Human cytomegalovirus (HCMV) infection appears to be linked to the pathogenesis of atherosclerosis. An association between HCMV infection and an enhanced restenosis rate as well as the induction of vasculopathies after solid organ transplantation has been documented. Knowledge of the cellular and molecular basis of these findings is limited, however. By Northern blot and RT-PCR analysis of human foreskin fibroblasts (HFF) and human coronary artery smooth muscle cells (SMC), we identified extracellular matrix (ECM) genes that were downregulated after HCMV infection, including collagen type I and fibronectin. Quantitative immunoassays showed a significant reduction of soluble collagen type I and fibronectin proteins in supernatants of both cell types. This was shown to be a direct effect of HCMV infection and not due to a response to interferons released from infected cells, since neutralization of alpha and beta interferon activity could not block virus-induced downregulation of matrix proteins. As the amount of ECM depends on both synthesis and degradation, we also assessed the influence of HCMV on the activity of matrix metalloproteinases (MMP). Interestingly, a significant difference in virus-induced matrix degradation could be shown between the two cell types. HCMV upregulated MMP-2 protein and activity in SMC but not in HFF. Thus, HCMV infection of SMC reduces ECM dramatically by inducing two independent mechanisms that influence synthesis as well as degradation of ECM. These may represent molecular mechanisms for HCMV–induced pathogenesis of inflammatory vasculopathies and may facilitate dissemination of HCMV by promoting the detachment of infected cells in vivo.
in human foreskin fibroblasts (HFF), the standard cell type used in vitro in HCMV studies. Also, in HFF infected with a laboratory-adapted HCMV strain (AD169), the results of DNA array analysis suggested an HCMV-associated reduction of collagen type I gene expression (Zhu et al., 1998). To our knowledge, neither confirmation of these data by Northern blot analysis nor data concerning collagen protein synthesis under the influence of HCMV infection is available. Furthermore, nothing is known about virus-induced modulation of ECM genes and protein expression in SMC. Since SMC are thought to be the major producer of the ECM scaffold of the vessel wall (Brooke et al., 2003) and are important cells in atherosogenesis, we analysed HCMV-induced effects on synthesis and degradation of ECM proteins in primary SMC obtained from human coronary arteries.

In the present study, we demonstrate a substantial HCMV-induced reduction of ECM in HFF as well as in SMC. The loss of intercellular ECM fibres is linked to direct virus-induced suppression of matrix gene expression, resulting in decreased protein production. This effect was observed in HFF and also in SMC, which are major target cells for HCMV in vivo (Sinzger et al., 1995). An additional matrix-degrading mechanism was identified in SMC, where increased amounts and activity of MMP-2 were detected. HCMV-induced modulation of ECM expression in SMC could trigger pathogenetic processes in inflammatory vasculopathies.

**METHODS**

**Cells and infection.** SMC (Clonetics) were cultured in SMC basal medium supplemented with 5% fetal calf serum (FCS) and growth factors (SmGM BulletKit; BioWhittaker). SMC were used in passage 5 or 6. HFF were prepared in our laboratory and were used in passages 13–20. HCMV strain AD169 was obtained from the ATCC. The low-passage, more 'wild-type'-like HCMV strain TB40E was obtained from the ATCC. HCMV strain AD169 was obtained from the ATCC.

**Northern blot analysis.** Total RNA was extracted and 20 μg total RNA was separated as described previously (Michel et al., 1996; Winkler et al., 1994). RNA was transferred to a nylon membrane and immobilized by baking for 2 h at 60°C. For hybridization, a specific oligonucleotide probe for collagen I (5'-GGGCCAGGGCGGGCTTCTGACCTCAAGCCTCTT-3') was used. The fibronectin probe was amplified using the primer pair 5'-TGGTCTGGTCTACCTGTGTGGG-3' and Fibro-M (5'-ATGCTTGGATGCTATGCT-3'). Membranes were stripped for 20 min with 0.1× SSC and 0.1% SDS for rehybridization with a probe specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Hybridization was carried out at 42°C for 18 h in the presence of 50% formamide, 2× Denhardt's solution, 5× SSPE, 0.05% SDS and 200 μg salmon sperm DNA ml⁻¹. Filters were washed at a stringency of 0.1× SSC, 0.1% SDS at 65°C. Bands were quantified using the Multianalyst software (Bio-Rad).

