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Maternal HIV Type 1 Infection Suppresses MMP-1 Expression in Endothelial Cells of Uninfected Newborns: Nonviral Vertical Transmission of HIV Type 1-Related Effects

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ABSTRACT

HIV-1 infection is associated with vascular alterations. This is accompanied by an increased risk of cardiovascular diseases and Kaposi's sarcoma, an endothelial cell-derived tumor. We investigated the impact of maternal HIV-1 infection on phenotype and gene expression of endothelial cells in newborns. For this reason endothelial precursor cells and differentiated endothelial cells were isolated from cord blood as well as from umbilical veins and arteries of noninfected infants born to HIV-1-infected (H-group) and noninfected (N-group) mothers. No apparent differences in proliferation, capillary formation, and expression of endothelial cell markers were detected in these cells. Interestingly, the expression of matrix metalloproteinase was repressed significantly (χ^2 analysis, $p < 0.002$) and consistently at the RNA, the protein, and the secretory levels in the H-group as compared to the N-group. Neither treatment with zidovudine (AZT), mutations in the matrix metalloproteinase-1 (MMP-1) promoter, nor epigenetic changes in the promoter methylation pattern were responsible for the repression of MMP-1 expression in H-group endothelial cells. The reduced MMP-1 expression may contribute to the impaired cardiac function that has been observed in children of HIV-1-infected women. Most interestingly, our findings indicate that HIV-1-related effects can be transferred from mother to child in the absence of HIV-1 transmission.

INTRODUCTION

Infection with human immunodeficiency virus (HIV)-1 leads to depletion of $CD4^+$ T cells and finally to acquired immunodeficiency syndrome (AIDS).[1] In uninfected children born to HIV-1-positive mothers decreased numbers of $CD4^+$ T cells were observed,[2] indicating that HIV-1-related effects may be vertically transmitted in the absence of virus transmission. In these children HIV-1-specific $CD4^+$ but rarely $CD8^+$ T cell activity was detected, suggesting that fetuses have been exposed to viral proteins.[2,3]

The vascular system is severely impaired in HIV-1-infected patients. For example, the incidence of cardiovascular diseases[4] and of an endothelial cell-derived tumor, Kaposi's sarcoma (KS),[5–8] is dramatically increased in these patients. In addition, chronic injury and cell activation have been detected at the aortic endothelium of HIV-1-infected patients *in vivo*.[9] Human herpesvirus-8 (HHV-8),[10] deregulation of the network of cytokines,[11] and angiogenic growth factors,[12,13] as well as the HIV-1-encoded transcriptional activator protein HIV-1 Tat[5] are regarded as key factors in the induction of HIV-1–associated vessel activation.

The aim of this study was to investigate whether maternal HIV-1 infection may influence circulating endothelial precursor cells (EPC) and differentiated endothelial cells of uninfected newborns, as has been observed for T cells.

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MATERIALS AND METHODS

Patients

Endothelial cells were isolated of nine HIV-1-negative infants born to HIV-1-infected women (hereafter referred as H-group) and of eight children born to uninfected women (N-group; Table 1). All mothers provided written informed consent. The study received ethical approval from the ethics committee of the University Hospital Grosshadern, LMU Munich.

Primary endothelial cell cultures

EPC were isolated from cord blood by enrichment with magnetic beads (anti-CD34, Miltenyi, Bergisch Gladbach, Germany) and adhesiveness to fibronectin-coated culture dishes (BD Becton Dickinson, Heidelberg, Germany). Differentiated endothelial cells of the H-group were isolated according to standard prodedures[14] from umbilical vein (HUVEC) or artery (HUAEC; Table 1). HUVEC of the N-group were commercially purchased from Clonetics (Verviers, France).

EPC were cultivated in endothelial basal medium (EBM) supplemented with 10% fetal bovine serum (FBS; Clonetics), 10% horse serum (Clonetics), and 50 ng/ml stem cell growth factor (PeproTech, Frankfurt, Germany). HUVEC and HUAEC were maintained in EBM with 5% FBS.

RT-PCR

RNA was isolated using RNeasy-Mini-Kit (Qiagen, Hilden, Germany), treated with DNase I (Clontech, Heidelberg, Germany), reverse transcribed, and subjected to polymerase chain reaction (PCR) with matrix metalloproteinase (MMP)-1, -2, and MT1-MMP specific primers (Clontech).

