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HIGH LEVELS OF CYTOMEGALOVIRUS ANTIBODY IN PATIENTS REQUIRING VASCULAR SURGERY FOR ATHEROSCLEROSIS

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Summary 157 caucasian male patients undergoing vascular surgery for atherosclerosis and a matched control group of patients with high cholesterol levels were screened for antibodies to cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV1) and type 2 (HSV2), indicative of persistent infection. The prevalence of CMV antibodies was higher in the surgical group than in the control group (90% and 74%, respectively), and a significantly greater percentage ($p < 0.001$) of surgical cases than controls had high titres of CMV antibodies (57% and 26%, respectively). Small but not significant differences in antibodies to HSV1 were observed, and there were no differences in HSV2 antibody titres. For each virus there was no correlation between antibody titre and blood levels of total cholesterol or triglycerides. It is suggested that periodically activated virus may have a role in the pathogenesis of atherosclerosis.

Introduction

OTHERS have investigated an association between viral infections and atherosclerosis,[1-4] but it was the finding of induction by a herpesvirus of atherosclerotic lesions in chickens[5-7] that prompted studies of a possible association between herpesviruses and human atherosclerosis. We found antigens of human cytomegalovirus (CMV) in smooth-muscle cells cultured from aortic punch biopsy samples and from plaque samples.[8] Benditt and colleagues[9] detected fragments of the herpes simplex viral genome in specimens of aortic tissue removed during coronary-artery-bypass surgery. Examination of similar specimens by electronmicroscopy showed herpesviruses in occasional smooth-muscle and endothelial cells of the proximal aorta.[10] We have also demonstrated CMV nucleic acid sequences in cell cultures from human arterial tissue.[11] This study was initiated to evaluate any association of infections by CMV and herpes simplex virus type 1 (HSV1) and type 2 (HSV2) with atherosclerosis, in a group of patients who underwent surgery for atherosclerotic vessel disease and a control group with high levels of cholesterol but no clinical evidence of disease on entry to the study. Follow-up lasted for 7 years or more.

Subjects and Methods

358 male patients who underwent vascular surgery in 1981–84 in the Methodist Hospital, Houston, and from whom surgical specimens and blood samples were taken for laboratory investigation, were available for this study. The indications for surgery were clinical symptoms of atherosclerotic vessel disease, and the extent and nature of lesions were verified before surgery by appropriate radiography.

The comparison group consisted of 218 male participants in the Baylor-Methodist Lipid Research Clinic program having their exit interviews for the Coronary Primary Prevention Trial (CPPT).[12-15] The CPPT had included men only. The socioeconomic background of this group of patients was similar to that of the surgical group.[13] On entry, the participants had no history or evidence of heart disease, but the plasma level of low-density-lipoprotein cholesterol was at least 175 mg/dl. At the end of the study (Aug 27, 1983) the cardiovascular events that occurred in each participant during the trial were classified by a committee of

TABLE I—LEVELS OF ANTIBODY TO CMV AND HSV1 IN GROUP A

—	Number of	
	Surgical patients (n = 113)	Controls (n = 113)
CMV positive/negative value:		
⩽1·99	11	29
2·00–2·99	14	20
3·00–3·99	10	16
4·00–4·99	14	19
⩾5·00	64	29
HSV1 positive/negative value:		
⩽2·92	38	44
2·93–3·99	13	12
4·00–4·99	10	14
⩾5·00	52	43

cardiovascular experts. The following definitions of events were used: definite myocardial infarction; coronary-artery-bypass surgery; suspected myocardial infarction—one or more of ischaemic pain, time-appropriate diagnostic enzymes (twice normal creatine phosphokinase [total], lactate dehydrogenase, or serum aspartate aminotransferase), equivocal electrocardiogram (ECG) and equivocal (raised) enzymes, and equivocal ECG alone provided that it was not based on ST or T wave changes only; positive exercise test; or positive Rose questionnaire[16]—chest pain defined by standard location, duration, and intensity.

Each consecutive surgical patient was matched by race and age (at exit) with a control. The people who carried out matching and antibody tests were unaware of cardiovascular events in the controls. Serum samples were obtained from the patients on admission for surgery and from the control group at the time of exit interview from CPPT. Samples were stored at −35°C.

