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Herpesviridae in the Coronary Arteries and Aorta of Young Trauma Victims

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The presence of herpes simplex virus (HSV) and cytomegalovirus (CMV) nucleic acid and/or antigen was demonstrated in the coronary arteries and thoracic aortas of young trauma victims by the *in situ* DNA hybridization and ABC immunoperoxidase methods, respectively. Epstein–Barr virus (EBV) nucleic acid and capsid antigen were not detected in the arteries sampled in this study. Of 8 subjects in which virus was detected in the coronary arteries, 6 were positive for HSV and 2 for CMV; of 7 cases positive in the thoracic aorta, 5 were identified as HSV and 2 as CMV. Viral DNA and/or antigen were found in occasional cells in the intact luminal surface and in focal clusters of spindle-shaped or "foamy" cells in the intimal layer. The histologic findings indicate that HSV and CMV are associated with areas showing early or advanced atheromatous changes in the coronary arteries and with lesion-free as well as lesion areas in the thoracic aorta. The virologic findings support the concept that herpesviruses may potentially play a direct or indirect role in the pathogenesis of human atherosclerosis. (Am J Pathol 1988, 130:71–79)

VIRUSES have long been suspected of playing a role in cardiovascular diseases, particularly the cardiomyopathies and certain chronic valvular diseases of unknown etiology. Recently, experimental studies by Fabricant et al[1,2] clearly established that infection of normocholesterolemic chickens with Marek's disease virus, a herpesvirus, leads to an arterial disease closely resembling chronic human atherosclerosis. Marek's herpesvirus infection was shown to alter aortic cholesterol metabolism and enhance cholesterol and cholesteryl ester accumulation in infected chickens and in cultured arterial smooth muscle cells.[3,4] These findings have stimulated interest in the possible role of herpesviruses as initiating or accelerating factors in human atherogenesis.

In 1983, Benditt et al reported the presence of herpes simplex virus (HSV) mRNA in specimens of thoracic aortic wall removed from patients undergoing coronary bypass surgery.[5] Presence of HSV genome was demonstrated by *in situ* hybridization in 13 aortic specimens, some of which appeared to represent early stages of atherogenesis. Cytomegalovirus (CMV) or Epstein–Barr virus (EBV) genome was not detected in any of the specimens examined. It was also demonstrated that HSV Type 1 (HSV-1) is capable of proliferating in cultured human fetal aortic smooth muscle cells. Benditt et al postulated that expression of at least a part of the herpesvirus genome in arterial smooth muscle cells may in some cases be instrumental in initiating or maintaining the enhanced cell proliferation observed in the pathogenesis of atherosclerosis. Melnick et al[6] demonstrated by immunofluorescence tests presence of CMV antigen in smooth muscle cells cultured from arterial tissues of patients undergoing blood vessel surgery. More than 25% of the cell cultures of arterial tissues derived from both carotid artery plaques and punch biopsy samples of uninvolved areas of the aorta were reported to contain antigens of CMV but not of HSV-1 or HSV-2. Replicating CMV was not detected by electron microscopy in the antigen-positive cells, and it was suggested that the artery wall may be a site of CMV latency. In 1984, Gyorkey et al[7] reported the presence of virions of the herpesviridae family on direct electron-microscopic examination of punch biopsy specimens from the proximal aorta of patients with atherosclerosis. Herpes-type virions were detected in occasional smooth muscle and rare endothelial cells in unin-

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volved areas of the aorta in 10 of 60 patients examined. Petrie et al[8] recently reported the presence of nucleic acid sequences of CMV in cells cultured from human arterial tissue, thus extending the earlier findings by Melnick et al[6] of CMV antigen detection in cultured arterial cells.

In this report we present evidence of the presence of HSV and CMV genome and antigen in the coronary arteries and aorta of young trauma victims by *in situ* DNA hybridization and immunohistochemical methods. The availability of fresh autopsy tissues from young trauma victims provided a unique opportunity to determine the presence and expression of these ubiquitous viruses in preclinical human atherosclerotic lesions.