**Quantitative RT-PCR.** For RT-PCR, cultures were harvested at 24, 48 and 72 h post-infection (p.i.). Total RNA was extracted by using a High Pure RNA isolation kit (Roche) according to the manufacturer's instructions. For detection of cyclophilin, collagen type I and fibronectin mRNA, 1 μg total RNA was reverse-transcribed into cDNA using Superscript reverse transcriptase (Gibco) according to the manufacturer's instructions. The following primers were used: cyclophilin, sense primer 5'-ATGGTCACACCCACGCGTGT-3' and antisense primer 5'-CTGCTGTCTTGTGGGACCTTGTC-3'; collagen type I, sense primer 5'-AAAGAAAGGCGCAAAGGT-3' and antisense primer 5'-ACGATCACCACCTCTGGCCA-3'; and fibronectin, sense primer 5'-TCCAGGATTCTGCTGCCC-3' and antisense primer 5'-CTGCAA-GCTTCTAAGTCA-3'. Forty nanograms of cDNA was amplified using LightCycler technology (Roche) as described previously (Bachem et al., 2005). FastStart Taq DNA polymerase (Roche) was activated by preincubation at 95°C for 10 min. Reactions were cycled 38 times. PCR products were quantified by using the LightCycler software.

**Immunofluorescence microscopy for detection of collagen type I and fibronectin.** Cells were grown on chamber slides (Becton Dickinson). At 24, 48 and 72 h p.i., cultures were washed, fixed with acetone and immunostained for collagen type I and fibronectin. Immunofluorescence microscopy was performed as described previously (Schmid-Kotsas et al., 1999; Luttenberger et al., 2000) with minor modifications. For collagen type I, the staining sequence was: rabbit anti-human collagen I (Dako; 1:20), anti-rabbit IgG–horseradish peroxidase (HRP) (Dako; 1:100), biotin-TSA reagent (Perkin Elmer; 1:500) and streptavidin–fluorescein isothiocyanate (FITC) (Dako; 1:1068) or Alexa Fluor 568 (Molecular Probes; 1:200). Fibronectin was stained using rat anti-fibronectin (DADE Behring; 1:50), biotin–anti-rabbit Ig (Dako; 1:50) and streptavidin–FITC (Dako; 1:100). HCMV was stained with an anti-immediate early 1 (IE1) antibody (Argene; 1:100) followed by an anti-mouse Ig–Alexa Fluor 488 antibody (Molecular Probes; 1:200). Counterstaining was performed using DAPI or bisbenzimide. Three washing steps with PBS followed each incubation step. Staining was observed using fluorescence microscopy (Olympus). Exposure time and aperture were identical for all pictures to allow comparison of staining intensities. Including rabbit non-immune serum instead of specific first antibody controlled non-specific staining. To quantify immunofluorescence intensity, three representative fields were evaluated using the Cell Imaging software (Olympus).

**Scanning electron microscopy (SEM).** For SEM, cells were fixed with 2.5% glutaraldehyde (v/v) in 0.1 M PBS with 1% sucrose (w/v), dehydrated in a graded series of ethanol and dried using carbon dioxide. Afterwards, cells were coated using a BAF 300 freeze-etching device (Bal-Tec). Cells were rotary-coated as described with a single layer of platinum-carbon (coating thickness 3 nm). The samples were imaged in a Hitachi S-5200 in-lens field emission scanning electron microscope at an accelerating voltage of 10 kV using the secondary electron signal (Walther & Muller, 1999).

