. 1999b). This notion is further supported by the fact that a high endothelial cell tropism is apparently associated with high infection efficiency also in monocyte-derived macrophages and dendritic cells, cells that are all assumed to mediate hematogenous dissemination of HCMV (Jahn et al. 1999).

Viral Genes and Proteins Contributing to Cell Tropism

Restriction fragment analyses of differentially propagated HCMV strains showed that the restriction of cell tropism during fibroblast adaptation is associated with multiple genetic modifications (Sinzger et al. 1999b). The introduction of BACmid technology and subsequent screening procedures for the effect of genetic deletions led to the identification of several open reading frames involved in endothelial cell and leukocyte tropism. Dunn et al. found that the residual endothelial cell tropism of HCMV strain Towne is further reduced by deletion of the viral tegument UL24-protein, a member of the US22 gene family (Dunn et al. 2003). The highly endotheliotropic phenotype of an endothelial propagated strain was found to depend on the UL128-131 gene region (Hahn et al. 2004). Deletion of either open reading frame within this region strongly reduced endothelial cell tropism, epithelial cell tropism, dendritic cell tropism and the virus transfer rate to granulocytes (Hahn et al. 2004; Wang and Shenk 2005a). UL128, UL130 and also UL131 were found in complex with glycoproteins gH and gL within virion particles (Wang and Shenk 2005b;

Adler et al. 2006). Endothelial cell infection of fibroblast-adapted, poorly endotheliotropic HCMV strains Merlin, Towne and AD169 were rescued by transient expression of intact UL128, UL130 and UL131, respectively (Hahn et al. 2004; Patrone et al. 2005). This suggests that loss of endothelial cell tropism during fibroblast adaptation is frequently caused by alterations in this gene region. In contrast, it is still unclear whether this gene region also contributes to the variability in endothelial cell tropism among naturally occurring HCMV isolates (Sinzger et al. 1999b), as a series of 34 clinical isolates appeared to contain intact copies of these genes (Baldanti et al. 2006).

Critical Events for Replication in Various Cell Types

Regarding the replication steps critical for successful infection of various cell types, interstrain comparisons in endothelial cells showed a particular role of initial postpenetration events. The efficiency of nuclear translocation of incoming virions and subsequent delivery of the viral genome to the nucleus of penetrated endothelial cells is very low with fibroblast-adapted strains (Sinzger et al. 2000), and a similar block occurs in monocyte-derived macrophages (Sinzger et al. 2006). In contrast, these steps are strain-independent in fibroblasts. The demonstration that pUL128-131 are part of glycoprotein complexes with gH and gL in the virion envelope (Wang and Shenk 2005b; Adler et al. 2006) fit well with the finding that endothelial cell tropism of HCMV is determined during entry, as gH is known to be involved in fusion events (see also the chapter by M.K. Isaacson et al., this volume). The particular importance of initial events is further emphasized by recent data suggesting infection of endothelial cells by an endocytic route, in contrast to direct fusion at the plasma membrane of fibroblasts (Sinzger 2008). It appears that, unlike previously assumed (Bodaghi et al. 1999), endocytosis of HCMV in endothelial cells is not necessarily an abortive pathway (Fig. 3). For Epstein Barr virus, different gH/gL complexes are engaged in different cell types, leading either to direct fusion in lymphocytes or endocytosis in epithelial cells. Interestingly, a cell-type-dependent cell-cell fusion activity induced by gH-gL complexes was found in a transient expression system (Kinzler and Compton 2005).

The susceptibility of other cell types may be regulated at later steps of the replication cycle. For example, infection of polymorphonuclear leukocytes is aborted after onset of IE gene expression (Grefte et al. 1994), independent of the virus strain. The exact nature of this block of progression toward the early phase of replication is unknown. In trophoblast cells, hepatocyte or macrophage HCMV can proceed through all phases of the replication cycle, formation and/or release of viral progeny. However, the production of progeny is up to 1,000-fold less efficient than in fibroblasts (Halwachs-Baumann et al. 1998; Sinzger et al. 1999a, 2006) and again the factors contributing to these differences in productivity are not known.