Materials and Methods

Donors

The subjects of this study were trauma victims without overt heart disease, mainly vehicular accident and gunshot or stabbing victims, from Cook County, Illinois. Donors of both sexes between 15 and 35 years of age were selected for this study on the basis of previous observations[9,10] of the frequent appearance of atheromatous lesions during the second and third decades of life. Cardiovascular specimens employed in this study were obtained 4½ to 36 hours (mean, 16.5 hours) post mortem by prosectors under the direction of Robert J. Stein, MD, Chief Medical Examiner of Cook County, Illinois.

Tissue Specimens

Cryostat cut frozen sections and formalin-fixed tissue sections of the proximal main right (MRC) and left anterior descending (LAD) coronary arteries and the descending thoracic aorta were employed in this study. For frozen sections, tissues mounted in OCT were snap-frozen in liquid nitrogen and stored at −80 C until tested. Tissues for paraffin sections were fixed in Ringer's formalin (or Carnoy's solution) and processed by standard techniques. All sections were cut at 4–6 μ thickness for histologic and *in situ* hybridization and immunohistochemical studies.

In Situ Hybridization

In situ hybridization using biotin-labeled viral DNA probes were performed on cryostat sections placed on poly-D-lysine (Sigma Chemical Co., St. Louis, Mo) coated slides. The frozen sections were fixed in cold absolute ethanol for 10 minutes and stored at 4 C before testing. Biotinylated DNA probes were prepared from specific nucleic acid sequences of HSV (1 and 2), CMV, and EBV (Enzo Biochem, Inc., New York, NY). The HSV probe was prepared from a mixture of three sequences, including two HSV-1 fragments and one HSV-2 fragment, each cloned into The Bam HI site of pBR322. The HSV (1 and 2) probe utilized the 3.0-kb fragment from the terminal repeat region and the 8.0-kb fragment from the long unique region of HSV-1 and the 16.0-kb fragment from the long unique region of HSV-2. The CMV probe used a mixture of two Bam HI DNA fragments, a 25.2-kb fragment near the 5′ end of the long unique region and a 17.2-kb fragment from the short unique region of the Towne strain of CMV. The EBV probe comprised a 3.1-kb Bam HI "V" (internal repeat 1) fragment of the EBV genome. The biotinylated DNA probes were used in hybridization solution consisting of 50% deionized formamide, 10% dextran sulfate, 2X SSC (0.3 M sodium citrate, pH 7.0), 400 μg/ml of carrier DNA, and 2 μg/ml of the biotinylated viral DNA probe mixture.

The hybridization procedure employed was a modification of the protocol suggested by the manufacturer and that described by Brigati et al.[11] All slide sections were treated with 0.01% hydrogen peroxide for 30 minutes to inactivate endogenous peroxidase and washed in phosphate-buffered saline (PBS), pH 7.2, for 20 minutes before hybridization. Approximately 10 μl of the "bio-probe" solution was applied to each section, coverslipped, and the slide was placed on wet filter paper in a Petri dish supported on a rack above the water level of an 80 C water bath. Following denaturation for 10 minutes, the dish containing the slide was immediately transferred to a humidified 37 C incubator for 30 minutes for hybridization to occur. After hybridization, the coverslip was removed by immersion of the slide in 0.05% Triton X-100 in 1X PBS and washed sequentially twice in 0.05% Triton X-100 for 5 minutes, once in 50% formamide in 0.1X SSC for 10 minutes at 37 C, and three times in 0.05% Triton X-100 at room temperature. The hybridized biotinylated probe was detected by addition of a streptavidin–biotinylated horseradish peroxidase detection complex for 30 minutes at 37 C followed by 0.05% diaminobenzidine tetrahydrochoride (DAB) and 0.02% H_2O_2 as the substrate solution. The chromogenic reaction was complete in 3–5 minutes and stopped by dipping the slide in water. The sections were counterstained with naphthol blue-black, air-dried, and mounted with Krystalon (EM Diagnostic Systems, Inc., Gibbstown, NY).

The specificity of the biotinylated viral DNA probes was evaluated with MRC-5 human embryonic lung monolayer cultures infected with American

Type Culture Collection reference strains of CMV (AD-169), HSV-1 (F strain), and HSV-2 (G strain) and with an EBV-infected Burkitt's lymphoma cell line (HR1-K). The biotinylated DNA probes were found to be specific and did not cross-hybridize with heterotypic viruses when used according to our modified protocol. Reference virus infected cell culture slides prepared in our laboratory were fixed in cold ethanol and stored at 4 C. They served as controls and were tested simultaneously with cryostat sections in all hybridization assays.

