Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues

Christian Sinzger,1 Annemarie Grefte,2 Bodo Plachter,3 Annette S. H. Gouw,4 T. Hauw The2 and Gerhard Jahn1

1Abteilung für Medizinische Virologie, Hygiene-Institut, Universität Tübingen, Tübingen, Germany, 2Department of Clinical Immunology, University of Groningen, Groningen, Netherlands, 3Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, Erlangen, Germany and 4Department of Pathology, University of Groningen, Groningen, Netherlands

High titre replication of human cytomegalovirus (HCMV) in cell culture is restricted to primary human fibroblasts. During acute infection in vivo, HCMV nucleic acids and antigens have been found in various organs. Using only morphological criteria, inconsistent data have been reported about the cell types that can be infected by HCMV. In particular, the role of fibroblasts in organ infections has remained unclear. To define accurately the target cells of HCMV in vivo, tissue sections from lung and gastrointestinal tract of patients suffering from acute HCMV infection were investigated using immunohistochemical double-labelling analyses. Monoclonal antibodies with defined specificity against immediate early (IE), early (E) and late (L) viral antigens and antibodies directed against cell marker proteins were employed to identify infected cells. The results demonstrated that a broad spectrum of cells was infected by HCMV in vivo. Consistent with their susceptibility in culture, fibroblasts formed a major population of HCMV-infected cells. In contrast, haemopoietic cells were only infrequently stained with virus-specific antibodies. Fibroblasts, epithelial cells, endothelial cells, smooth muscle cells and macrophages appeared to be permissive for HCMV replication. Contrary to this, polymorphonuclear cells showed only IE gene expression, indicating that these cells were abortively infected. The analysis of the distribution of infected cells in tissue supported the hypothesis that endothelial cells and monocytes/macrophages may play a crucial role in the haematogenous spread of HCMV; in contrast, fibroblasts, smooth muscle cells and epithelial cells may form the cell populations important for the multiplication and spread of the virus in infected tissues.

Introduction

Infection with human cytomegalovirus (HCMV) is a major cause of morbidity and mortality in immuno-compromised hosts such as AIDS patients (Jacobson & Mills, 1988; Peters et al., 1991; Aukrust et al., 1992; Ullrich et al., 1992) or transplant recipients (Schulman et al., 1991; Duncan et al., 1991; Ljungman et al., 1992; Sakr et al., 1992). Furthermore, it causes severe sequelae in newborns after prenatal transmission (Alford et al., 1990). A lot of information has been gathered throughout the past years on the molecular biology of the virus (reviewed by Stinski, 1991; Mocarski, 1993; Gibson, 1993). Most of these studies have been carried out in culture experiments using primary human fibroblasts. Compared with the rich knowledge about the molecular biology of HCMV in cell culture systems, only limited information is available on the mechanisms that regulate permissive and latent infection in vivo.

One reason for this is the lack of an animal model, which would help to define, as a first step, the cell types that support HCMV in its natural host. Several studies have focused on the identification of infected cells using HCMV-specific monoclonal antibodies (MAbs) in immunohistochemical analyses of patient material (Francis et al., 1989; Roberts et al., 1989; Agel et al., 1991; Reynolds-Kohler et al., 1993). In these investigations, infected cells have been tentatively assigned to the endothelial, epithelial, stromal and smooth muscle cell populations by their morphology including their localization within the analysed tissues. However, morphological criteria alone appear to be insufficient to unequivocally identify infected cells for several reasons. Firstly, processing of tissue may result in dislocation of cells in a given section thus providing a possible pitfall in...
the identification of cell types simply by virtue of their location. Furthermore, the well-known cytomegaloviral alteration of permissively HCMV-infected cells renders the identification of such cells solely by their morphology impossible. Staining of cell-specific marker proteins and analysis of sorted blood cell fractions have improved the analysis of HCMV cell tropism. Recent reports described the presence of infected monocytes, endothelial cells and polymorphonuclear cells in the peripheral blood (Gerna et al., 1992b; Grefte et al., 1993a, b; Percivalle et al., 1993). However, the role of these cell types in the infection of organ tissues remains unclear. A promising way to clearly identify infected cells is the simultaneous use of cell type-specific and virus-specific antibodies. In sections of lung and gastrointestinal tissues, only infection of endothelial cells was unequivocally demonstrated by simultaneous detection of HCMV DNA and an endothelial cell marker (Myerson et al., 1984; Roberts et al., 1989). Most of the cell types that are involved in solid organ infection by HCMV have not been defined using this technique. It is well established that multiple organs can be severely infected during active HCMV disease. Therefore, solid organ cells are suspected to play a key role in multiplication and distribution of the virus. In addition, reports on murine CMV indicate that certain populations of lung cells may also be targets during latent HCMV infection (Balthesien et al., 1993).