Western blot analysis

Cell stimulation was carried out in EBM with 0.5% FBS by adding either VEGF (R&D Systems, Heidelberg, Germany) and bFGF (Roche, Mannheim, Germany), 10 ng/ml each, or zidovudine (AZT, Glaxo Smith Kline, Munich, Germany). Monoclonal mouse anti-MMP-1 (1:500; R&D Systems) and anti-GAPDH (1:50,000; Chemicon, Hofheim, Germany) antibodies were used as described.[15]

Immunocytochemistry

Cells were fixed in 2% paraformaldehyde and permeabilized in 0.1% Triton X-100. An anti-MMP-1 monoclonal mouse primary antibody (1:100; Oncogene, Schwalbach, Germany) and an AlexaFluor 488–conjugated anti-mouse secondary antibody (1:500; Molecular Probes, Leiden, the Netherlands) were used.

Table 1. MMP-1 Expression in Endothelial Cells of Children in Relation to HIV-1-Infection and Therapy Regimen of the Mothers[a]

Culture name	*Origin of endothelial cells*	*MMP-1 expression*	*Antiretroviral therapy during pregnancy*			
			AZT	*NRTI*	*NNRT*	*PI*
		Children of HIV-1-infected mothers (H-group)				
H1-HUVEC	Vein	−	X			
H1-EPC	Cord blood	−				
H2-HUVEC	Vein	−	X	X		X
H2-EPC	Cord blood	−				
H3-HUAEC	Artery	+	X			
H3-EPC	Cord blood	+				
H4-HUVEC	Vein	+	X	X	X	X
H5-EPC	Cord blood	−	X	X	X	
H6-EPC	Cord blood	−	X	X	X	
H7-EPC	Cord blood	+	X	X		X
H8-EPC	Cord blood	+	X	X	X	
H9-EPC	Cord blood	−	X	X	X	
		Children of HIV-1-negative mothers (N-group)				
N1-HUVEC	Vein	+++				
N2-HUVEC	Vein	+++				
N3-EPC	Cord blood	+++				
N4-EPC	Cord blood	+++				
N5-EPC	Cord blood	−				
N6-EPC	Cord blood	+++				
N7-EPC	Cord blood	+++				
N8-EPC	Cord blood	+++				

[a]AZT, zidovudine; NRTI, nucleoside reverse transcriptase inhibitor in addition to AZT; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; EPC, endothelial precusor cells; HUVEC, human umbilical vein endothelial cells; HUAEC, human umbilical aorta endothelial cells. −, no MMP-1 expression detectable; +, low MMP-1 expression; +++, high MMP-1 expression. (*n*) in H(*n*) and N(*n*) indicates the number of the donor. X indicates treatment.

Gelatin-zymography

Substrate gel electrophoresis (zymography) with 1 mg/ml gelatin was carried out as described.[15,16]

Promoter methylation

Methylation of the MMP-1 promoter was analyzed using the bisulfite method.[17]

RESULTS

MMP-1 expression and release are decreased in endothelial cells of children born to HIV-1-infected mothers

EPC were isolated from cord blood and differentiated endothelial cells were isolated from umbilical vein (HUVEC) or artery (HUAEC) derived from children of HIV-1-infected (H-group, $n = 9$) and noninfected (N-group, $n = 8$) mothers (Table 1).

EPC, HUVEC, and HUAEC were consistently positive for the endothelial cell markers von Willebrand factor, CD31, CD105, and uptake of DiI-Ac-LDL. Treatment with interleukin (IL)-1β (200 U/ml) induced expression of ICAM-1 and E-selectin and treatment with tumor necrosis factor (TNF)-α (300 U/ml) induced the expression of VCAM-1 in all cell populations. All cells revealed a typical cobblestone morphology, comparable growth rates, and formed capillaries when seeded on Matrigel (data not shown).

Gene expression of HUVEC and EPC of two donors of the N-group and one donor of the H-group was compared by cDNA array technology (Clontech Atlas Human 1.2 Array). Of the 1176 genes analyzed most were found to be similarly expressed in the different cells. However, MMP-1 expression was clearly