Immunohistochemical Studies

Cryostat cut frozen sections of coronary arteries and aorta were tested directly for presence of viral antigen(s) utilizing the avidin–biotin complex (ABC) immunoperoxidase method.[12] Monoclonal antibodies directed against the following viral antigens were employed according to the procedure outlined in the Vectastain anti-mouse IgG ABC kit (Vector Laboratories, Inc., Burlingame, Calif): CMV early nuclear protein (DuPont NEN Products, North Billerica, Mass), HSV-1 nucleocapsid protein (Cooper Diagnostics, Inc., Westchester, Pa), HSV-2 nucleocapsid protein (DuPont NEN Products), and EBV capsid antigen (DuPont NEN Products). The monoclonal antibodies were tested against reference viruses and did not cross-react with heterotypic viruses when employed at predetermined working titers. Monoclonal antibody to CMV was applied at 1:320, HSV-1 at 1:80, and HSV-2 or EBV at 1:160 dilutions. Normal mouse ascitic fluid served as a negative control for the antibodies. Positive controls consisting of CMV-, HSV-, and EBV-infected cell cultures as well as buffer controls were included in all immunoperoxidase tests.

Virus Isolation Studies

Virus cultivation studies were conducted on selected autopsy cases in which tissue specimens could be processed immediately without delay or temporary storage. Approximately 2–4-mm sections of the coronary arteries (LAD, MRC) and aorta were removed aseptically and placed into sterile screw-cap vials containing transport medium consisting of Eagle's minimum essential medium (E-MEM) containing 10% fetal calf serum and antibiotics (penicillin, 1000 U/ml; streptomycin, 1000 μg/ml; fungizone, 25 μg/ml). A 10% homogenate of each tissue was prepared, and 0.1 ml of supernatant was inoculated into duplicate monolayer tube cultures (M.A. Bioproducts, Walkersville, Md) of primary rhesus monkey kidney, primary human embryonic kidney (HEK), and diploid human embryonic lung (WI-38) cells. All cultures were placed on maintenance medium consisting of E-MEM plus 2% fetal calf serum and incubated at 36 C and examined microscopically at 2–3-day intervals for viral cytopathic effects. Primary rhesus monkey kidney cultures were tested at 7 and 14 days for hemagglutinating viruses by the hemadsorption technique utilizing guinea pig erythrocytes. Primary HEK and the WI-38 cell cultures were observed during 3–4 weeks' incubation.

Results

We previously reported as abstracts preliminary findings demonstrating the presence of HSV antigen and genome in the coronary arteries of 2 of 4 subjects with severe atherosclerosis.[13,14] Microscopic examination of Carnoy's-fixed tissue sections by the ABC immunoperoxidase method using polyvalent rabbit HSV antiserum (Dako Corp., Santa Barbara, Calif) revealed positive staining intimal cells in the subendothelial region of the LAD coronary of one subject and in the wall of the main right coronary artery of the second. Frozen sections of the HSV antigen-positive tissues from the above cases also exhibited positive staining intimal cells when tested with biotinylated HSV DNA probes. These preliminary studies were extended to a search for HSV, CMV, and EBV genome and antigen in coronary arteries and the thoracic aorta of 20 subjects with specific viral DNA probes and monoclonal antibodies.[14] The results of the above study are summarized in this report.

Serial cryostat sections of each tissue sample were examined sequentially for viral DNA, antigen, and histologic changes for correlation of virologic results with microscopic findings. The former are summarized in Table 1. Herpesviridae were detected in the coronary arteries of 8 of 20 subjects assayed by both *in situ* hybridization and ABC immunoperoxidase methods. Of the 8 cases in which virus was detected in

Table 1—Detection of Herpesviridae in Coronary Arteries and Aorta by *In Situ* Hybridization and ABC Immunoperoxidase Methods