In conclusion, genes UL128-131 classified as nonessential in fibroblast cultures have been shown to contribute to interstrain differences regarding infection of

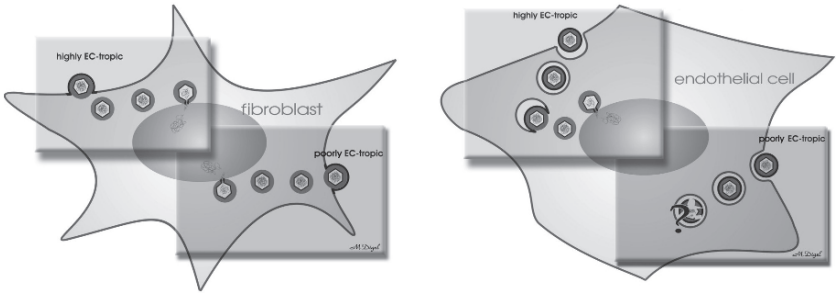

Fig. 3 Hypothetical mechanism mediating interstrain differences in endothelial cell tropism. While all HCMV strains can release their capsids into fibroblasts by direct fusion of their envelope with the plasma membrane, cell type differences are assumed for viral entry into endothelial cells. Both highly endotheliotropic and poorly endotheliotropic strains are internalized by endocytosis, but only highly endotheliotropic can escape from endocytic vesicles and release their capsid into the cytoplasm

endothelial cells, epithelial cells and macrophages. As the respective proteins are structural components of the envelope of virion particles, it is not unexpected that they exert their effects on the level of viral entry. The cellular counterparts mediating the cell-type-specificity of these virion components are to be defined.

Cell Tropism of Other Cytomegaloviruses

The tendency toward systemic dissemination resulting in infection of various organs is not unique to human CMV but has also been reported for animal CMVs. Apparently, a broad organ tropism is a hallmark of cytomegaloviruses, which is based on a similarly broad cell tropism.

Under conditions of severe immunosuppression, murine CMV-infected cells were found in lung, liver, spleen, kidneys, adrenals, gastrointestinal tract, brain, salivary gland, and fibroblasts, epithelial cells, neuronal cells, glial cells, ependymal cells hepatocytes and endothelial cells were identified as predominantly infected cell types within these tissues (Reddehase et al. 1985; Podlech et al. 1998; van Den Pol et al. 1999; Podlech et al. 2000). Likewise, a broad target cell range including fibroblasts, SMC, EC, macrophages was found with rat CMV (Kloover et al. 2000; van der Strate et al. 2003; Streblow et al. 2007).

While the histological distribution of HCMV and MCMV appears almost indistinguishable, the underlying mechanisms regulating cell tropism are apparently not completely conserved between cytomegaloviruses from different species. Deletion of the m45 gene abrogated replication of MCMV in endothelial cell cultures (Brune et al. 2001) by sensitizing infected endothelial cells to apoptosis. In contrast, deletion of UL45 did not influence the endothelial cell tropism of HCMV strain

FIXBAC (Hahn et al. 2002). On the other hand, involvement on US22 family members in cell tropism regulation of both HCMV and MCMV indicated a certain degree of conservation. The contribution of UL24 to endothelial cell tropism of HCMV strain Towne (Dunn et al. 2003) has already been mentioned. US22-family members of MCMV, namely m139, m140 and m141 contribute to macrophage tropism and promote MCMV replication in the spleen of infected mice (Hanson et al. 2001; Menard et al. 2003). M36, another US22 family gene of MCMV, also contributes to efficient replication in macrophages again through its anti-apoptotic function (Menard et al. 2003). Obviously, cell-type-specific inhibition of virus-induced apoptosis is a more general theme with cytomegaloviruses, suggesting that similar tropism-relevant anti-apoptotic genes may also exist in the HCMV genome (see also the chapter by A.L. McCormick, this volume). The HCMV counterpart of M36, UL36, has a known antiapoptotic function (Skaletskaya et al. 2001), which has not yet been tested in the context of cell tropism. UL45, the HCMV homolog of M45, exhibited a weak antiapoptotic activity only upon application of strong proapoptotic stimuli (Patrone et al. 2003) and was dispensable for viral replication in endothelial cells (Hahn et al. 2002). However, in vivo the situation may be different depending on the microenvironment within the infected tissues. In the presence of strong proapoptotic stimuli, UL36 and UL45 may be essential for successful completion of viral replication in a cell-type-dependent fashion, as reported for their murine CMV homologs. Complex cell culture systems reflecting the situation of an inflamed tissue are required to test this hypothesis.