**Immonoassay to quantify synthesis of collagen type I and fibronectin.** Starting with infection, HFF and SMC were cultivated under serum-reduced conditions (0.1% FCS) to avoid unspecific results. Each experiment was performed in triplicate. Six hours after HCMV infection or mock infection, ascorbic acid (100 μg ml⁻¹) and β-aminopropionitrile (100 μg ml⁻¹) and, when indicated, EDTA (1.5 mM) were added. For neutralization of alpha and beta interferon (IFN-α and -β), neutralizing or irrelevant monoclonal antibodies were added (BPL Biomedical; 1 μg ml⁻¹) (Zimmermann et al., 2005). Supernatants were harvested 48 h p.i. and cleared of cellular debris by centrifugation. The fibronectin concentration in culture supernatants was measured by time-resolved fluorescence immunoassay as described previously (Schmid-Kotsas et al., 1999; Luttenberger et al., 2000). To measure collagen type I, 96-well plates (Nunc) were coated with 50 μl rabbit anti-mouse IgG per well (6 μg ml⁻¹; Dako) and with mouse anti-human collagen type I (15 μg ml⁻¹; Sigma) for 4 h at room temperature. After blocking for 2 h at room temperature, samples (100 μl per well) and standards
(7.8–1000 ng human placental collagen type 1 ml⁻¹; Biozol) were added and incubated overnight at 4°C. After three washing steps, the biotinylated second antibody (goat anti-human collagen type I, 1:300; Biozol) was added (4 h). After further washing and incubation for 2 h at room temperature with streptavidin–europium (1:500; Delfia Wallac), another five washing steps were performed, enhancement solution (Delfia Wallac) was added and, after 45 min, time-resolved fluorescence of the europium chelate was measured with a Victor multiwell-counter. All measurements were done in duplicate. Variations of duplicate measurements were between 0.5 and 5%. Statistical analysis was performed using a paired t-test.

Quantification of MMP-2 enzyme activity and concentration. Zymography. During HCMV infection and mock infection, cells were maintained under serum-reduced conditions (0.1% FCS) to avoid unspecific signals. At indicated time points, supernatants were harvested, cleared of cellular debris by centrifugation and UV-inactivated (366 nm, 2 min, 200 kJ). To correct for loss of cells due to virus-induced lysis, supernatants were adjusted to equal cell numbers of the corresponding wells and were mixed with sample buffer (0.98 M Tris/HCl, pH 6.8, 1% SDS, 4% glycerol, 0.006% bromophenol blue) and separated on a 7% polyacrylamide gel containing 1 mg gelatin or casein ml⁻¹. Gels were soaked in 2.5% Triton X-100 for 1 h, developed (50 mM Tris/HCl, pH 7.5, 200 mM NaCl, 5 mM CaCl₂, 0.2% Brij-35) at 37°C for 16 h and stained using Coomassie blue (Bio-Rad).

ELISA. To measure MMP-2 protein concentrations, 96-well plates (Nunc) were coated with 50 μl rabbit anti-mouse-IgG per well (6 μg ml⁻¹; Dako) overnight at 4°C. Wells were washed three times and then coated with mouse anti-MMP-2 antibody for 4 h at room temperature. After three washing steps, wells were blocked for 2 h at room temperature. Samples (100 μl per well) and standards (Chemicon) were added and incubated overnight at 4°C. Following three washing steps, biotinylated second antibody (goat anti-human MMP-2, 1:500; R&D Systems) was added (4 h). After washing and incubation for 1 h at room temperature with streptavidin–europium (1:500; Delfia Wallac), another five washing steps followed, enhancement solution (Delfia Wallac) was added and, after 45 min, time-resolved fluorescence of the europium chelate was measured with a Victor multiwell-counter. All measurements (standards, controls and samples) were done in duplicate. Variations of duplicate measurements were between 0.5 and 5%. Active MMP-2 protein present in the supernatants of SMC cultures was quantified using a commercially available assay (Calbiochem) following the manufacturer’s instructions.