Sample site	Virus assayed	No. cases positive (n = 20)	No virus positive		
			DNA probe only	ABC-IP only	Both methods
Coronary artery	HSV	6	2	1	3
	CMV	2	2	0	0
	EBV	0	0	0	0
Thoracic aorta	HSV	5	3	2	0
	CMV	2	1	0	1
	EBV	0	0	0	0

the coronary arteries, 6 were positive for HSV and 2 for CMV. The biotinylated probes detected HSV in 5 of 6 and CMV in 2 of 2 coronary artery sections. Presence of HSV in the coronary arteries was confirmed in 3 of the 5 DNA probe-positive sections in adjacent sections reacted with HSV-1 and HSV-2 monoclonal antibody probes. The HSV was identified in all cases as HSV-2. In one of the 6 HSV-positive cases, HSV-2 was demonstrated in the coronary vessels by only the ABC immunoperoxidase method and was negative by *in situ* hybridization. CMV early nuclear protein antigen was not detected in any of the coronary artery sections assayed by the ABC immunoperoxidase method. EBV DNA and viral capid antigen were not detected in any of the sections studied.

Herpesviridae were detected in cryostat sections of the thoracic aorta of 7 of 20 subjects; 5 were identified as HSV and 2 as CMV. Presence of HSV or CMV in the aorta sections were demonstrated by *in situ* hybridization in 5 cases and by the ABC immunoperoxidase method in 3 cases. None of the HSV detected in aortic tissue sections were positive by both methods, whereas both CMV DNA and early nuclear protein antigen were detected in 1 of 2 CMV-positive cases. The HSV was typed as HSV-2 by the ABC immunoperoxidase method. EBV was not detected in any of the thoracic aorta sections studied.

Table 2 shows the distribution of viruses in the coronary arteries and aortas. HSV and/or CMV were detected in either the coronary arteries or aortas of 4 of 8 and 4 of 7 subjects, respectively. In 3 subjects HSV and/or CMV were present in both sites. HSV was detected more frequently in the LAD than in the main right coronary artery.

Table 2—Localization of Herpesvirus in Coronary Arteries and Thoracic Aorta

Sample site	No. cases virus-positive	Virus detected		
		HSV only	CMV only	Both viruses
Coronary artery				
LAD only	4	3	1	0
MRC only	2	1	1	0
Both LAD and MRC	2	1	0	1
Thoracic aorta only	3	1	0	2
Both aorta and LAD	3	2	0	1
Both aorta and MRC	0	0	0	0
Aorta and LAD and MRC	1	0	0	1

Positive staining reactions observed in the various arterial tissue sections with the biotinylated DNA probe and immunohistochemical methods are illustrated in Figures 1 through 3. HSV and CMV DNA were demonstrated in occasional cells in the intact luminal surface and in focal clusters of spindle-shaped or "foamy" cells in the subendothelium as well as deeper intimal layers. Viral antigen was detected in rare luminal or intimal cells. Although the histogenesis of these cells has not been definitely established, they corresponded to endothelial cells, smooth muscle cells, or monocyte/macrophages on the basis of morphology and tissue location.

The relationship between the virologic findings and histologic observations in serial H&E-stained sections of the coronary arteries and thoracic aorta are summarized in Tables 3 and 4. HSV and CMV DNA or antigen was found in the coronary vessels of 5 of 16 subjects showing early atheromatous changes consisting of focal or eccentric intimal thickening, duplication of internal elastic lamina, and/or the presence of scattered foam cells. Both subjects with atheromatous plaques in the coronary vessels were positive for HSV. In 7 of the 8 virus-positive cases, HSV and CMV DNA or antigen was usually found in focal areas of intimal thickening with smooth muscle cell proliferation and the presence of lymphocytic infiltrates.