A specific contribution of rat CMV concerns the role of vascular endothelial cells and smooth muscle cells in CMV-associated pathogenesis. Under the well-defined conditions of this animal model, a contribution of vascular CMV infection to the development of atherosclerotic lesions is clearly shown and the molecular mechanisms are partially deciphered, including oxLDL uptake, altering monocyte adhesion or increasing the production of pro-inflammatory cytokines (Stassen et al. 2006). In the human system, it may be impossible to prove the contribution of HCMV to a multifactorial disease such as atherosclerosis under natural clinical conditions. However, similarities between RCMV and HCMV regarding cytopathic effects in vascular cell types nevertheless suggest CMV as a proatherosclerotic agent also in humans.

Impact of Cell Tropism Analyses

The ability of CMV species to infect a variety of different cell types in their respective host appears to be central for successful entry, dissemination, persistence, reactivation and excretion. Analyzing CMV replication in various cell culture systems is therefore an absolute requirement for a comprehensive understanding of their biology and will in itself create additional value. Particularly, many of the genes still classified nonessential with regard to replication in the standard cell culture system will turn out to be essential if tested in other cell types or in complex tissues composed of several interacting cell types.

The practical impact of cell tropism issues may concern the design of cytomegalovirus vaccines. Given the limited success of vaccination with the highly adapted strain Towne, it appears as if a higher level of replication within the vaccinee is necessary for induction of a more robust immune response. Therefore, a more moderate attenuation with partial preservation of endothelial and epithelial cell tropism may be desirable. The introduction of small nonlethal mutations within known tropism genes is one possible approach to achieve such intermediate phenotypes.

Finally, an exact definition of entry pathways in diverse cell types may allow for the development of novel antiviral intervention strategies. At present, all anti-CMV chemotherapies target viral DNA replication. By analogy to HIV, entry inhibitors may complement the available drugs and allow for synergistic effects in combination therapies. Such an approach should certainly consider the possibility of different entry pathways in major target cell types such as fibroblasts and endothelial cells.

Acknowledgements This work was supported by the German research foundation (DFG SI 779/3-2).

References

Adler B, Scrivano L, Ruzcics Z, Rupp B, Sinzger C, Koszinowski U (2006) Role of human cytomegalovirus UL131A in cell type-specific virus entry and release. J Gen Virol 87:2451-2460

Albrecht T, Weller TH (1980) Heterogeneous morphologic features of plaques induced by five strains of human cytomegalovirus. Am J Clin Pathol 73:648-654

Balazs M (1984) Electron microscopic examination of congenital cytomegalovirus hepatitis. Virchows Arch A Pathol Anat Histopathol 405:119-129

Baldanti F, Paolucci S, Campanini G, Sarasini A, Percivalle E, Revello MG, Gerna G (2006) Human cytomegalovirus UL131A, UL130 and UL128 genes are highly conserved among field isolates. Arch Virol 151:1225-1233

Bissinger AL, Sinzger C, Kaiserling E, Jahn G (2002) Human cytomegalovirus as a direct pathogen: correlation of multiorgan involvement and cell distribution with clinical and pathological findings in a case of congenital inclusion disease. J Med Virol 67:200-206

Bissinger AL, Oettle H, Jahn G, Neuhaus P, Sinzger C (2004) Cytomegalovirus infection after orthotopic liver transplantation is restricted by a pre-existing antiviral immune response of the recipient. J Med Virol 73:45-53