The goal of this study was to identify the cell types that were targets of HCMV infection in solid organs in vivo. For this, thin sections from the lungs and gastrointestinal tract of patients suffering from acute HCMV infection were investigated by immunohistochemical double-labeling techniques. Both lungs and gastrointestinal tract are known to be heavily involved during HCMV disease and may exemplify the situation in other organs. The results showed that, in contrast to what was thought before, fibroblasts constitute a major target cell population of HCMV in vivo. Endothelial cells, epithelial cells and smooth muscle cells were also frequently found to be infected, whereas haematopoietic cells formed only a minor fraction of the HCMV-positive cells. The implications of these findings for our understanding of viral replication and spread are discussed.

Methods

Tissue samples. Formalin-fixed paraffin-embedded tissue sections of HCMV-infected lung and gastrointestinal specimens were studied (Table 1). The presence of an active HCMV infection in the patients was confirmed by the presence of characteristic cytomegaloviral cells in haematoxylin–eosin-stained tissue samples, as well as by positive virus isolation from blood and/or the appearance of a significant rise in HCMV-specific antibodies as described previously (van der Bij et al., 1988). Tissue samples, from biopsy and autopsy material, originated from 10 different patients. Underlying disorders included infection with

the human immunodeficiency virus (HIV; four patients), previous organ transplantation (four patients), chronic non-specific respiratory disease (one patient), or were unknown (one patient).

Antibodies. MAbs directed against HCMV proteins characteristic for the immediate early, early and late stage of infection were used in this study. MAb E13 (Biosoft) was directed against the non-structural immediate early antigens (IEA) IE1-pp72 (UL123) and IE2-pp86 (UL122) (Mazeron et al., 1992). MAb CCH2 (generously provided by B. Zweyenberg Wirgart and L. Grillner, Stockholm, Sweden) was directed against the non-structural early 52 kDa DNA-binding protein (EA) (UL44) (Plachter et al., 1992). MAb XPI (NCNL 03; Behringwerke) directed against the cytoplasmic tegument protein pp150 (UL32) of HCMV, was used to detect late-stage infected cells (Jahn et al., 1990). This antibody was the only available MAb directed against late-stage protein of HCMV that binds in paraffin-embedded tissue sections.

To identify the cell types infected by HCMV, a set of monoclonal and polyclonal antibodies against different cell-specific marker proteins was used. These reagents were tested on sections from normal tissues prior to analyses (Table 2) to verify their reactivity. Anti-keratin MAb AE1/3 (Boehringer) was used to detect cells of epithelial origin. Only epithelial cells were stained by this antibody. Labelling by anti-actin MAb (Ortho Diagnostics) was used to detect smooth muscle cells and resulted in cytoplasmic staining of this cell type only. Anti-vimentin polyclonal antibody (PAb) was directed against mesenchymal cells. This antiserum stained spindle-shaped cells in the interstitium and some endothelial cells. MAb anti-F VIII (Dako) was directed against factor VIII-related antigen on endothelial cells and stained the cytoplasm of endothelial cells of arteries, veins and capillaries. Mabs HAM56 (Ortho-Diagnostics) and KP1 (Dako) were used as macrophage markers and showed cytoplasmic staining of tissue macrophages and intravascular monocytes. MAb HAM56 crossreacted faintly with endothelial cells. MAb KP1 reacted weakly with polymorphonuclear leukocytes (PMNL). However, this cross reaction was clearly distinguishable from the intense staining of macrophages. Anti-lymphocyte common antigen (LCA) MAb (Dako) was used as a pan-lymphocyte marker. Anti-neutrophil elastase MAb (Dako) was used to detect PMNL. Anti-LCA MAb stained the cytoplasm of lymphocytes. Macrophages or PMNL were only rarely stained by this MAb in the formalin-fixed tissue sections investigated, which agrees with the supplier’s specification. MAb against neutrophil elastase stained PMNL. A faint staining was also found in macrophages, which was clearly distinguishable from the intense staining of PMNL.