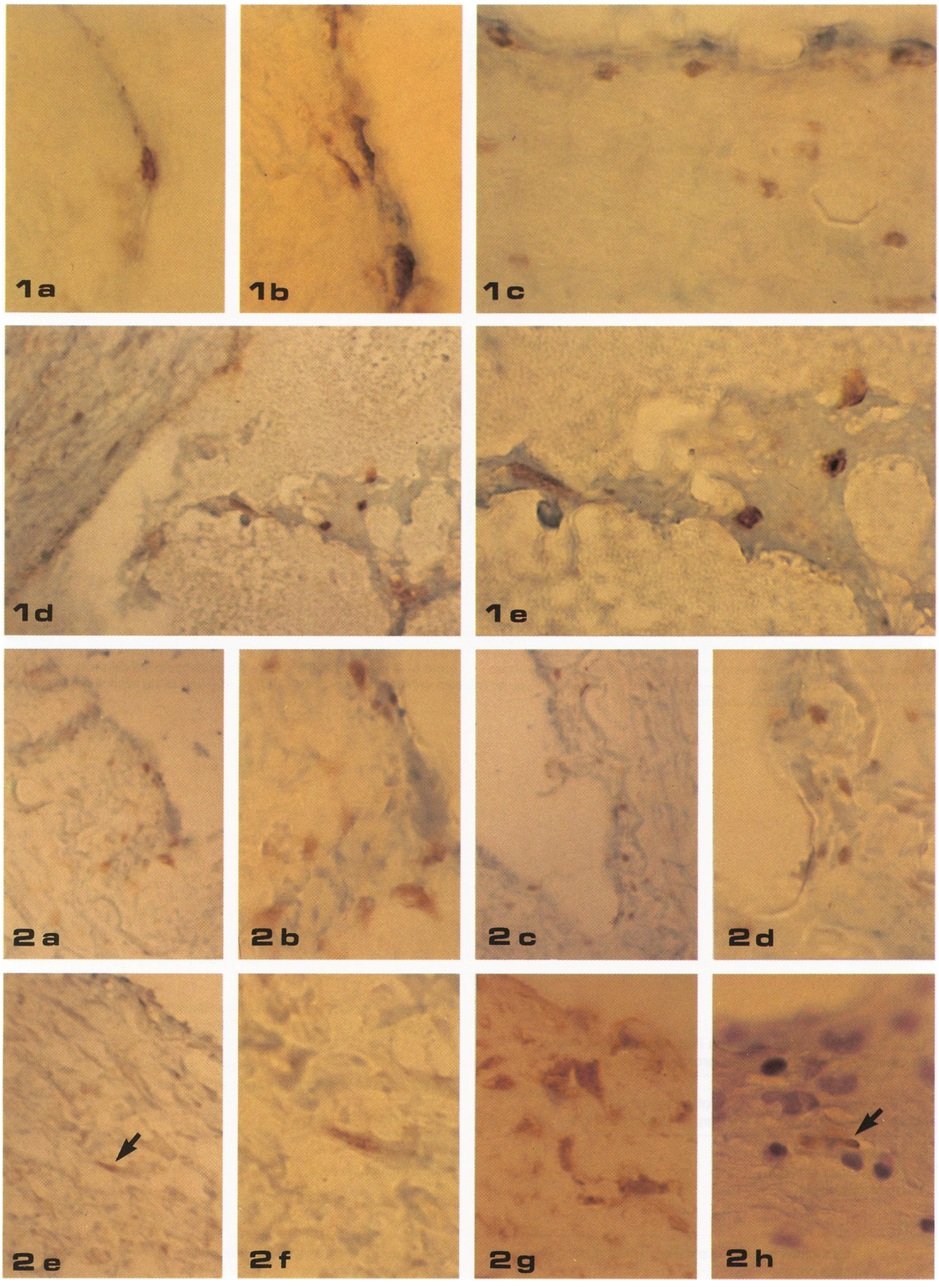
In contrast to the above findings, HSV and CMV DNA or antigen was associated with lesion-free areas of the thoracic aorta in 4 of 7 subjects, in 2 or 4 cases with minimal atheromatous changes and in 1 of 5 cases with atheromatous lesions (Table 4). Lymphocytic infiltrates were evident in the intima of all 3 cases with early or advanced atheromatous changes.

Cytopathic and hemadsorbing viruses were not detected in the indicator monolayer cell cultures inoculated with arterial samples from 16 donors, including 8 cases positive for HSV and/or CMV in the LAD, MRC, and/or aorta by viral DNA probe and immunohistochemical analyses.