RESULTS

HCMV infection induces a decline in ECM mRNAs

Using differential display RT-PCR analysis, we had previously identified several host cell genes encoding ECM proteins that were modulated by HCMV in infected HFF (Schaarschmidt et al., 1999). We confirmed these results by Northern blot analysis. In HFF, a dramatic virus-induced decrease in mRNA was observed within the first 24 h p.i. In infected HFF compared with mock-infected HFF after 24 h p.i., the strongest reduction in mRNA was observed for tenascin, with a complete loss of specific mRNA. Thrombospondin, fibronectin, decorin and collagen type I mRNAs were reduced by more than 3-fold and lectin and fibulin mRNAs were reduced 2-fold. Compared with mock-infected HFF 48 h p.i., collagen type I, lectin and fibulin specific mRNAs were reduced by more than 3-fold. For fibronectin, decorin, tenascin and thrombospondin, a complete loss of mRNA expression was observed in HCMV-infected HFF (Fig. 1a).

In subsequent experiments, we focused on the HCMV-induced modulation of collagen and fibronectin, two major components of the ECM that form the fibrous cap of atherosclerotic plaques. To exclude the possibility that the HCMV-induced reduction of collagen and fibronectin gene expression was confounded by differences in cell proliferative activity, we infected HFF cultures 2 and 5 days after seeding of cells, resulting in different cell densities. However,
cell density did not influence the HCMV-induced reduction of matrix gene expression (not shown).

In HCMV-infected compared with mock-infected SMC, a decrease in collagen type I mRNA to approximately 6% was demonstrated 24 h p.i. using quantitative PCR (Fig. 1b). The virus-induced downmodulation at 48 h p.i. was comparable after infection with the 'wild-type'-like strain TB40E and the laboratory strain AD169. Downregulation of fibronectin mRNA was somewhat delayed and less pronounced compared with the reduction of collagen type I gene expression. Following infection of SMC with TB40E, the level of fibronectin mRNA was reduced to approximately 50, 12 and 2%, respectively, at 24, 48 and 72 h p.i.

Fibrillar collagen type I and extracellular fibronectin are reduced in infected HFF and SMC

Indirect immunofluorescence studies of HCMV-infected and mock-infected cells showed that the decreased gene expression in virus-infected cells correlated well with a decrease of matrix protein expression. A clear reduction of extracellular, insoluble collagen type I fibres was seen in HFF and SMC. Fig. 2(a) demonstrates the fluorescence intensities of a representative experiment of five. Visible changes were observed as early as 24 h p.i. (not shown) and were fully developed at 48 h p.i. Compatible with the Northern blot and RT-PCR results, the HCMV-induced downregulation of collagen type I was more pronounced than

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**Fig. 2.** Expression of extracellular collagen type I and fibronectin in HCMV-infected HFF and SMC. (a) Indirect immunofluorescence staining of collagen type I and fibronectin (green fluorescence) was performed on mock-infected and HCMV-infected HFF and SMC 48 h p.i. HFF and SMC were infected at an m.o.i. of 1 with HCMV strains AD169 and TB40E, respectively. Cells were stained in parallel with a rabbit anti-human collagen I or a rabbit anti-human fibronectin antibody. Nuclei were stained with DAPI, demonstrating a comparable number of cells in infected and mock-infected HFF and SMC 48 h p.i. (×200). (b)–(g) The presence of ECM fibres in mock-infected and HCMV-infected cultures was analysed 48 h p.i. in HFF (AD169) (b, c) and in SMC (TB40E) by scanning (d, e) and transmission (f, g) electron microscopy. Arrows indicate the intercellular spaces with (mock-infected HFF and SMC) or without (HCMV-infected HFF and SMC) the presence of ECM fibres. Bars, 10 μm (b, c), 2 μm (d, e) and 1 μm (f, g).
that of fibronectin. To exclude virus strain-specific effects, we performed cross-over experiments, where similar results were obtained following infection of HFF with the TB40E strain as well as following infection of SMC with the AD169 strain. No HCMV strain-specific modulation was found (data not shown).