Double-staining immunohistochemistry. An indirect immunostaining method combining the streptavidin–biotin–peroxidase and streptavidin–biotin–alkaline phosphatase techniques was employed to detect HCMV antigens and the respective cell marker simultaneously. In a first step, HCMV immediate early antigen was stained using MAb E13 as previously described (Sinzer et al., 1993b). Predigestion was done with 0.1% protease (Merck). Binding of the primary MAb was followed by incubation with biotinylated rabbit anti-mouse Ig antibody (Dako) and streptavidin–biotin–peroxidase complex (Dako). Washing was done with PBS. After diaminobenzidine (DAB; Sigma) staining of HCMV antigens, the respective cell marker proteins were labelled in the same way with the following modifications: Tris–HCl buffer was used for all washing steps. Biotinylated swine anti-rabbit Ig (Dako) was used as a secondary antibody if the cell marker-specific antibody was polyclonal. Streptavidin–biotin–alkaline phosphatase complex (Dako) was used and staining was performed with a Fast red (Sigma) solution. If the cell marker-specific antibody was polyclonal, swine non-immune serum was used instead of rabbit non-immune serum for the whole double-staining procedure. Predigestion was done with 0.4% pepsin (Sigma) at 37 °C for 30 min when anti-F VIII was the cell-specific

1990). Most of the cell types that are involved in solid organ infection by HCMV have not been defined using this technique. It is well established that multiple organs can be severely infected during active HCMV disease. Therefore, solid organ cells are suspected to play a key role in multiplication and distribution of the virus. In addition, reports on murine CMV indicate that certain populations of lung cells may also be targets during latent HCMV infection (Balthesien et al., 1993).

The goal of this study was to identify the cell types that were targets of HCMV infection in solid organs in vivo. For this, thin sections from the lungs and gastrointestinal tract of patients suffering from acute HCMV infection were investigated by immunohistochemical double-labeling techniques. Both lungs and gastrointestinal tract are known to be heavily involved during HCMV disease and may exemplify the situation in other organs. The results showed that, in contrast to what was thought before, fibroblasts constitute a major target cell population of HCMV in vivo. Endothelial cells, epithelial cells and smooth muscle cells were also frequently found to be infected, whereas haematopoietic cells formed only a minor fraction of the HCMV-positive cells. The implications of these findings for our understanding of viral replication and spread are discussed.

Methods

Tissue samples. Formalin-fixed paraffin-embedded tissue sections of HCMV-infected lung and gastrointestinal specimens were studied (Table 1). The presence of an active HCMV infection in the patients was confirmed by the presence of characteristic cytomegaloviral cells in haematoxylin–eosin-stained tissue samples, as well as by positive virus isolation from blood and/or the appearance of a significant rise in HCMV-specific antibodies as described previously (van der Bij et al., 1988). Tissue samples, from biopsy and autopsy material, originated from 10 different patients. Underlying disorders included infection with
Table 1. Clinical data of the cases investigated by immunohistochemical double staining