Bodaghi B, Slobbe-van Drunen ME, Topilko A, Perret E, Vossen RC, van Dam-Mieras MC, Zipeto D, Virelizier JL, LeHoang P, Bruggeman CA, Michelson S (1999) Entry of human cytomegalovirus into retinal pigment epithelial and endothelial cells by endocytosis. Invest Ophthalmol Vis Sci 40:2598-2607

Brune W, Menard C, Heesemann J, Koszinowski UH (2001) A ribonucleotide reductase homolog of cytomegalovirus and endothelial cell tropism. Science 291:303-305

Digel M, Sinzger C (2006) Determinants of endothelial cell tropism of human cytomegalovirus. In: Reddehase MJ (ed) Cytomegaloviruses: molecular biology and immunology. Caister Academic Press, Norfolk, pp 445-464

Donnellan WL, Chantra-Umporn S, Kidd JM (1966) The cytomegalic inclusion cell. An electron microscopic study. Arch Pathol 82:336-348

Dunn W, Chou C, Li H, Hai R, Patterson D, Stolc V, Zhu H, Liu F (2003) Functional profiling of a human cytomegalovirus genome. Proc Natl Acad Sci USA 100:14223-14228

Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD (1999) The dynamics of human cytomegalovirus replication in vivo. J Exp Med 190:177-182

Francis ND, Boylston AW, Roberts AH, Parkin JM, Pinching AJ (1989) Cytomegalovirus infection in gastrointestinal tracts of patients infected with HIV-1 or AIDS. J Clin Pathol 42:1055-1064

Gerna G, Zipeto D, Parea M, Revello MG, Silini E, Percivalle E, Zavattoni M, Grossi P, Milanesi G (1991) Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia, and DNAemia. J Infect Dis 164:488-498

Gerna G, Zipeto D, Percivalle E, Parea M, Revello MG, Maccario R, Peri G, Milanesi G (1992) Human cytomegalovirus infection of the major leukocyte subpopulations and evidence for initial viral replication in polymorphonuclear leukocytes from viremic patients. J Infect Dis 166:1236-1244

Genta RM, Bleyzer I, Cate TR, Tandon AK, Yoffe B (1993) In situ hybridization and immunohistochemical analysis of cytomegalovirus-associated ileal perforation. Gastroenterology 104:1822-1827

Gerna G, Percivalle E, Baldanti F, Sozzani S, Lanzarini P, Genini E, Lilleri D, Revello MG (2000) Human cytomegalovirus replicates abortively in polymorphonuclear leukocytes after transfer from infected endothelial cells via transient microfusion events. J Virol 74:5629-5638

Gilbert GL, Hayes K, Hudson IL, James J (1989) Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leucocytes. Neonatal Cytomegalovirus Infection Study Group. Lancet 1:1228-1231

Grefte A, Blom N, van der Giessen M, van Son W, The TH (1993) Ultrastructural analysis of circulating cytomegalic cells in patients with active cytomegalovirus infection: evidence for virus production and endothelial origin. J Infect Dis 168:1110-1118

Grefte A, Harmsen MC, van der Giessen M, Knollema S, van Son WJ, The TH (1994) Presence of human cytomegalovirus (HCMV) immediate early mRNA but not ppUL83 (lower matrix protein pp65) mRNA in polymorphonuclear and mononuclear leukocytes during active HCMV infection. J Gen Virol 75:1989-1998

Grigoleit U, Riegler S, Einsele H, Laib Sampaio K, Jahn G, Hebart H, Brossart P, Frank F, Sinzger C (2002) Human cytomegalovirus induces a direct inhibitory effect on antigen presentation by monocyte-derived immature dendritic cells. Br J Haematol 119:189-198

Hahn G, Khan H, Baldanti F, Koszinowski UH, Revello MG, Gerna G (2002) The human cytomegalovirus ribonucleotide reductase homolog UL45 is dispensable for growth in endothelial cells, as determined by a BAC-cloned clinical isolate of human cytomegalovirus with preserved wild-type characteristics. J Virol 76:9551-9555