Electron microscopy confirmed the results of the immunofluorescence studies, since a dramatic HCMV-induced loss of fibrillar matrix was demonstrated in HFF and SMC. Hardly any fibres were detectable between adjacent HCMV-infected cells, whereas, in the mock-infected control, matrix fibres interlaced the intercellular gaps (Fig. 2b).

**HCMV influences the equilibrium of synthesis and degradation of ECM**

Immunofluorescence assays do not allow exact quantification of the proteins studied. Therefore, we performed quantitative immunoassays for collagen type I and fibronectin proteins in the supernatants of HCMV-infected and mock-infected HFF and SMC. Furthermore, by adding EDTA, as an inhibitor of MMPs, we wanted to determine whether HCMV, besides influencing synthesis, also modulates degradation of matrix proteins. Therefore, soluble collagen type I and fibronectin were quantified by ELISA with and without added EDTA. A reduction of mean fibronectin concentrations to 65 and 48 % was observed in HCMV-infected cultures compared with uninfected HFF and SMC, respectively. Although some further decrease in fibronectin concentrations was observed, addition of EDTA to the cultures did not alter levels of soluble fibronectin significantly in HCMV-infected HFF and SMC (Fig. 3a, b). It is reasonable to assume that proteases uninfluenced by EDTA are involved in the reduction of fibronectin protein following HCMV infection, since an increased casein-digesting activity was observed in supernatants of HCMV-infected SMC (see Fig. 6a).

The loss of free collagen was most notable ($P<0.0001$) in infected HFF, where a reduction to 3 % of the mean collagen concentration of the uninfected control was observed within 24 h p.i. (Fig. 3c). A significant ($P=0.0006$; results from three independent experiments, using different SMC donors) reduction of collagen to 38 % compared with the uninfected control could also be demonstrated in HCMV-infected SMC. Addition to the cell cultures of antibodies that neutralize IFN-α and -β activity did not alter the extent of HCMV-induced reduction of fibronectin and collagen expression in the supernatants of infected cells (Fig. 4). Thus, the observed downmodulation appears to be a direct effect of HCMV.

With respect to matrix degradation, we observed an interesting difference between the two cell types (Fig. 3c, d). In contrast to HFF, inhibition of MMPs by addition of...
EDTA antagonized the reduction of soluble collagen in HCMV-infected SMC cultures partially, but significantly \( (P = 0.0009) \). This indicates that, in SMC, HCMV infection modulates matrix expression by influencing synthesis as well as degradation.

To analyse these findings further, we first performed indirect immunofluorescence on HCMV-infected SMC and HFF with and without added EDTA during infection. In HCMV-infected SMC cultivated with EDTA, the specific red fluorescence signal for insoluble extracellular collagen type I fibres (Fig. 5a) was significantly stronger compared with infected SMC cultures without inhibition of degradation \( (P = 0.01) \) (Fig. 5b). In contrast, addition of EDTA to HCMV-infected HFF did not alter extracellular collagen type I staining noticeably (Fig. 5a). Comparing the numbers of cells positive for nuclear staining with the anti-viral IE1 antibody and with bisbenzimide demonstrated that, following infection with an m.o.i. of 1, all cells of the cultures were infected with HCMV (Fig. 5a) (Reinhardt et al., 2005a).