<table>
<thead>
<tr>
<th>Case number</th>
<th>Tissue</th>
<th>Source</th>
<th>Inflammatory reaction</th>
<th>Sex, age, clinical data*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lung</td>
<td>Autopsy</td>
<td>Mixed cellular interstitial infiltrate</td>
<td>Male, 47, HIV +, AIDS; multiple opportunistic infections</td>
</tr>
<tr>
<td>2</td>
<td>Lung</td>
<td>Autopsy</td>
<td>Mixed cellular infiltrate</td>
<td>Male, 31, HIV +, Kaposi sarcoma of lymph nodes and skin</td>
</tr>
<tr>
<td>3</td>
<td>Lung</td>
<td>Autopsy</td>
<td>Polymorphonuclear infiltrate</td>
<td>HCMV infection of lung and gut, invagination ileum to caecum</td>
</tr>
<tr>
<td>4</td>
<td>Lung</td>
<td>Autopsy</td>
<td>No inflammatory infiltrations</td>
<td>Female, 48, OLT, systemic HCMV infection (gut, oesophagus, spleen, lung)</td>
</tr>
<tr>
<td>5</td>
<td>Lung</td>
<td>Autopsy</td>
<td>Mixed cellular infiltrate</td>
<td>Female, 37, HELLP syndrome post partum, intensive care, multiple organ failure, HCMV infection of multiple organs</td>
</tr>
<tr>
<td>7</td>
<td>Duodenum</td>
<td>Biopsy</td>
<td>Mixed cellular infiltrate</td>
<td>Male, 28, RTx, pneumonia, arthralgia</td>
</tr>
<tr>
<td>8</td>
<td>Stomach</td>
<td>Biopsy</td>
<td>Mixed cellular infiltrate</td>
<td>Male, 63, CARD, respiratory insufficiency, multiple infections (HCMV, fungi, atypical mycobacteria)</td>
</tr>
<tr>
<td>9</td>
<td>Stomach</td>
<td>Biopsy</td>
<td>Mixed cellular infiltrate</td>
<td>Male, 29, HIV +, nausea, vomiting</td>
</tr>
<tr>
<td>10</td>
<td>Stomach</td>
<td>Biopsy</td>
<td>Mixed cellular infiltrate</td>
<td>Male, 69, RTx</td>
</tr>
</tbody>
</table>

* Key: HIV, human immunodeficiency virus; OLT, orthotopic liver transplantation; RTx, renal transplantation; CARD, chronic aspecific respiratory disease; HELLP, haemolysis, elevated liver enzymes, low platelet count.

Table 2. Specificity of MAbs and PAbs used in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Species</th>
<th>Specificity</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13</td>
<td>Mouse MAb</td>
<td>HCMV immediate early antigen</td>
<td>Biosoft</td>
</tr>
<tr>
<td>CCH2</td>
<td>Mouse MAb</td>
<td>HCMV early protein p52</td>
<td>Dako</td>
</tr>
<tr>
<td>XP1</td>
<td>Mouse MAb</td>
<td>HCMV late protein pp150</td>
<td>Behringwerke</td>
</tr>
<tr>
<td>Cytokeratin AE1/3</td>
<td>Mouse MAb</td>
<td>Epithelial cells</td>
<td>Boehringer</td>
</tr>
<tr>
<td>Actin</td>
<td>Mouse MAb</td>
<td>Smooth muscle cells</td>
<td>Ortho</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Rabbit PAb</td>
<td>Cells of mesenchymal origin</td>
<td>Dako</td>
</tr>
<tr>
<td>F VIII</td>
<td>Mouse MAb</td>
<td>Endothelial cells</td>
<td>Dako</td>
</tr>
<tr>
<td>HAM56</td>
<td>Mouse MAb</td>
<td>Macrophages</td>
<td>Ortho</td>
</tr>
<tr>
<td>KP1</td>
<td>Mouse MAb</td>
<td>Macrophages</td>
<td>Dako</td>
</tr>
<tr>
<td>LCA</td>
<td>Mouse MAb</td>
<td>Lymphocytes</td>
<td>Dako</td>
</tr>
<tr>
<td>Neutrophil elastase</td>
<td>Mouse MAb</td>
<td>Polymorphonuclear cells</td>
<td>Dako</td>
</tr>
</tbody>
</table>

antibody. After the second staining, slides were counterstained in haematoxylin (Merck) and mounted in Kaiser’s glycerol gelatin (Merck). A positive result consisted of brown nuclear staining (HCMV antigens) and red cytoplasmic staining (cell marker proteins). No red staining of HCMV antigens occurred even if both antibodies were mouse monoclonals. DAB staining is well known to form a complex that is insoluble even in organic solvents. Obviously, this complex seems to prevent binding of anti-mouse Ig antibodies in the second staining step by covering antigenic sites.

In addition, double stainings were performed to study early viral gene expression in PMNL: HCMV early antigen (MAb CCH2) and neutrophil elastase were detected simultaneously in the same way as described for immediate early antigen and cell marker-specific antibodies. Control stainings included HCMV-infected tissue stained with anti-β-galactosidase MAb as a negative control for the primary antibodies; normal lung and gastrointestinal tissue as a negative control for HCMV infected tissue and HCMV-infected lung tissue as a positive control for the HCMV staining. To determine the proportion of the various cell types of overall HCMV-infected cells, a maximum of 1000 HCMV IE antigen-positive cells per section were analysed.