Solid-phase radioimmunoassays were used for detection of antibodies. The whole antigen of CMV strain AD169 and the major type-specific glycoproteins VP123 for HSV1 and VP119 for HSV2 were used as capture antigens for detection of the respective antibodies. Positive/negative values for CMV represent the ratio of the average counts of the test serum with antigen from CMV-infected cells to the average counts of the test serum with control antigen from uninfected cells. The positive/negative values for HSV1 and HSV2 represent the ratio of the average counts of the test serum to the average counts of the negative control serum samples both corrected for background activity.[17,18] Titration of serum showed that the positive/negative values were directly related to antibody concentration. The matched serum pairs (diluted 1 in 20) were always tested on the same microtitre plate. Each microtitre plate also contained positive and negative controls. Positive/negative values exceeding 2·93 for HSV1, 1·77 for HSV2, and 2·0 for CMV were regarded as positive for antibodies to the respective antigen because these levels were found to be cut-off points for distinguishing known positive and negative control serum samples.[17,18]

The difference in prevalence of antibodies to CMV and HSV between the groups of patients was evaluated and comparisons were made for different antibody levels as determined by positive/negative values. The significance of differences was evaluated by the chi-square test for matched pairs.[19] For the estimation of odds ratio and its confidence limits, the method for paired samples or, where applicable, the method of Haldane[20] was used. The *t* test was used to evaluate differences in mean values.

Results

157 surgical patients could be suitably matched with participants in the CPPT study. 45 of these patients had coronary-bypass surgery, 106 carotid endarterectomy, and 6 femoral, iliac, or abdominal aortic surgery. The mean age of the surgical patients was 58·2 (SD 6·9) years and that of the control group 56·9 (6·5) years. The surgical patients had a significantly lower mean total cholesterol level than the controls (219 [42] mg/dl *v* 276 [31] mg/dl; p = 0·003). The mean triglyceride level was significantly higher in the surgical group (199 [166] mg/dl *v* 142 [52] mg/dl; p < 0·001).

The 157 case-control pairs were classified into two groups according to cardiovascular events that occurred after the controls entered the study. The 113 controls in group A had no recorded cardiovascular events. Group B comprised 10 controls in whom disease requiring coronary-bypass surgery developed and 34 in whom myocardial infarction (7 subjects), suspected myocardial infarction (3), a positive cardiac exercise test (22), or a positive response to the Rose questionnaire (2) occurred.

Levels of antibody to CMV and HSV1 in group A are given in table I. There was a higher prevalence of high CMV antibody levels among the surgical cases. In contrast there was no substantial difference between the cases and controls in levels of antibody to HSV1 (table I) or HSV2 (data not shown).

The prevalence of antibodies in concordant and discordant matched pairs of surgical cases and controls is shown in table II. 90% of surgical cases compared with 74% of controls had antibodies to CMV (p < 0·01). The prevalence of antibodies to HSV1 and HSV2 was similar in cases and controls.

57% of surgical cases but only 26% of controls had high anti-CMV levels (positive/negative greater than 5·00) (p < 0·001; table III). The differences in prevalence of high-titre antibodies to HSV1 and to HSV2 between the cases and controls were not significant.

In group B, in which the controls suffered cardiovascular events, 28 of the 44 surgical patients (64%) had high levels of antibodies to CMV, whereas 20 of the 44 matched controls (45%) had anti-CMV in high titre (p < 0·1). High levels of

TABLE II—PREVALENCE OF ANTIBODIES IN GROUP A

Virus	Antibodies				Number (%) with antibody		χ^2 for matched pairs
	Present in cases and in controls	Present in cases; absent in controls	Absent in cases; present in controls	Absent in cases and in controls	Surgical cases	Controls	
CMV	75	27	9	2	102 *(90)*	84 *(74)*	8·03
HSV1	46	29	23	15	75 *(66)*	69 *(61)*	0·5
HSV2	11	19	20	63	30 *(26)*	31 *(27)*	0

TABLE III—HIGH LEVELS OF ANTIBODIES (POSITIVE/NEGATIVE > 5) TO CMV AND HSV1 IN GROUP A

Virus	P/N > 5				Number (%) with P/N > 5		χ^2 for matched pairs
	In cases and controls	In cases; not in controls	Not in cases; in controls	Not in cases or in controls	Surgical cases	Controls	
CMV	15	49	14	35	64 *(57)*	29 *(26)*	18·3
HSV1	22	30	21	40	52 *(46)*	43 *(38)*	1·25

antibodies to HSV1 were present in 47% of surgical cases and in 32% of the controls ($p > 0.3$).

