PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **147** 357-360 (1974)

Evidence For Chronic Viral Infections In Human Arteries (38341)

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Development of atherosclerosis is a complex process which involves changes in the intima and media of arteries. Factors known to hasten the development of atherosclerosis are hypertension, hyperlipemia, hemodynamic stresses, hypoxia and vessel injury. Atherosclerotic lesions in the larger arteries consist of patchy accumulations of lipids, including cholesterol and its esters, phospholipids and triglycerides. Many investigators have sought to determine the biochemical mechanisms for deposition of these lipids in vascular tissues. However, it is still unclear what factors are responsible for *initiating* lipid deposits in arteries at certain foci. Subtle injury to the endothelial lining seems to be one of the better possibilities to explain the genesis of degenerative changes in the larger vessels, changes which sometimes progress to atherosclerotic lesions. It is difficult to determine the exact cause, time of appearance or extent of *initial* injuries in humans because lesions of the arteries are usually not seen until they produce catastrophic complications, such as severe hemorrhage in the wall or complete occlusion of a strategic vessel.

We postulate that a significant cause of chronic injury to vascular tissues in humans may be infectious agents. A number of observations are consistent with this hypothesis. Several of the common childhood viral infections involve viremia, hence viral exposure and possible infection of the vascular tissues themselves. Common childhood viral diseases which involve transitory viremia at some point during the course of the acute disease include measles, varicella, mumps, and rubella. Available published information (1) and our own experimental work suggest that cell cultures derived from arteries are fully susceptible to infection by several viruses. Therefore, it seems reasonable to believe that viruses could infect vascular tissues *in vivo* during periods of viremia. Some viruses are easily recognized as having a predilection for blood vessels. Other agents may well infect the intima of blood vessels but not be recognized because the initial tissue damage is not severe.

We have sought evidence for "quiet" viral infections in large vessels of humans in two ways: (1) by examination of these vascular tissues for the presence of viral antigen–antibody complexes and (2) by attempting to isolate viruses directly from vascular tissues obtained at autopsy. The present report describes results we obtained using the first approach.

Methods. Excellent cooperation was obtained from the Chiefs of autopsy services in four local hospitals. The 40 subjects studied included both females and males. Ages ranged from 11 to 87 years; the mean age was 45 years. Cause of death was variable, but did not include subjects with chronic, deteriorating diseases which were known to involve terminal episodes of septicemia. Subjects who had been dead for periods longer than about 6 hr before refrigeration or who could not be autopsied within less than 48 hr after death were also excluded. Time between deaths and autopsies ranged from 5 to 48 hr, with a mean time of about 17 hr.

We selected the thoracic aorta for these studies because the vessel is thick-walled and, most importantly, is distant from the organs of the lower digestive tract which contain an abundance of bacteria. Arterial tissues were taken before any other autopsy procedures were performed. The thoracic cavity was opened and the thoracic aorta from about the sixth to about the tenth thoracic vertebra was bluntly dissected and clamped in two places to provide a length of approximately 8 cm between the clamps. The vessel was then cut at points distal to the clamps, the aorta segment removed aseptically from the thoracic cavity and placed in a sterile container

for transport to the laboratory. All remaining procedures were conducted in a closed cubicle used for tissue culture work. The vessel was cut free of the clamps, gently washed in saline and opened longitudinally. The intima and part of the media were stripped from a section of the vessel having dimensions of about 4 × 6 cm (the remaining media and external adventitia of the vessel wall were discarded).The intima–media portion (handled aseptically to this point) was divided into two equal parts, one for isolation of infectious agents and the other for antibody elution studies. Elution of immunoglobulins was accomplished as follows: the intima–media was placed in a colander-like plastic basket and washed for 15 min in 200 ml isotonic saline (phosphate buffered at pH 7.2); thorough washing was accomplished by continuous rotation of the tissue on a magnetic stirrer. The fluid was discarded, and the procedure repeated 3–5 times. Ten milliliter of PBS were added, the tissue was rinsed for 15 min and this fluid was saved as the "last PBS wash." The intima–media was then treated, according to the method of Edgington (2), with 10 ml of 2.5 *M* KI for 15 min to dissociate any immunoglobulins complexed with antigens in the tissues. This "KI eluate" was saved and the tissue discarded. The last PBS wash and the KI eluate were centrifuged at about 2000*g* for 10 min and the supernatant fluid from each was dialyzed for 16–24 hours at 4° in several changes of PBS. Materials were usually stored at −70° prior to performance of serologic studies.