Results

Tissue distribution of HCMV infected cells

Autopsy samples from lung and colonic tissue (cases no. 1 to 6) and biopsy samples from duodenal and gastric tissue (cases no. 7 to 10; Table 1) were included in this study. HCMV infection was proven histologically by the presence of cytomegalic cells with nuclear inclusion bodies in these specimens. The distribution of HCMV-infected cells was studied using MAbs E13, CCH2, and XP1. Staining of uninfected control sections with these antibodies never yielded a positive signal. MAb E13 is directed against IE antigens of HCMV (Mazeron et al., 1992). These antigens are synthesized very early after HCMV infection of culture cells. In all tissue sections studied, a characteristic nuclear staining pattern was
found with MAb E13. Viral IEA was detected not only in cytomegalic cells but also in a number of morphologically unaltered cells (Fig. 1 and 2). Another MAb, CCH2, had been demonstrated to be directed against the DNA-binding protein p52 of HCMV (Plachter et al., 1992). The initiation of the synthesis of this protein requires the presence of viral immediate early proteins. It is expressed at early and, more abundantly, at late times after infection with HCMV. In tissue sections, MAb CCH2 showed nuclear as well as cytoplasmic staining (data not shown). In most cases, CCH2 predominantly stained cytomegalic cells. Only few of the cells stained by CCH2 showed no morphological signs of HCMV infection. MAb XP1 was directed against the late tegument protein pp150 (Jahn et al., 1990). This antibody exclusively stained the cytoplasm of characteristically altered cytomegalic cells in tissue sections (data not shown). It can be used as an indicator of de novo synthesis of viral particles and permissiveness of cells for cytomegalovirus replication. It is therefore obvious that cytomegalic cells with nuclear inclusions represent late-stage infected cells and might be assumed to be permissively infected.

In lung sections, HCMV-infected cells were multifocally distributed in the alveolar walls, the alveolar spaces, and the interstitium. Infection of bronchi or vessels was rarely seen. In gastrointestinal tissue, various patterns of distribution of infected cells were found. In some specimens, infected cells were tightly associated with capillaries and venules with a multifocal pattern. In other specimens, the infected cells were homogeneously or irregularly distributed in the mucosal layer, the muscularis mucosae, the submucosal layer or the muscularis propria. No pattern of distribution was unique to colonic or gastric tissue, respectively. These experiments showed that HCMV-infected cells were broadly distributed in lung and gastrointestinal tissue and that apparently different cell populations were involved.

Identification of HCMV-infected cells

To identify the target cells for HCMV infection in solid organs in vivo, an immunohistochemical double-staining technique was employed on tissue sections from lung and gastrointestinal tract. In all cases, the characteristic brown nuclear staining pattern with the HCMV-specific MAb could be easily distinguished from the red cytoplasmic staining for cell markers. The specificity of cell-specific antisera was verified on sections from normal tissues prior to analyses.

In lung specimens, the majority of infected cells were of epithelial origin, indicated by the binding of the antikeratin antibody (Table 3). These cells were almost exclusively alveolar lining cells and were thus identified as being alveolar epithelial cells. Some of them showed no signs of morphological alteration, whereas others were cytomegalic with or without inclusion bodies (Fig. 1a). Furthermore, a small number of cytomegalic cells had apparently lost contact with the alveolar membrane and were located in the alveolar spaces. Infected mesenchymal cells, indicated by the detection of the intermediate filament vimentin, were also found in all lung specimens investigated. These cells were located in the alveolar interstitium, and included spindle-shaped cells as well as cytomegalic cells with the characteristic 'owl's eye' appearance (Fig. 1b). Since these cells lacked cell markers other than vimentin, they were identified by exclusion as being fibroblasts. In four out of six lung specimens, infected endothelial cells were identified by double-staining with factor VIII antibody. These cells were always located in the endothelial layer of capillaries and venules (Fig. 1c), including cells of typical endothelial morphology as well as cytomegalic cells. Smooth muscle cells, detected by anti-actin MAb binding, were a rare finding in lung tissue. These cells were located in the wall of small bronchi. Infected macrophages, indicated by binding of MAbs HAM56 or KP1, were also only rarely found and were located in the alveolar spaces. If present, they displayed either an unaltered morphology or a characteristic 'owl's eye' appearance. Infected cells stained by the anti-LCA antibody were not observed. In three out of six lung specimens, infected PMNL were found, indicated by detection of neutrophil elastase. These cells were either of typical PMNL morphology or showed a slightly enlarged nucleus. A cytomegalic phenotype was never observed in PMNL. In contrast to the observation of immediate early antigen in PMNL, no early or late viral antigen was detected in these cells (data not shown).