Discussion

In this study we show evidence for the presence of HSV and CMV nucleic acid or antigen in the LAD

Figure 1—Main right coronary artery showing the presence of viral DNA. **a**—Positive-staining luminal cell reacted with CMV DNA probe (×100) **b**—Positive-staining surface and subendothelial cells reacted with HSV DNA probe. (×100) **c**—Positive-staining subendothelial cells and mononuclear infiltrates reacted with HSV DNA probe. (×100) **d** and **e**—Detached intimal cells showing positive staining with CMV DNA probe. (**d**, ×40; **e**, ×100) **Figure 2**—Left anterior descending coronary artery showing the presence of viral DNA and antigen. **a** and **b**—Positive-staining superficial and deep intimal cells, including foam cells reacted with CMV DNA probe. (**a**, ×40; **b**, ×100) **c** and **d**—Positive-staining intimal cells resembling mononuclear infiltrates reacted with CMV DNA probe. (**c**, ×40; **d**, ×100) **e** and **f**—Positive-staining intimal cells resembling smooth muscle cells in advanced atheromatous plaque reacted with HSV DNA probe. (**e**, ×40; **f**, ×100) **g**—Positive-staining intimal cells reacted with polyclonal HSV antibody. (×100) **h**—Same as above reacted with monoclonal HSV-2 antibody. (×100)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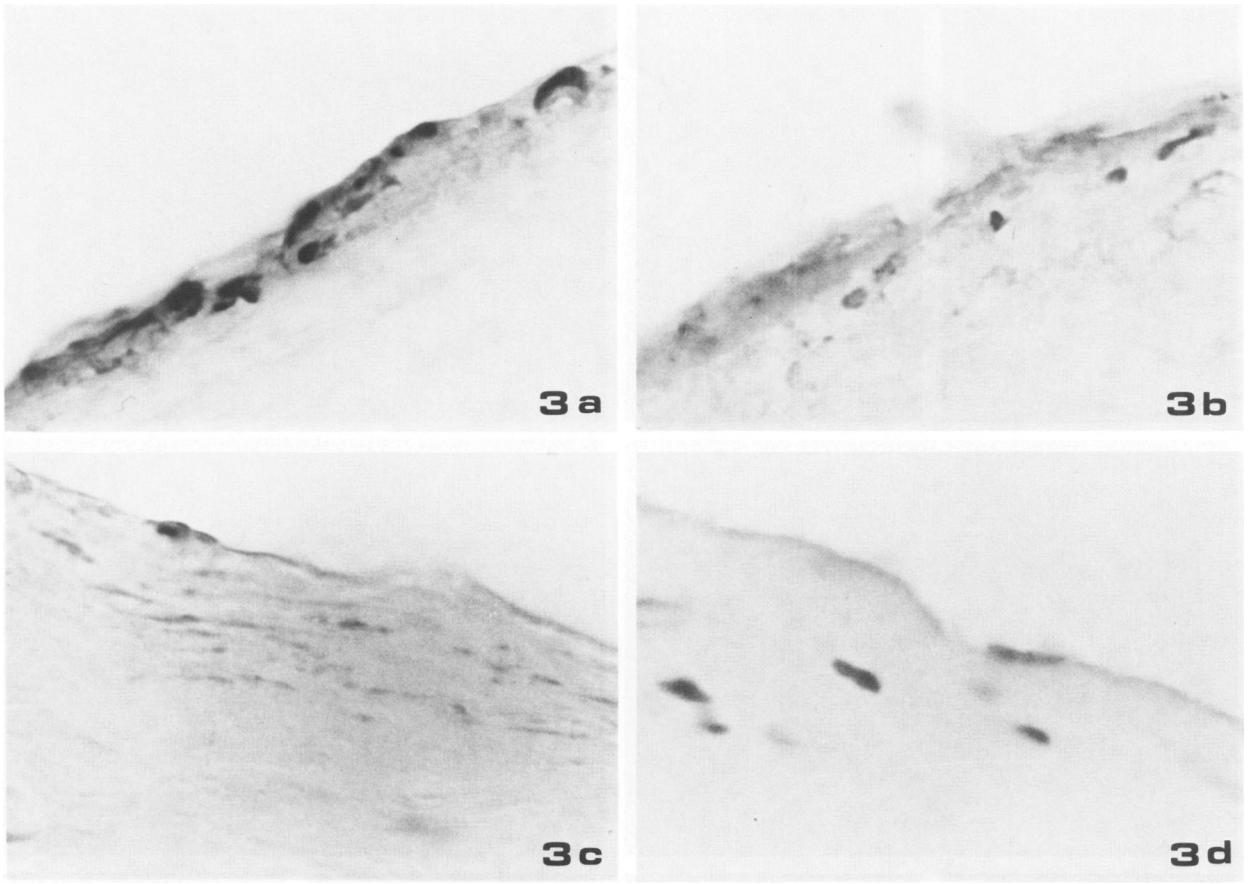