The prevalence of high levels of antibodies to CMV was the most prominent difference between the groups. Therefore, we calculated odds ratios for frequency of high levels of antibodies to CMV associated with surgery for atherosclerotic vascular disease. The odds ratio for surgical patients and controls in group A was 3·5 (95% confidence interval 1·4–5·6). The frequency of high antibody levels in the group-A surgical patients did not differ significantly ($p > 0.3$) from that of the group-B controls (57% *v* 45%; odds ratio = 1·56 [0·78–3·12]) or the group-B surgical cases (57% *v* 64%; odds ratio = 1·32 [0·65–2·65]). The frequency of high CMV antibody levels in the group-B surgical cases was significantly higher than that in the group-A controls (64% *v* 26%; odds ratio = 4·95 [2·36–10·05]; $p < 0.001$). Group-B controls had a higher frequency of high levels of antibodies to CMV than group-A controls (45% *v* 26%; $p < 0.05$; odds ratio = 2·4 [1·16–4·91]).

The type of surgery, such as coronary-artery-bypass or carotid endarterectomy did not affect the differences found between surgical cases and their matched controls (data not shown). There was no correlation between antibody titres and the levels of total cholesterol or triglycerides. There was no correlation between levels of antibody to CMV and to HSV1 or HSV2.

Discussion

Selection of a proper control group for a retrospective study is very difficult. Ideally, a control group should have similar diagnostic arteriograms to the surgical cases. Obviously, it was not possible to carry out diagnostic arteriograms in a retrospective study and, further, it would be unethical in a cohort study.

The association of high levels of cholesterol with atherosclerosis is widely accepted. However, no correlation was observed between antibody levels to CMV and HSV and total cholesterol or triglycerides in either of the study groups. Thus, the viral infections studied were not covariates of higher cholesterol or triglyceride levels. Our findings in the various study groups suggest that high levels of antibodies to CMV are associated with clinically manifest atherosclerotic vascular disease.

In an experimental model, the infection of pathogen-free chickens with an avian herpesvirus induced atherosclerosis; however, arterial disease was not observed in a control group of uninfected chickens fed high levels of dietary cholesterol.[6] Increased accumulation of lipids, particularly cholesterol, saturated types of cholesteryl esters, and non-esterified fatty acids, was observed in cultured smooth-muscle cells infected with the avian herpesvirus. Similarly, CMV induced cell proliferation and produced latent infection in smooth-muscle-cell cultures of human fetal origin.[21] The infected smooth-muscle cells accumulated crystalline formations resembling cholesterol in polarised-light microscopy.[21,22] Thus, it was postulated that virus infection could cause atherosclerosis by inducing lipid accumulation.

The detection of fragments of herpesviruses in arterial tissue[8-11] does not conclusively indicate a pathogenetic role for these viruses in the development of atherosclerosis. Any causative association can be only speculative at this time. The viral infection may be secondary in a tissue already affected by the pathological process. It remains to be shown that the virus is able to participate in arterial cell proliferation, plaque formation, and/or increased lipid accumulation in human beings. However, our serological findings suggest that in a large proportion of patients with clinical evidence of atherosclerotic disease, a periodically activated or continuously active virus infection stimulates higher levels of antibody production. Since CMV antigen, but not replicating virus, is found in cells cultured from the walls of atherosclerotic vessels,[8] the artery itself may be the site of viral latency. Tumilowicz et al[23] have suggested that a vascular lesion may develop as latent CMV is activated and destroys intimal cells, provoking division of smooth-muscle cells and inducing more damage to the modified or differentiated smooth-muscle cells; it may resolve again as immune factors re-establish homoeostasis. Their view is compatible with "occurrence of atypical proliferation of intimal smooth-muscle cells and of degenerative processes in the evolution of atherosclerosis". Such a recurring phenomenon would account for the high levels of CMV antibody observed in our study.