In gastrointestinal tissue specimens, the pattern of infected cells was more variable (Table 3). In the three cases with vessel-associated HCMV infection, endothelial cells (factor VIII-positive) were the major fraction of infected cells. They were always located in capillaries (Fig. 2a) and venules, some of them being cytomegalic. When cytomegalic alteration occurred, the factor VIII staining was weaker than in unaltered cells and was concentrated at the margin of the cytoplasm (Fig. 2b). Some of the cytomegalic endothelial cells appeared to be losing adherence to the vessel wall. Other endothelial 'owl's eye' cells completely filled the vascular lumen. In two gastrointestinal tissue specimens epithelial cells (Fig. 2c) were the major fraction of infected cells. Some of these epithelial cells were cytomegalic. In one gastrointestinal tissue specimen with a deep ulceration, smooth muscle cells (actin-positive) of the muscularis propria were the predominantly infected cells, including
Cell types infected by HCMV

745

typical elongated smooth muscle cells (Fig. 2d) as well as elongated cytomegalic cells with inclusion bodies. Infected mesenchymal cells (vimentin-positive) (Fig. 2e), either spindle-shaped or cytomegalic, were located in the lamina propria. In contrast, infected PMNL (elastase-positive), which composed up to 20% of the infected cells in one specimen, were always non-cytomegalic (Fig. 2f). No early or late viral antigen could be detected in PMNL (data not shown). Infected macrophages (HAM56-, KPI-positive), either cytomegalic or non-cytomegalic, were a rare finding in gastrointestinal tissue (Fig. 2g). Anti-LCA binding was never observed in HCMV-infected cells.

In summary, there was a rather constant pattern of HCMV-infected cells in lung tissue, with epithelial cells and fibroblasts being the predominantly infected cell types. In contrast, the pattern was more variable in gastrointestinal tissue, where fibroblasts, endothelial, epithelial and smooth muscle cells were the main targets. In all these cell populations, cytopathogenic alterations characteristic for HCMV infection were found, indicating that these cells were permissive for HCMV replication. However, in PMNL, which formed a significant number of the infected cells in lung and gastrointestinal tissues, signs of cytomegalic alteration were never found.

Discussion

As no animal model is available for HCMV, the mechanisms that regulate viral multiplication, spread and latency in vivo have been difficult to study. Defining the cell populations that are infected during natural infection is a prerequisite in the process of understanding the viral and host factors that influence HCMV infection. In the past, conflicting data about the cell types infected by HCMV in vivo have been published (Schrier et al., 1985; Gnann et al., 1988; Francis et al., 1989; Roberts et al., 1989; Aqel et al., 1991; Reynolds-Kohler et al., 1993). Owing to the property of HCMV to alter the morphology of infected cells, earlier investigations of HCMV-infected tissues, based mainly on morphology, had failed to define the majority of infected cells.

This study was designed to identify the cell types involved in acute HCMV infection in vivo. Employing a double-immunolabelling technique for the simultaneous detection of viral antigens and cell-specific proteins, we could demonstrate that a variety of cell types were susceptible to HCMV infection. In general, fibroblasts, epithelial cells, endothelial cells and smooth muscle cells were predominantly infected by the virus, whereas polymorphonuclear cells and macrophages formed only a minor infected fraction in tissue sections.
Fig. 1.