The last PBS rinse and KI eluate were tested at the undiluted level for the presence of IgG, IgM and IgA by single radial immunodiffusion (3) using "low level" plates (Meloy Lab., Inc., Springfield, VA.). Immunoglobulin levels are expressed in international units/ml as recommended by WHO (4). Undiluted last PBS wash and KI eluate pairs were screened for the detection of specific viral antibody by a complement fixation (CF) test (5) modified for use in microtiter apparatus, and by a radioimmunoassay (RIA) method described originally by Goldfield (6) and modified by us for use with viral antigens (7).

Imitation pearls (Grieger's of Pasadena, CA., W773–6) were used to adsorb viral antigens ("sensitized") for the detection of antibody in human sera by RIA. The following mechanical scheme was used in the RIA procedure:

(1) Pearls rinsed twice with distilled water and sensitized with antigen at 4°.

(2) Sensitized pearls transferred with forceps to a beaker containing 150 ml PBS. Each pearl rapidly dipped 6 times in this beaker of PBS and 6 times in a second beaker of PBS. (After washing ten pearls, PBS changed.)

(3) Each pearl dropped into a test tube containing 1.0 ml of appropriately diluted human serum, incubated for 30 min at 23°.

(4) Diluted serum aspirated, pearls washed as in step 2.

(5) Each pearl dropped into a test tube containing 1.0 ml of ^{125}I–labeled antihuman globulin, incubated for 30 min at 23°.

(6) Fluid aspirated, pearls washed as in step 2.

(7) Pearls placed in appropriate container for measuring activity in a scintillation counter.

Dilutions of human sera applied to pearls coated with antigens from virus infected cell cultures were similarly applied to pearls coated with antigens from control (uninfected) cell cultures. The counts obtained with the latter were subtracted from counts obtained with infected cultures and the differences taken as the virus–specific counts for these serum dilutions. The specific activity was calculated by dividing the virus specific counts by the control counts. The RIA titer of a serum was read as the reciprocal of the highest serum dilution giving a specific activity of approximately 1 (a statistically significant reaction at an alpha level of 0.01).

Further details of the RIA test are described elsewhere (7). If *any* antibody was detected in the last PBS wash (by radial immunodiffusion or by RIA), we assumed that the aortic tissue had been insufficiently washed to remove noncomplexed antibody and these pairs were discarded. The data we report here include only information obtained on KI eluates which had a *negative* last PBS wash.

Results. Table I summarizes our results at this point in the study. Eluates from about 1/3 of the aortic tissues contained IgG concentrations of 0.1 International Unit (IU) or greater (range was 0.11–25.0 IU/ml). No IgA or IgM was detected in any of 40 eluates. Measlesvirus antibody was found in 23% (7/30) and herpesvirus antibody in 9% (2/22) of the eluates. No antibody to mumpsvirus or varicella-zoster (V-Z) virus could be detected (positive controls included sera with specific antibody to these viruses).

TABLE I. Antibody Eluted from Human Aortic Tissues.

Incidence of antibody	by radial diffusion	Antibody measured by radioimmunoassay			
	IgG	Measlesvirus	Herpesvirus	Mumpsvirus	V-Z virus
No. aortas positive[a]					
No. aortas examined	13/40	7/30	2/22	0/19	0/15
% aortas positive	33	23	9	<5	<7

[a] "Positive" is defined as follows:
(1) Radial immunodiffusion method: eluate contained 0.1 IU of IgG or greater.
(2) Radioimmunoassay method: eluate contained sufficient antibody to give a positive reaction at the undiluted level, or greater.
Eluates were scored "positive" only if the last tissue wash contained no detectable antibody.

Four aortic tissue eluates which were RIA positive for measlesvirus antibody were (a) serially diluted to determine their end-points by the RIA technique and (b) titrated by the microtiter technique for complement fixing (CF) activity. Two human sera known to be CF antibody positive were similarly tested. It can be seen in Table II that the RIA determination for measles antibody in sera is 40–80 times more sensitive than the CF test. It is not surprising, therefore, that CF reactions with the aortic tissue eluates were negative, since an RIA titer of about 40–80 would be required to give a minimal CF reaction.

One of the KI eluates containing herpesvirus antibody showed a low but significant virus neutralizing activity (75% plaque reduction in the undiluted material). This eluate gave an end-point of 1:4 in the RIA, which is consistent with our estimate that the RIA technique is about 10 times more sensitive than the neutralization test for herpesvirus antibody (paper submitted for publication).