Figure 3—Thoracic aorta showing the presence of viral DNA and antigen. **a** and **b**—Positive-staining surface and subendothelial cells reacted with HSV DNA probe. (**a**, ×40; **b**, ×100) **c**—Positive-staining subendothelial cells reacted with CMV DNA probe. (×100) **d**—Sequential section showing positive staining with monoclonal CMV antibody. (×100)

Table 3—Relationship Between Histologic Findings and Herpesvirus Detection in the Coronary Arteries

Microscopic findings	No. of cases	No. virus-positive	Virus detected		
			HSV	CMV	EBV
No apparent changes	2	1	1	0	0
Minimal histologic changes*	16	5	3	3	0
Atheromatous lesions	2	2	2	0	0
Total	20	8	6	3	0

*Focal intimal thickening and/or duplication of internal elastic lamina and/or scattered foam cells.

Table 4—Relationship Between Histologic Findings and Herpesvirus Detection in the Thoracic Aorta

Microscopic findings	No. of cases	No. virus-positive	Virus detected		
			HSV	CMV	EBV
No apparent changes	7	4	3	1	0
Minimal histologic changes*	8	2	1	1	0
Atheromatous lesions	5	1	1	1	0
Total	20	7	5	3	0

*Focal intimal thickening and/or duplication of internal elastic lamina and/or scattered foam cells.

and main right coronary arteries and thoracic aorta of young trauma victims by *in situ* DNA hybridization and ABC immunoperoxidase methods, respectively. EBV nucleic acid and viral capsid antigen were not detected in the coronary arteries and aorta sampled in this study. These findings confirm and extend those of Benditt et al[5] and Melnick et al[6] of the presence of herpesviruses not only in the proximal aorta but also in the coronary arteries of human subjects. HSV was encountered more frequently than CMV in both sample sites.

Both HSV and CMV were detected more frequently by *in situ* hybridization with the biotinylated DNA probes, compared with direct antigen detection with monoclonal antibodies by the ABC immunoperoxidase method. Replicating HSV or CMV was not demonstrated by direct inoculation of tissue homogenates into susceptible indicator monolayers. Although explant cultures of the arterial tissues and cocultivation techniques were not attempted in this study, the above findings support the thesis of Melnick et al[6] and Gyorkey et al[7] that the artery wall may be a site of latency of CMV and possibly HSV. However, our inability to demonstrate infectious virus in the arterial tissue samples may in part be due to low virus levels in the infected cells or possible inactivation of virus due to postmortem alterations.

The identification of HSV-2 in the arterial tissues is of epidemiologic interest because of the high prevalence of primary infection with this virus in sexually active adolescents and young adults. Presence of HSV-1 in the arterial tissues examined in this study cannot be excluded, because 5 of the 11 HSV detected in coronary artery and aorta samples were positive with the generic HSV DNA probe only and did not react with the HSV-1 and HSV-2 monoclonal antibody probes. The site of latency of CMV has not been established, and primary infection with this virus is also prevalent in the age group under study. In disseminated CMV infection local viral spread is believed to occur via endothelial cell infection.[15] Whether the presence of HSV and CMV in the coronary arteries and aorta represents primary infection with hematogenous spread to lesion predilection sites or secondary seeding following reactivation of latent infection at a distant site remains to be established.

The mere presence of herpesviruses in the arterial wall does not necessarily signify a causal relationship to atherosclerosis. However, HSV and/or CMV could play a significant role in the initiation or progression of human atherosclerosis by several mechanisms, depending on the particular cell type involved and whether the infection is permissive or nonpermissive. Productive infection in permissive cells may result in alterations of aortic lipid metabolism and/or direct injury of endothelial or intimal cells. In Marek's disease herpesvirus infection of chickens, lipid metabolism has been shown to be altered with significant accumulation of cholesterol and cholesteryl esters in infected aortic smooth muscle cells.[3,4] CMV-infected human fetal aortic cells have also been reported to proliferate and accumulate cholesterol *in vitro*.[4] Both HSV-1 and HSV-2 are cytopathic in cultured human fetal abdominal aortic smooth muscle cells.[5] CMV has also been shown to replicate in smooth muscle cell cultures derived from human umbilical arteries.[16] CMV can infect endothelial cells *in vivo*, in support of the concept that endothelial cell integrity may play an important role in the initial stages of atherogenesis.