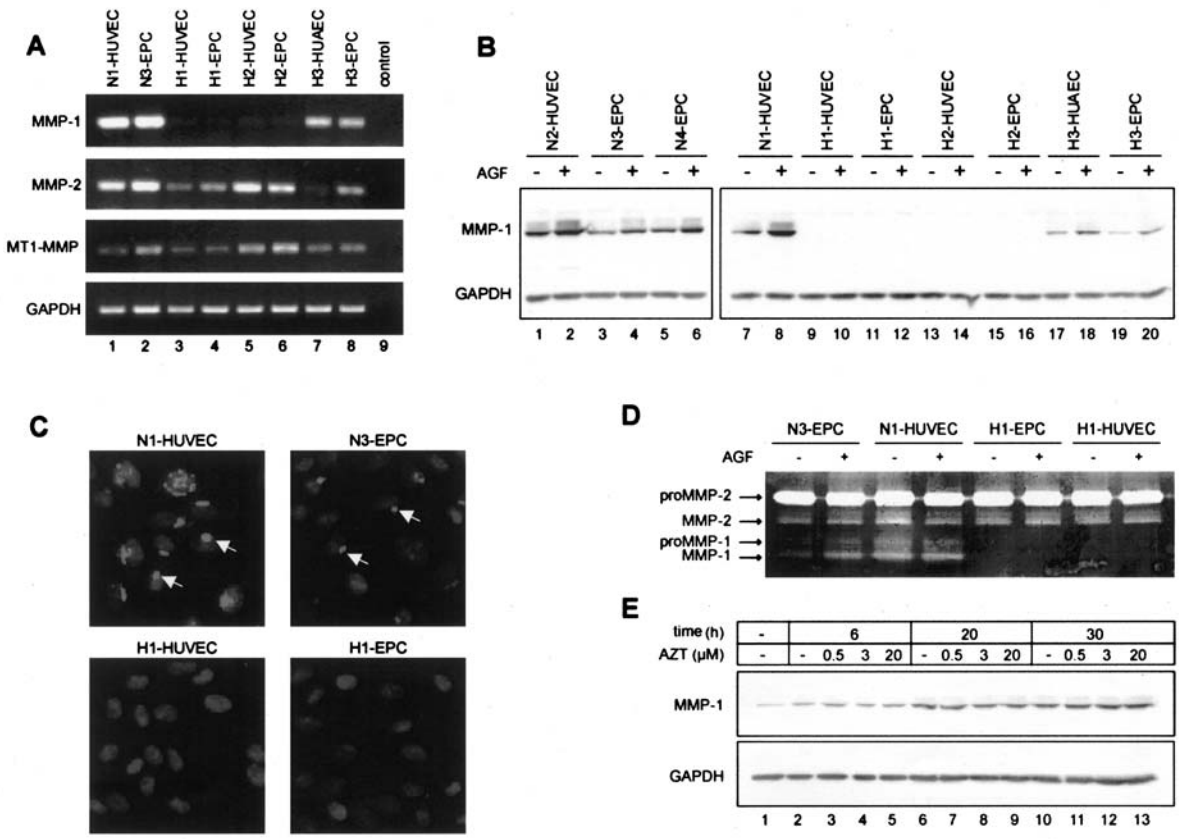

FIG. 1. MMP-1 expression in EPC, HUVEC, and HUAEC. (**A**) RT-PCR analysis of MMP-1 (upper panel), MMP-2 (upper middle panel), MT1-MMP (lower middle panel), and GAPDH (lower panel) expression in EPC (lanes 2, 4, 6, and 8), HUVEC (lanes 1, 3, and 5), and HUAEC (lane 7) of different donors of the N-group (lanes 1 and 2) and of the H-group (lanes 3–8). (*n*) in N(*n*) and H(*n*) indicates the number of the donor. (**B**) Western blot analysis of MMP-1 expression in HUVEC (lanes 1, 2, 7–10, 13, and 14), HUAEC (lanes 17 and 18), and EPC (lanes 3–6, 11, 12, 15, 16, 19, and 20) of different donors of the N-group (lanes 1–8) and of the H-group (lanes 9–20). Cells were either unstimulated (−) or stimulated (+) with AGF (VEGF and bFGF, 10 ng/ml each) for 20 hr. GAPDH served as loading control. (**C**) Immunocytochemical detection of MMP-1 in HUVEC (left panels) and EPC (rigth panels) of the N-group (top) and the H-group (bottom). MMP-1 expressing cells are labeled by an arrow. Nuclei were visualized with DAPI. Magnification ×40. (**D**) Gelatin-zymography of cell culture supernatants of EPC and HUVEC of donors of the N- and of the H-group with (+) and without (−) AGF stimulation for 20 hr. Supernatants were size-separated on a polyacrylamide–SDS gel containing 1 mg/ml gelatin. After staining with Coomassie brilliant blue proteolytic activity was indicated by clear, nonstained bands. (**E**) Western blot analysis of MMP-1 expression in HUVEC of donor N1 that were either treated with AGF alone [(−), lanes 1, 2, 6, and 10] or in combination with 0.5 μM AZT (lanes 3, 7, and 11), 3 μM AZT (lanes 4, 8, and 12), and 20 μM AZT (lanes 5, 9, and 13) for the indicated time range. GAPDH was detected as loading control.

detectable in HUVEC and EPC of the N-group (N-HUVEC, N-EPC), but not in HUVEC and EPC of the H-group (H-HUVEC, H-EPC; data not shown).