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REFERENCES

1. Fabricant CG, Krook L, Gillespie JH. Virus induced cholesterol crystals. *Science* 1973; **181:** 566–67.
2. Smith KO, Gehle WD, Sanford BA. Evidence for chronic viral infections in human arteries. *Proc Soc Exp Biol Med* 1974; **147:** 357–60.
3. Burch GE. Viruses and atherosclerosis. *Am Heart J* 1974; **87:** 407–12.
4. Fabricant CG, Fabricant J, Litrenta MM, Minick CR Virus induced atherosclerosis. *J Exp Med* 1978; **148:** 335–40.
5. Minick CR, Fabricant CG, Fabricant J, Litrenta MM. Atherosclerosis induced by infection with a herpesvirus. *Am J Pathol* 1979; **96:** 673–706.
6. Fabricant CG, Fabricant J, Minick CR, Litrenta MM. Herpesvirus induced atherosclerosis. In: Cold Spring Harbor Conference on Cell Proliferation. Viruses in naturally occurring cancers, vol 7. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1980: 1251–57.
7. Fabricant CG, Hajjar DP, Minick CR, Fabricant J. Herpesvirus infection enhances cholesterol and cholesteryl ester accumulation in cultured arterial smooth muscle cells. *Am J Pathol* 1981; **105:** 176–84.
8. Melnick JL, Dreesman GR, McCollum CH, Petrie BL, Burek J, DeBakey ME. Cytomegalovirus antigen within human arterial smooth muscle cells. *Lancet* 1983; ii: 644–47.
9. Benditt EP, Barrett T, McDougall JK. Viruses in the etiology of atherosclerosis. *Proc Natl Acad Sci USA* 1983; **80:** 6386–89.
10. Gyorkey F, Melnick JL, Guinn GA, Gyorkey P, DeBakey ME. Herpesviridae in the endothelial and smooth muscle cells of the proximal aorta in atherosclerotic patients. *Exp Mol Pathol* 1984; **40:** 328–39.
11. Petrie BL, Melnick JL, Adam E, Burek J, McCollum CH, DeBakey ME. Nucleic acid sequences of cytomegalovirus in cells cultured from human arterial tissue. *J Infect Dis* 1987; **155:** 158–59.
12. The Lipid Research Clinics Program. The Coronary Primary Prevention Trial: Design and implementation. *J Chron Dis* 1979; **32:** 609–31.
13. The Lipid Research Clinics Program. Pre-entry characteristics of participants in the Lipid Research Clinics Coronary Primary Prevention Trial. *J Chron Dis* 1983; **36:** 467–79.
14. The Lipid Research Clinics Coronary Primary Prevention Trial Results. I. Reduction in incidence of coronary heart disease. *JAMA* 1984; **251:** 351–64.
15. The Lipid Research Clinics Coronary Primary Prevention Trial Results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* 1984; **251:** 365–74.
16. Rose JA, Blackburn H. Cardiovascular survey methods. Geneva: World Health Organisation, 1968.
17. Matson DO, Adler-Storthz K, Adam E, Dreesman GR. A micro solid-phase radioimmunoassay for detection of herpesvirus type-specific antibody: parameters involved in standardization. *J Virol Methods* 1983; **6:** 71–83.
18. Adler-Storthz K, Matson DO, Adam E, Dreesman GR. A micro solid-phase radioimmunoassay for detection of herpesvirus type-specific antibody: Specificity and sensitivity. *J Virol Methods* 1983; **6:** 85–97.
19. McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages *Psychometrika* 1947; **12:** 153–57.
20. Haldane JBS. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956; **2:** 309–11.
21. Hajjar DP, Fabricant CG, Minick CR, Fabricant J. Virus induced atherosclerosis. Herpesvirus infections alters aortic cholesterol metabolism and accumulation. *Am J Pathol* 1986; **122:** 62–70.
22. Fabricant CG. Viruses: causal factors in atherosclerosis. *Microbiol Sci* 1986; **3:** 50–52.
23. Tumilowicz JJ, Gawlik ME, Powell BB, Trenton JJ. Replication of cytomegalovirus in human arterial smooth muscle cells. *J Virol* 1985; **56:** 839–45.