Immunologic injury as a consequence of productive or lytic infection with HSV and CMV may also play a role in atherogenesis. In 1974, Smith et al[17] reported the presence of measles and herpesvirus antigen–antibody complexes in thoracic aorta from autopsy cases and postulated that a significant cause of vascular tissue injury in humans may be chronic viral infection of the blood vessels *per se* and/or the deposition of antigen–antibody complexes in the vascular tissues. Cines et al[18] have demonstrated the induction of Fc and C3 receptors by HSV-1 on cultured human endothelial cells and have suggested that these receptors may promote the deposition of immune complexes in vascular tissue. Several human viruses, including HSV and CMV, have been shown to induce formation of Fc receptors in a variety of cultured cells.[19,20] Whether Fc and C3 receptors are induced in arterial endothelial and smooth muscle cells *in vivo* by HSV and/or CMV remains to be established.

In addition to lytic infection, latency or persistence, or less frequently, acquisition of a transformed phenotype are pathogenetic features of human herpesvirus infection.[21] Both HSV-2 and CMV are transforming viruses associated with human neoplasia.[22,23] Benditt et al[5] have suggested that expression of at least a part of the herpesvirus genome in arterial smooth muscle cells may initiate or maintain enhanced smooth muscle cell proliferation leading to monotypic atheromatous plaque formation. In support of Benditt and Benditt's hypothesis that human atherosclerotic plaques are monoclonal in nature,[24] recently Penn et al[25] demonstrated that plaque cells exhibit molecular alterations similar to that observed in oncogenic transformation and have proposed that one or more unidentified transforming genes may play a role in the proliferation of smooth muscle cells in atherogenesis. Human atherosclerotic plaque DNA was shown to complete transformation of NIH-3T3 cells via DNA transfection and that the transformed

cells were tumorigenic in nude mice. Of obvious interest in this regard is whether HSV or CMV infection of arterial tissues induce somatic cell gene alterations leading to smooth muscle cell proliferation and atherosclerotic plaque development. Both HSV and CMV genomes contain sequences that are homologous to human cellular DNA sequences.[26,27] The extent to which the viral DNA sequences represent functional homology with proto-oncogenes or alternatively regions of preferred interaction with the cellular chromosome remains to be established.[21]

The histologic findings in our study indicate that HSV and CMV infections are associated mainly with areas showing early or advanced atheromatous changes in the coronary arteries and with lesion-free as well as lesion areas in the thoracic aorta. Lymphocytic infiltrates were evident in both sites in the majority of virus-positive cases with early or advanced atheromatous changes. Because of the small number of cases sampled in this study, a definite conclusion cannot be made as to the relationship of viral infection to atheromatous changes in the various segments of the coronary artery and thoracic aorta. Concurrent studies conducted by Emeson et al[28] in our department have demonstrated mononuclear cell infiltrates in the coronary arteries and thoracic aorta and have identified some of the inflammatory cells as T cells and subsets and monocyte/macrophages. The relationship of the mononuclear infiltrates to viral infection requires elucidation. CMV has been shown to abortively infect peripheral human lymphocytes of T- and B-cell lineage and monocytes with virus expression limited to synthesis of immediate–early viral polypeptides.[29]

In conclusion, the virologic and histologic findings presented in this report further substantiate the possible role of herpesviruses in the pathogenesis of human atherosclerosis. Studies are in progress to identify the specific cell types (endothelial cells, smooth muscle cells, foam cells, mononuclear cells) harboring viral genome and/or antigen and to define their relationship to the pathogenesis of atherosclerosis. Because atherosclerosis is a multifactorial disease, the relationship of these findings to epidemiologic features, such as age, sex, race, and other risk factors will be assessed in an expanded study population as part of a multicenter study.

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