Fig. 2.
Fig. 2. Identification of infected cell types in HCMV-infected gastrointestinal tissue by immunohistochemical double-staining. Detection of HCMV immediate early antigen (IEA) by immunoperoxidase technique yielded brown nuclear staining. Detection of specific cell markers by immunoalkaline phosphatase technique resulted in red cytoplasmic staining. Counterstaining was performed with haematoxylin. (a) Detection of endothelial marker factor VIII in an infected gastric capillary (case no. 8). (b) Detection of endothelial marker factor VIII in an infected gastric venule (case no. 8). (c) Detection of epithelial cell marker keratin in HCMV-infected cells of duodenal glands (case no. 7). (d) Detection of smooth muscle cell marker actin in infected cells of the muscularis propria (case no. 1). (e) Detection of mesenchymal marker vimentin in HCMV-infected interstitial cells (case no. 7). (f) Detection of polymorphonuclear cell marker neutrophil elastase in a colonic stromal cell (case no. 2). The nucleus of this polymorphonuclear cell is enlarged. (g) Detection of macrophage marker CD68 in a colonic stromal cell (case no. 1). Bar markers represent 30 μm. (all parts are at the same magnification).

Fig. 1. Identification of HCMV-infected cell types in HCMV-infected lung tissue by immunohistochemical double-staining. Detection of HCMV immediate early antigen (IEA) by immunoperoxidase technique yielded brown nuclear staining. Detection of specific cell markers by immunoalkaline phosphatase technique resulted in red cytoplasmic staining. Counterstaining was performed with haematoxylin. (a) Detection of epithelial cell marker keratin in HCMV-infected alveolar cells (case no. 1). (b) Detection of mesenchymal marker vimentin in HCMV-infected interstitial cells (case no. 1). (c) Detection of endothelial marker factor VIII in an infected endothelial cell (case no. 1). Bar markers represent 30 μm. (all parts are at the same magnification).
Fibroblasts were one of the predominant cell populations infected by HCMV in vivo. This finding appears to be of particular interest, as fibroblast cell cultures are widely used for the isolation and propagation of the virus from patient specimens. These cells are the only culture system available at present that can be used to grow the virus to high titres. Therefore, most studies on the molecular biology and replication of HCMV have been carried out in fibroblasts. Up to now it has been assumed that fibroblasts were not a natural target cell or made up only a minor fraction of cells supporting growth of HCMV in vivo (Alford & Britt, 1990). The experiments presented here suggest that experimental knowledge obtained through studies of HCMV in fibroblast cell cultures is likely to reflect biologically relevant mechanisms operative during in vivo infection.

Another issue important for the interaction between HCMV and its host is the definition of cells that allow permissive infection as opposed to those cell populations which are only abortively infected. Using MAbs against immediate early, early and late viral proteins, we have recently shown that cytomegalic cells in tissue sections represent the late stage of the viral replication. This indicates that these cells are fully permissive for HCMV infection (Sinzger et al., 1993a, b). In this study, a cytomegalic phenotype has been found in fibroblasts, epithelial cells, endothelial cells, smooth muscle cells, and macrophages suggesting that these cell populations are permissive for HCMV replication. This corresponds well with earlier reports showing that endothelial cells (Lathey et al., 1990; Waldman et al., 1991), smooth muscle cells (Tumilowicz et al., 1985), and macrophages (Ibanez et al., 1991; Lathey & Spector, 1991) are permissive for HCMV in cell culture. In contrast, the lack of morphological alterations as well as the absence of any detectable early and late viral antigens in infected PMNL indicates that these cells are only abortively infected. In agreement with this are analyses on PMNL from peripheral blood, in which no synthesis of late viral antigen could be demonstrated (Taylor-Wiedeman et al., 1993; Grefte et al., 1992). Taken together, compared with the conflicting data from earlier in vivo studies based on histopathology alone, the immunohistochemical double staining analysis corresponded much better with the replication of HCMV in cell culture systems.

The distribution of HCMV-infected cells found in our analyses may have implications for the spread of the virus during acute infection. Alveolar epithelial cells appeared to be the main target for HCMV in the lung tissues. A large number of them showed cytopathic alterations characteristic of late-stage infected cells. In addition, these cells seemed to have lost adherence when they became cytomegalic, resulting in detachment from the alveolar basement membrane. It is tempting to speculate that detection of infectious virus, viral antigen, or viral DNA in bronchoalveolar lavage fluid (Cordonnier et al., 1987; Masih et al., 1991; Weiss et al., 1991; Clarke et al., 1992) may be due to infected alveolar epithelial cells and that infectious virus may be shed by those cells. Epithelial cells were also the predominantly infected cell population in two gastrointestinal tissue specimens taken from duodenum and stomach. Although completely detached infected epithelial cells were never observed, a few cytomegalic cells were found in the process of losing connection to the epithelial layer, similar to the findings in lung epithelium. This supports the hypothesis of Aqel et al. (1991), who suggested a luminal route of viral spread, based on the finding that spread of HCMV infection occurred along a gastrocolic fistula.