Discussion. These data demonstrate that viral antibody is complexed in some human aortic tissues in such a way that it is not released by rinsing with saline; it can be released by a method known to be effective in dissociating antigen–antibody complexes. We tentatively conclude, therefore, that measles antigen–antibody complexes are present in about one quarter of the human aortic tissues we examined and that herpesvirus antigen–antibody complexes can be found occasionally. Two interpretations can be made for the presence of these complexes: (1) complexes were formed at sites other than blood vessels, circulated through the vessels and were merely deposited in the vessel walls; or (2) complexing occurred in the blood vessel wall itself as a result of focal viral replication in the aortic tissues and consequent viral antigen interaction with circulating antibody bathing the sites of infection. We do not now have sufficient data which would clearly differentiate between these two alternatives. Antigen–antibody complexes are known to trigger focal tissue damage by immune-mediated mechanisms, hence can initiate damage in arterial tissues regardless of the mechanics of their formation or deposit.

Viruses having pronounced vascular tissue tropisms or which produce severe damage to the vascular system include those causing the human hemorrhagic fevers (8), simian hemorrhagic fever (9), equine arteritis (10) and hemorrhagic encephalopathy (11, 12). The damaging effects of fetal rubella infections upon the cardiovascular system are well known. Forrest *et al.* (13) stated that "congenital rubella should be considered in *adults* presenting with symptoms of coronary, cerebral and peripheral vascular disease," because many rubella infections during pregnancy may be undetected. Gocke *et al.* (14)

TABLE II. Sensitivity Comparison of RIA and CF Assays for Measlesvirus Antibody.

Specimen assayed	Reciprocal of endpoint dilution	
	RIA	CF
Serum #1	640	16
Serum #2	1280	16
Aortic eluate #1	2	<1
Aortic eluate #2	2	<1
Aortic eluate #3	4	<1
Aortic eluate #4	1	<1

found an interesting association between serum hepatitis antigen (Australia antigen) and human polyarteritis, which suggests a possible etiologic relationship between the virus causing serum hepatitis and polyarteritis lesions. Paramyxovirus-like structures have been found in the synovial vascular endothelium of patients with arthritis (15). Measlesvirus causes damage to capillaries in the skin and, in severe infections, can cause a hemorrhagic rash. A paramyxovirus has been isolated from human atherosclerotic arteries (16); Coxsackie B virus infection has been postulated as an initiating factor in atherosclerosis (17). These findings suggest that viral infections of the human vascular system can produce signs or symptoms ranging from the obvious to the subtle. It is the subtle injuries in the larger vessels which could escape early detection, yet play an important role in *initiating* chronic, degenerative changes in the human cardiovascular system.

Several of the common viral infections in man produce long-lasting immunity. A probable explanation for this is that each virus establishes a latent infection somewhere in the host, with a resultant slow release of antibody-stimulating antigens over a period of years. The anatomic locations of many of these latent infections are unknown. Our data suggests that large blood vessels are potential sites of viral latency.

A satisfactory hypothesis to explain the etiology of chronic cardiovascular disease must take into account the fact that degenerative changes begin early in life, affect large numbers of people and develop progressively until old age. Measlesvirus and herpesvirus hominis infections usually occur early in life, most people are sooner or later infected, both viruses are capable of establishing latency and, according to our data, they may infect the vascular system. Such viral infections in the lining of arteries could provide the *initial insult* leading to the sequence of events associated with induction of atherosclerosis. If this is true, unusually severe cardiovascular disease in later life might be initiated by particularly extensive, persistent viral infections of vascular tissues which are established early in life.

Summary. Evidence is presented that measlesvirus antibody and herpesvirus antibody are sometimes present in human aortic tissues in a state not readily dissociable by extensive washing with buffered physiologic saline. This antibody is readily released from the tissues by high molarity potassium iodide solution, a treatment known to dissociate antigen–antibody complexes. We postulate: (1) that viral antigen–antibody complexes are sometimes present in aortic tissues; and (2) that a significant cause of vascular tissue injury in humans may be viral infection of the blood vessels themselves and/or deposit of viral antigen–antibody complexes in the vascular tissues.

We thank Mary M. Ryan and John Tuley for excellent technical assistance. The work was funded by a grant from the Heart and Lung Institute, National Institutes of Health.

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Received April 15, 1974. P.S.E.B.M., 1974, Vol. 147.