Hahn G, Revello MG, Patrone M, Percivalle E, Campanini G, Sarasini A, Wagner M, Gallina A, Milanesi G, Koszinowski U, Baldanti F, Gerna G (2004) Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. J Virol 78:10023-10033

Halwachs-Baumann G, Wilders-Truschnig M, Desoye G, Hahn T, Kiesel L, Klingel K, Rieger P, Jahn G, Sinzger C (1998) Human trophoblast cells are permissive to the complete replicative cycle of human cytomegalovirus. J Virol 72:7598-7602

Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G (2001) Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet 357:513-518

Hamprecht K, Maschmann J, Vochem M, Speer CP, Jahn G (2003) Transmission of cytomegalovirus to preterm infants by breast-feeding. In: Proesch S, Cinatl J, Scholz M (eds) New aspects of CMV-related immunopathology, vol 24. Karger, Basel, pp 33-42

Hanson LK, Slater JS, Karabekian Z, Ciocco-Schmitt G, Campbell AE (2001) Products of US22 genes M140 and M141 confer efficient replication of murine cytomegalovirus in macrophages and spleen. J Virol 75:6292-6302

Heieren MH, Kim YK, Balfour HH Jr (1988) Human cytomegalovirus infection of kidney glomerular visceral epithelial and tubular epithelial cells in culture. Transplantation 46:426-432

Hertel L, Lacaille VG, Strobl H, Mellins ED, Mocarski ES (2003) Susceptibility of immature and mature Langerhans cell-type dendritic cells to infection and immunomodulation by human cytomegalovirus. J Virol 77:7563-7574

Ho DD, Rota TR, Andrews CA, Hirsch MS (1984) Replication of human cytomegalovirus in endothelial cells. J Infect Dis 150:956-957

Ibanez CE, Schrier R, Ghazal P, Wiley C, Nelson JA (1991) Human cytomegalovirus productively infects primary differentiated macrophages. J Virol 65:6581-6588

Jahn G, Stenglein S, Riegler S, Einsele H, Sinzger C (1999) Human cytomegalovirus infection of immature dendritic cells and macrophages. Intervirology 42:365-372

Jonjic S, Mutter W, Weiland F, Reddehase MJ, Koszinowski UH (1989) Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. J Exp Med 169:1199-1212

Kahl M, Siegel-Axel D, Stenglein S, Jahn G, Sinzger C (2000) Efficient lytic infection of human arterial endothelial cells by human cytomegalovirus strains. J Virol 74:7628-7635

Kasnic G Jr, Sayeed A, Azar HA (1982) Nuclear and cytoplasmic inclusions in disseminated human cytomegalovirus infection. Ultrastruct Pathol 3:229-235

Kinzler ER, Compton T (2005) Characterization of human cytomegalovirus glycoprotein-induced cell-cell fusion. J Virol 79:7827-7837

Kloover JS, Hillebrands JL, de Wit G, Grauls G, Rozing J, Bruggeman CA, Nieuwenhuis P (2000) Rat cytomegalovirus replication in the salivary glands is exclusively confined to striated duct cells. Virchows Arch 437:413-421

Lathey JL, Spector SA (1991) Unrestricted replication of human cytomegalovirus in hydrocortisone-treated macrophages. J Virol 65:6371-6375

Martin AM Jr, Kurtz SM (1966) Cytomegalic inclusion disease. An electron microscopic histochemical study of the virus at necropsy. Arch Pathol 82:27-34

Menard C, Wagner M, Ruzsics Z, Holak K, Brune W, Campbell AE, Koszinowski UH (2003) Role of murine cytomegalovirus US22 gene family members in replication in macrophages. J Virol 77:5557-5570

Mocarski ES, Shenk T, Pass RF (2006) Cytomegaloviruses. In: Knipe DM (ed) Fields virology. Lippincott Williams and Wilkins, Philadelphia, pp 2701-2772