Endothelial cells and macrophages seem to be involved in the haematogeneous spread of HCMV. Circulating cytomegalic inclusion cells were recently found to be present in the peripheral blood of patients with an active HCMV infection (Grefte et al., 1993a). These cells were shown to contain viral antigens from all three stages of the viral replication cycle, indicative of a productive HCMV infection. In addition, immunocytochemical studies demonstrated that they were of endothelial origin (Grefte et al., 1993a). Using transmission electron microscopy, it was recently shown that these circulating cytomegalic endothelial cells contained numerous mature HCMV particles (Grefte et al., 1993b). Taken together, these findings suggest a role for these cells in the spread of HCMV via the bloodstream. Consistent with these data, we observed infected endothelial cells in capillaries and venules. Some of them showed cytomegalic alteration and seemed to be the non-circulating counterpart of the circulating cytomegalic inclusion cells. It could be speculated that with the development of a cytopathic effect, some of these cells detach from the vessel wall and are carried away in the bloodstream. Finally, they may become trapped in the terminal microvessels and distribute infectious virus to adjacent tissues.

Our finding of late-stage infected tissue macrophages is in agreement with and further extends reports demonstrating productive HCMV infection of primary differentiated macrophages in vitro (Ibanez et al., 1991; Lathey & Spector, 1991). Circulating monocytes were reported to contain viral DNA but show only restricted expression of viral genes (Taylor-Wiedeman et al., 1991). After tissue invasion and differentiation to become macrophages, they seem to support a complete cycle of viral replication and thus may play an important role in the spread of HCMV into various organs. After the virus has reached the stroma of lung and gastrointestinal tract by the haematogeneous route, it seems to spread easily by productively infecting fibroblasts and smooth muscle.
cells, thus eventually reaching the epithelial layer. In contrast to the permissively infected cells discussed before, PMNL found in tissues during acute infection solely expressed HCMV immediate early antigens and showed no cytomegalic phenotype. Up to now, the presence of infected PMNL cells in tissue sections has not been reported. In peripheral blood PMNL, the structural viral protein pp65 was demonstrated to be the predominantly detectable antigen. This is assumed to be a result of uptake rather than of synthesis (Grefte et al., 1992).

The non-structural immediate early antigens which are likely to reflect new synthesis were detectable only in a minor fraction of PMNL in peripheral blood (Gerna et al., 1992a; Grefte et al., 1992). The HCMV immediate early antigen-positive PMNL described here in tissue sections probably represent the counterpart of those immediate early antigen-positive PMNL circulating in peripheral blood. Their role in the spread of the virus remains to be further elucidated.

In conclusion we have shown here that HCMV is able to infect a broad spectrum of target cells during acute infection in its natural host. Fibroblasts, epithelial and endothelial cells, smooth muscle cells and tissue macrophages appeared to promote the production of progeny virus in vivo. Based on these results, additional studies will have to focus on the role that each of these cell populations bears for virus multiplication, spread and latency.

We are indebted to Prof H. K. Müller-Hermelink, Prof H. Stöß and Dr P. Ortloff for providing tissue sections of case numbers 1, 7, 8 and 9. We thank Dr J. Nelson for helpful discussion and Mrs B. M. Schilizzi for revision of the English. This work was supported by the Deutsche Forschungsgemeinschaft, Forschergruppe 'DNA-Viren des hämatopoetischen Systems', by the Bundesministerium für Forschung und Technologie (Projektnummer 01 KI 9305), and by grant no. 506-9. We thank Dr J. Nelson for helpful discussion and Mrs B. M. Schilizzi for revision of the English. This work was supported by the Deutsche Forschungsgemeinschaft, Forschergruppe 'DNA-Viren des hämatopoetischen Systems', by the Bundesministerium für Forschung und Technologie (Projektnummer 01 KI 9305), and by grant no. 506-9.

References


(Received 7 July 1994; Accepted 21 November 1994)