**HCMV infection induces MMP-2 activity in SMC**

To identify the activity responsible for the collagen degradation present in the supernatants of HCMV-infected SMC, zymographic assays were performed. An increase in gelatin-digesting activity corresponding to the molecular size of MMP-2 and activated MMP-2 was detected. HCMV-induced elevation of MMP-2 activity could be demonstrated in five independent experiments using SMC from different donors and two distinct HCMV isolates. Zymographs from representative experiments are shown (Fig. 6a). The activated form of the MMP-2 protein was quantified in supernatants from the same experiment using a commercial assay. A 2- to 3-fold increase compared with the uninfected control was observed within 72 h of HCMV infection (Table 1). These results corresponded well with the zymographic pattern (Fig. 6a; right panel). Interestingly, in supernatants from HCMV-infected HFF, a decrease rather than an increase in MMP-2 activity was detected by zymography, independent of the HCMV isolate used (Fig. 6b). Using an ELISA, we quantified MMP-2 protein in HFF and SMC following infection with AD169 and TB40E. In HCMV-infected SMC, we could show a 1.5- to 2-fold increase in MMP-2 protein concentration within 72 h p.i., whereas the amount of MMP-2 protein in HFF decreased to 60–80% when compared with mock-infected cells (Fig. 6c). Again, these results are in good accordance with the quantitative results obtained by immunoassay (Fig. 3). Thus, these data support the notion that, depending on the cell type, HCMV modulates different mechanisms that lead to a reduction of ECM proteins.

**DISCUSSION**

HCMV is a strictly species-specific virus, and animal CMVs differ in their biological behaviour and sometimes even in
the function of homologous genes (Wagner et al., 2000). Thus, animal models using animal CMVs are not completely suitable to verify findings observed in vitro with HCMV. A wide spectrum of cell types, such as fibroblasts, endothelial cells and SMC, can be infected by HCMV in vivo (Sinzger et al., 1995). However, there are differences in susceptibility towards distinct HCMV strains and HCMV might interfere with different pathways of the cell machinery depending on the infected cell type. In experimental models, it is therefore of particular importance to use target cells that are relevant for pathogenesis in vivo. In order to meet this criterion, we included coronary artery SMC in our studies. Further, the HCMV strain used is important, since the commonly used laboratory strain AD169 carries large gene deletions and has a restricted cell tropism relative to clinical strains, which call into question its de facto role as a prototype (Gerna et al., 2003). Therefore, other, more 'wild-type'-like HCMV strains, such as TB40E, have to be used in parallel when studying HCMV-induced modulations of cellular genes.

Our study shows that, early after HCMV infection, ECM gene expression and the amount of the respective proteins is reduced significantly, independent of the cell type and HCMV strain used. Neutralization of IFN-α and -β activity did not influence the HCMV-induced modulation of ECM protein expression, suggesting that the downregulation is due directly to the HCMV infection and not a response to IFN (Fig. 4) (Zimmermann et al., 2005). The data demonstrate that HCMV affects both synthesis and degradation of ECM only in SMC, which in fact proves differential gene modulations in different cell types by the same virus. To our knowledge, this is the first study to demonstrate an HCMV-induced effect on fibronectin and collagen gene expression and protein synthesis in SMC, a cell
type highly relevant for vascular disease. The reduction of ECM proteins is not just a uniform effect due to a generalized virus-induced shutdown of the host cell machinery. On the contrary, the expression of several cellular genes in SMC is even induced by HCMV, such as the functionally active vascular endothelial growth factor (VEGF) (Reinhardt et al., 2005b) or the platelet-derived growth factor receptor-β (PDGFR) (Reinhardt et al., 2005a). Further, proving the viability of HCMV-infected SMC, a greater responsiveness to PDGF stimulation (Reinhardt et al., 2005a) as well as an increase in chemotaxis (Streblow et al., 1999) could be shown in HCMV-infected SMC.

Whereas ECM has traditionally been regarded as an inert scaffold that merely provides mechanical support for functional cells within an organ or tissue, in recent years it has become increasingly evident that ECM regulates pivotal processes such as growth, death, adhesion, migration, invasion, gene expression and differentiation of cells. In addition, the sponge-like architecture of the ECM may serve as a reservoir for vasoactive growth factors, such as VEGF or PDGF (Streuli, 1999). Finally, modulating the amount or composition of ECM may be relevant for the detachment and spreading of cells that harbour infectious agents and for the pathogenesis of several disease entities, among them inflammatory processes of vessels. Vascular remodelling, consisting of degradation and reorganization of ECM, allows adaptation and repair-healing of adult vessels. Incorrect remodelling or lack thereof leads to vascular disease (Ivan et al., 2001). This process can result in a clinically stable (Schoenhagen et al., 2000) or unstable angina, the latter related to plaque instability and rupture (Cullen et al., 2003; Naghavi et al., 2003). A potential pathomechanism for plaque rupture appears to be a reduction of the ECM scaffold, especially of collagens that confer the tensile strength to the cap. Influences on SMC, the