Moutaftsi M, Mehl AM, Borysiewicz LK, Tabi Z (2002) Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. Blood 99:2913-2921

Myerson D, Hackman RC, Nelson JA, Ward DC, McDougall JK (1984) Widespread presence of histologically occult cytomegalovirus. Hum Pathol 15:430-439

Ng Bautista CL, Sedmak DD (1995) Cytomegalovirus infection is associated with absence of alveolar epithelial cell HLA class II antigen expression. J Infect Dis 171:39-44

Patrone M, Percivalle E, Secchi M, Fiorina L, Pedrali-Noy G, Zoppe M, Baldanti F, Hahn G, Koszinowski UH, Milanesi G, Gallina A (2003) The human cytomegalovirus UL45 gene product is a late, virion-associated protein and influences virus growth at low multiplicities of infection. J Gen Virol 84:3359-3370

Patrone M, Secchi M, Fiorina L, Ierardi M, Milanesi G, Gallina A (2005) Human cytomegalovirus UL130 protein promotes endothelial cell infection through a producer cell modification of the virion. J Virol 79:8361-8373

Plachter B, Sinzger C, Jahn G (1996) Cell types involved in replication and distribution of human cytomegalovirus. Adv Virus Res 46:195-261

Podlech J, Holtappels R, Wirtz N, Steffens HP, Reddehase MJ (1998) Reconstitution of CD8 T cells is essential for the prevention of multiple-organ cytomegalovirus histopathology after bone marrow transplantation. J Gen Virol 79:2099-2104

Podlech J, Holtappels R, Pahl-Seibert MF, Steffens HP, Reddehase MJ (2000) Murine model of interstitial cytomegalovirus pneumonia in syngeneic bone marrow transplantation: persistence of protective pulmonary CD8-T-cell infiltrates after clearance of acute infection. J Virol 74:7496-7507

Poland SD, Costello P, Dekaban GA, Rice GP (1990) Cytomegalovirus in the brain: in vitro infection of human brain-derived cells. J Infect Dis 162:1252-1262

Potena L, Holweg CT, Chin C, Luikart H, Weisshaar D, Narasimhan B, Fearon WF, Lewis DB, Cooke JP, Mocarski ES, Valantine HA (2006) Acute rejection and cardiac allograft vascular disease is reduced by suppression of subclinical cytomegalovirus infection. Transplantation 82:398-405

Raftery MJ, Schwab M, Eibert SM, Samstag Y, Walczak H, Schonrich G (2001) Targeting the function of mature dendritic cells by human cytomegalovirus: a multilayered viral defense strategy. Immunity 15:997-1009

Reddehase MJ, Weiland F, Munch K, Jonjic S, Luske A, Koszinowski UH (1985) Interstitial murine cytomegalovirus pneumonia after irradiation: characterization of cells that limit viral replication during established infection of the lungs. J Virol 55:264-273

Reeves MB, Lehner PJ, Sissons JG, Sinclair JH (2005) An in vitro model for the regulation of human cytomegalovirus latency and reactivation in dendritic cells by chromatin remodelling. J Gen Virol 86:2949-2954

Reinhardt B, Vaida B, Voisard R, Keller L, Breul J, Metzger H, Herter T, Baur R, Luske A, Mertens T (2003) Human cytomegalovirus infection in human renal arteries in vitro. J Virol Methods 109:1-9

Reinhardt B, Winkler M, Schaarschmidt P, Pretsch R, Zhou S, Vaida B, Schmid-Kotsas A, Michel D, Walther P, Bachem M, Mertens T (2006) Human cytomegalovirus-induced reduction of extracellular matrix proteins in vascular smooth muscle cell cultures: a pathomechanism in vasculopathies? J Gen Virol 87:2849-2858

Rice GP, Schrier RD, Oldstone MB (1984) Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. Proc Natl Acad Sci USA 81:6134-6138

Riegler S, Hebart H, Einsele H, Brossart P, Jahn G, Sinzger C (2000) Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. J Gen Virol 81:393-399