These results were confirmed by RT-PCR with HUVEC, EPC, and HUAEC isolated from different donors (N-group, $n = 2$; H-group, $n = 3$). N-HUVEC and N-EPC strongly expressed MMP-1 (Fig. 1A, top, lanes 1 and 2). In contrast, MMP-1 was not detectable in H-HUVEC and H-EPC of two donors and was clearly reduced in H-HUAEC and H-EPC of a third donor (Fig. 1A, top, lanes 3–8). In contrast, the expression of MMP-2 and MT1-MMP was not related to maternal HIV-1 infection (Fig. 1A, middle panels). Detection of GAPDH served as a standard (Fig. 1A, lower panel).

At the protein level endothelial cells of all donors were tested for MMP-1 expression. In the N-group seven of the eight donors were strongly positive for MMP-1 while one donor was negative [Fig. 1B, lanes 1, 3, 5, and 7 (example of Western blot of four donors); Table 1 (overview of all results)]. In contrast, five donors of the H-group were negative [Fig. 1B, lanes 9, 11, 13, and 15 (example of four donors); Table 1] and four donors showed a weak expression of MMP-1 [Fig. 1B, lanes 17 and 18 (example of two donors); Table 1]. In each case MMP-1 protein expression of the cells closely reflected the results obtained by RT-PCR (compare Fig. 1A and B). Angiogenic growth factor (AGF) treatment of the cells increased MMP-1 expression, but only in those cells that already expressed MMP-1 before stimulation (Fig. 1B, lanes 2, 4, 6, 8, 18, and 20). AGF did not increase MMP-1 expression to detectable levels in cells that were negative before stimulation (Fig. 1B, lanes 10, 12, 14, and 16). χ^2 analysis revealed that significantly ($p < 0.002$) fewer children of the H-group expressed MMP-1 in endothelial cells.

In agreement with the Western blot analysis MMP-1 was detected by immunocytochemistry in N-HUVEC and N-EPC, whereas H-HUVEC and H-EPC were negative (representative examples in Fig. 1C) or exhibited only very weak signals (data not shown).

Finally, gelatinolytic activities released by HUVEC and EPC were analyzed by gel zymography. All donors tested ($n = 4$) showed gelatinolytic activities corresponding to pro-MMP-2 and MMP-2[15,16] (Fig. 1D). Gelatinases with molecular weights corresponding to pro-MMP-1 and MMP-1 were detected only in N-HUVEC and N-EPC but not in cells of the H-group, irrespective of stimulation with AGF (Fig. 1D).

Therapy does not influence MMP-1 expression

Antiretroviral therapy may have pathogenic effects on the vascular system.[4,18] Of all drugs commonly used for therapy only zidovudine (AZT) was used by all HIV-1-infected mothers (Table 1). AZT was applied immediately before cesarean section and partly also as continuous treatment starting at week 32 of pregnancy. Treatment of N-HUVEC with AGF and increasing concentrations of AZT for 6–30 hr showed that AZT does not influence AGF-induced MMP-1 expression (Fig. 1E). This indicated that an antiretroviral regimen during pregnancy is unlikely to cause inhibition of MMP-1.

Sequence analysis of the MMP-1 promoter region (nt −550 to +290 relative to transcription start +1) in two donors of the H-group revealed wild-type sequences and excluded promoter mutation as a cause for MMP-1 down-regulation (data not shown). Epigenetic effects are often mediated by methylation of CpG islands in promoter sequences. The proximal MMP-1 promoter contains a cluster of five CpG islands. Analysis of the methylation status in two donors of the H-group and in one donor of the N-group revealed that all five CpG islands were methylated in all donors independently of the MMP-1 expression (data not shown).

DISCUSSION

We indicated that MMP-1 expression is inhibited consistently in different types of endothelial cells derived from non-infected children born to HIV-1-infected mothers. MMP-1 influences the angiogenic capability of endothelial cells.[15] The observed down-regulation of MMP-1 expression in endothelial cells may be related to impaired cardiovascular function, which has been observed in uninfected children of HIV-1-positive mothers.[19] Most importantly, our study provides an interesting example of a vertically transmitted effect of HIV-1 infection on the endothelium of newborns that is manifested in the absence of virus transmission.

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