**Table 1.** Quantification of MMP-2 activity in supernatants of HCMV-infected and mock-infected SMC

<table>
<thead>
<tr>
<th>Infection</th>
<th>24 h p.i.</th>
<th>48 h p.i.</th>
<th>72 h p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock</td>
<td>1.3</td>
<td>5.1</td>
<td>8.93</td>
</tr>
<tr>
<td>TB40E</td>
<td>3.44</td>
<td>15.6</td>
<td>17.35</td>
</tr>
</tbody>
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main producer of collagens within the vessel wall (Brooke et al., 2003), are therefore of particular importance. In this light, our data demonstrating a substantial HCMV-induced loss of ECM in SMC (Figs 1, 2, 3 and 4) may represent a potential HCMV-induced pathomechanism towards a high-risk phenotype of unstable atherosclerotic plaques, facilitating plaque rupture. The loss of matrix fibres in SMC was based on both a reduction in matrix gene expression and an increase in matrix degradation by MMP-2 activity (Fig. 6). Degradation of the ECM scaffold by specialized enzymes is an important prerequisite for the reshaping of tissue. Regarding vascular disease in vivo, local overexpression of matrix-degrading activity has been shown in the vulnerable shoulder regions of plaques (Galis et al., 1994). Disruption of the equilibrium of the ECM content in vivo is especially associated with an increase in MMP-2 activity (Pasterkamp et al., 2000) and seems to be related to plaque instability. Endothelial cells and SMC produce MMP-2 constitutively (Galis et al., 1994). By influencing matrix production and matrix degradation, HCMV infection of SMC may alter vascular remodelling in infectious vasculitis. In an artery organ culture model, we could identify SMC in the vascular media and intima as prime target cells of HCMV infection (Reinhardt et al., 2003), underlining the potential pathogenetic relevance of HCMV-induced modulations of gene expression in SMC.

Modulating the quantity and quality of ECM may also facilitate virus spread during primary and recurrent infections in the infected organism. A small number of highly infectious, HCMV-containing cells has been observed in circulation during active HCMV infection of immunosuppressed patients, and it was proposed that circulating infected cells may serve as a vehicle for virus dissemination (Greffte et al., 1993). Here, we present an HCMV-induced molecular mechanism that potentially promotes cell rounding and loss of contact to the underlying tissue. As a consequence, HCMV-infected intima cells could detach from the vessel wall because of virus-induced reduction of ECM proteins, enter the blood circulation and thus lead to cell-bound virus spread.

In conclusion, we have shown that HCMV infection of SMC and HFF leads to a significant loss of collagen type I and fibronectin. Two independent, directly HCMV-induced mechanisms for ECM reduction were identified in SMC, being major players in the pathogenesis of vascular diseases: namely, an HCMV-associated decrease in matrix synthesis and an increase in matrix degradation through MMP-2. As for the pathophysiological relevance of this virus-induced effect, we can only hypothesize that the reduction of the ECM scaffold might on the one hand lead potentially to plaque instability, with all its clinical consequences, and on the other hand to a promotion of cell-associated, systemic virus spread.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of T. Schmid and I. Bennett in preparing the manuscript. Further, we want to thank A. Lüske, M. Adam and M. de Groot for excellent technical assistance. This study was supported by the Deutsche Forschungsgemeinschaft ‘Sonderforschungsbereich 451’, project A2, and the Bundesministerium für Bildung und Forschung ‘IZKF’, project H4 (01KS9605/2). P. S. was supported by the Novartis-Stiftung.

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