Roberts WH, Sneddon JM, Waldman J, Stephens RE (1989) Cytomegalovirus infection of gastrointestinal endothelium demonstrated by simultaneous nucleic acid hybridization and immunohistochemistry. Arch Pathol Lab Med 113:461-464

Rummelt V, Rummelt C, Jahn G, Wenkel H, Sinzger C, Mayer UM, Naumann GO (1994) Triple retinal infection with human immunodeficiency virus type 1, cytomegalovirus, and herpes simplex virus type 1. Light and electron microscopy, immunohistochemistry, and in situ hybridization. Ophthalmology 101:270-279

Schafer P, Tenschert W, Cremaschi L, Schroter M, Gutensohn K, Laufs R (2000) Cytomegalovirus cultured from different major leukocyte subpopulations: association with clinical features in CMV immunoglobulin G-positive renal allograft recipients. J Med Virol 61:488-496

Schmidbauer M, Budka H, Ulrich W, Ambros P (1989) Cytomegalovirus (CMV) disease of the brain in AIDS and connatal infection: a comparative study by histology, immunocytochemistry and in situ DNA hybridization. Acta Neuropathol (Berl) 79:286-293

Schwartz DA, Walker B, Furlong B, Breding E, Someren A (1990) Cytomegalovirus in a macerated second trimester fetus: persistent viral inclusions on light and electron microscopy. South Med J 83:1357-1358

Shelbourn SL, Sissons JG, Sinclair JH (1989) Expression of oncogenic ras in human teratocarcinoma cells induces partial differentiation and permissiveness for human cytomegalovirus infection. J Gen Virol 70:367-374

Sinclair J, Sissons P (2006) Latency and reactivation of human cytomegalovirus. J Gen Virol 87:1763-1779

Sinzger C (2008) Entry route of HCMV into endothelial cells. J Clin Virol 41:174–179

Sinzger C, Muntefering H, Loning T, Stoss H, Plachter B, Jahn G (1993) Cell types infected in human cytomegalovirus placentitis identified by immunohistochemical double staining. Virchows Arch A Pathol Anat Histopathol 423:249-256

Sinzger C, Plachter B, Grefte A, The TH, Jahn G (1996) Tissue macrophages are infected by human cytomegalovirus in vivo. J Infect Dis 173:240-245

Sinzger C, Grefte A, Plachter B, Gouw AS, The TH, Jahn G (1995) Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. J Gen Virol 76:741-750

Sinzger C, Jahn G (1996) Human cytomegalovirus cell tropism and pathogenesis. Intervirology 39:302-319

Sinzger C, Bissinger AL, Viebahn R, Oettle H, Radke C, Schmidt CA, Jahn G (1999a) Hepatocytes are permissive for human cytomegalovirus infection in human liver cell culture and In vivo. J Infect Dis 180:976-986

Sinzger C, Schmidt K, Knapp J, Kahl M, Beck R, Waldman J, Hebart H, Einsele H, Jahn G (1999b) Modification of human cytomegalovirus tropism through propagation in vitro is associated with changes in the viral genome. J Gen Virol 80:2867-2877

Sinzger C, Kahl M, Laib K, Klingel K, Rieger P, Plachter B, Jahn G (2000) Tropism of human cytomegalovirus for endothelial cells is determined by a post-entry step dependent on efficient translocation to the nucleus. J Gen Virol 81:3021-3035

Sinzger C, Eberhardt K, Cavignac Y, Weinstock C, Kessler T, Jahn G, Davignon JL (2006) Macrophage cultures are susceptible to lytic productive infection by endothelial-cell-propagated human cytomegalovirus strains and present viral IE1 protein to CD4+ T cells despite late downregulation of MHC class II molecules. J Gen Virol 87:1853-1862

Skaletskaya A, Bartle LM, Chittenden T, McCormick AL, Mocarski ES, Goldmacher VS (2001) A cytomegalovirus-encoded inhibitor of apoptosis that suppresses caspase-8 activation. Proc Natl Acad Sci USA 98:7829-7834

Spiller OB, Borysiewicz LK, Morgan BP (1997) Development of a model for cytomegalovirus infection of oligodendrocytes. J Gen Virol 78:3349-3356

Stagno S, Cloud GA (1994) Working parents: the impact of day care and breast-feeding on cytomegalovirus infections in offspring. Proc Natl Acad Sci USA 91:2384-2389

Stassen FR, Vega-Cordova X, Vliegen I, Bruggeman CA (2006) Immune activation following cytomegalovirus infection: more important than direct viral effects in cardiovascular disease? J Clin Virol 35:349-353

Streblow DN, van Cleef KW, Kreklywich CN, Meyer C, Smith P, Defilippis V, Grey F, Fruh K, Searles R, Bruggeman C, Vink C, Nelson JA, Orloff SL (2007) Rat cytomegalovirus gene expression in cardiac allograft recipients is tissue specific and does not parallel the profiles detected in vitro. J Virol 81:3816-3826

Tabi Z, Moutaftsi M, Borysiewicz LK (2001) Human cytomegalovirus pp65- and immediate early 1 antigen-specific HLA class I-restricted cytotoxic T cell responses induced by cross-presentation of viral antigens. J Immunol 166:5695-5703

The TH, van der Bij W, van den Berg AP, van der Giessen M, Weits J, Sprenger HG, van Son WJ (1990) Cytomegalovirus antigenemia. Rev Infect Dis 12 Suppl 7: S734-S744

Tugizov S, Maidji E, Pereira L (1996) Role of apical and basolateral membranes in replication of human cytomegalovirus in polarized retinal pigment epithelial cells. J Gen Virol 77:61-74

Tumilowicz JJ, Gawlik ME, Powell BB, Trentin JJ (1985) Replication of cytomegalovirus in human arterial smooth muscle cells. J Virol 56:839-845

Valantine HA (2004) The role of viruses in cardiac allograft vasculopathy. Am J Transplant 4:169-177

van Den Pol AN, Mocarski E, Saederup N, Vieira J, Meier TJ (1999) Cytomegalovirus cell tropism, replication, and gene transfer in brain. J Neurosci 19:10948-10965

van der Strate BW, Hillebrands JL, Lycklama a Nijeholt SS, Beljaars L, Bruggeman CA, Van Luyn MJ, Rozing J, The TH, Meijer DK, Molema G, Harmsen MC (2003) Dissemination of rat cytomegalovirus through infected granulocytes and monocytes in vitro and in vivo. J Virol 77:11274-11278

Variend S, Pearse RG (1986) Sudden infant death and cytomegalovirus inclusion disease. J Clin Pathol 39:383-386

Waldman WJ, Sneddon JM, Stephens RE, Roberts WH (1989) Enhanced endothelial cytopathogenicity induced by a cytomegalovirus strain propagated in endothelial cells. J Med Virol 28:223-230

Waldman WJ, Roberts WH, Davis DH, Williams MV, Sedmak DD, Stephens RE (1991) Preservation of natural endothelial cytopathogenicity of cytomegalovirus by propagation in endothelial cells. Arch Virol 117:143-164

Waldman WJ, Knight DA, Huang EH, Sedmak DD (1995) Bidirectional transmission of infectious cytomegalovirus between monocytes and vascular endothelial cells: an in vitro model. J Infect Dis 171:263-272

Wang D, Shenk T (2005a) Human cytomegalovirus UL131 open reading frame is required for epithelial cell tropism. J Virol 79:10330-10338

Wang D, Shenk T (2005b) Human cytomegalovirus virion protein complex required for epithelial and endothelial cell tropism. Proc Natl Acad Sci USA 102:18153-18158

Wiley CA, Nelson JA (1988) Role of human immunodeficiency virus and cytomegalovirus in AIDS encephalitis. Am J Pathol 133:73-81

Yamane Y, Furukawa T, Plotkin SA (1983) Supernatant virus release as a differentiating marker between low passage and vaccine strains of human cytomegalovirus. Vaccine 1:23-25