

## Infections and Atherosclerosis: New Clues from an Old Hypothesis?

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The inability of “traditional” risk factors such as hypercholesterolemia, hypertension, and smoking to completely explain the incidence and trends in cardiovascular diseases has resulted in repeated calls for a search for “new risk factors” (1, 2). Recently, infections have been placed among these *new* putative risk factors (3–16). This commentary reviews the historical antecedents of the infectious hypothesis, which is actually one of the oldest etiologic theories of atherosclerosis. Because inflammation may mediate the putative atherogenic role of infections (8, 10, 13–15), the historical antecedents that have discussed a possible role of inflammation in atherogenesis are also briefly reviewed.

This review demonstrates that inflammation and infection were considered as possibly atherogenic more than a century ago. However, these hypotheses were all but completely abandoned early in the present century, coinciding with a shift in paradigms in the pathogenetic theories of atherogenesis, i.e., with the development of the multifactorial model of causation of chronic (“non-infectious”) diseases (17, 18).

Striking resemblances are apparent between current research on the putative atherogenic role of infections and research on the topic that was conducted up to one century ago. The synthesis of past and current evidence reviewed here suggests the following hypotheses and clues for future studies: 1) that the study of a possible atherogenic role of infections should be extended to infectious agents other than the three that have been the focus of research in recent years (herpesvirus—mainly cytomegalovirus (CMV), *Chlamydia pneumoniae*, and *Helicobacter pylori*) (6, 7); 2) that the introduction of antibiotic therapies in the 1940s and 1950s may have contributed to the decline in coronary heart disease incidence and mortality ob-

served in the Western hemisphere in the last three decades; 3) that infections could be one of the mediating factors in the association between low socioeconomic status (SES) and coronary disease; and 4) that infections in childhood could also be one of the confounding factors that explain the alleged association between birth/childhood weight and coronary disease in adulthood.

### INFLAMMATION AND ATHEROGENESIS: HISTORICAL PRECEDENTS

While there is currently little doubt that inflammatory changes are present in atherosclerosis (19–23), it is not entirely clear whether these changes are a consequence or part of the pathogenesis of the disease (19, 21). This current controversy is more than one-and-a-half centuries old. As early as 1823, Rayer (24) compared the calcification (“ossification morbide”) observed in the arteries to that observed in inflammatory processes elsewhere:

Morbid ossification of the arteries [is] the result of the inflammation of their fibrous layer. It is accompanied by bright redness of its internal layer, . . . and frequently surrounded by a *yellow matter*, soft and solid, non transparent. (24, p. 330).

Nineteenth century pathologists, however, disagreed on the pathogenetic significance of these findings. Rokitansky (25) considered the inflammatory changes secondary to the atheromatous deposit. On the other hand, in his 1859 *Cellular Pathology*, Virchow (26) explicitly distinguished the “simple fatty metamorphosis” of the arteries, without inflammation, from another form:

. . . in which we can distinguish a stage of irritation preceding the fatty metamorphosis, comparable to the stage of swelling, cloudiness, and enlargement which we see in other inflamed parts. I have therefore felt no hesitation in siding with the old view in this matter, and in admitting an inflammation of the inner arterial coat to be the starting point of the so-called atheromatous degeneration. (26, p. 396).

At the turn of the century, there was a lively debate on the possible linkage between “coronary sclerosis” or “arterial degeneration” and inflammatory processes

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Abbreviations: CMV, cytomegalovirus; SES, socioeconomic status.

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due to systemic infections (27). However, with only a few exceptions (28), consideration of the relation of inflammation to atherosclerosis all but disappeared from the literature until recently (20, 29).

### HISTORY OF THE ENUNCIATION AND ABANDONMENT OF THE INFECTIOUS HYPOTHESES OF ATHEROSCLEROSIS

In the context of the debate regarding the inflammatory component of atherosclerosis during the last decades of the nineteenth century, French and German pathologists did extensive experimental work on the possible role of infectious diseases in diverse forms of *endarteritis* and *arteriosclerosis* (30–32). One of the first reports was a 1889 two-page article by Gilbert and Lion (33) (see figure 1), which described how,

after inflicting slight mechanical injury to the arterial wall of the aorta of a rabbit, the injection of pathogenic bacteria (*B. typhosus*) resulted in fatty sclerotic changes. Similar results were observed after inoculation of streptococci without injury. The authors concluded that

... thus, this is an experimental verification of this medical concept, relatively new, suggesting that infections merit an important place in the etiology of human atheromatous arteritis. (33, p. 584).

This “relatively new” concept was soon verified in larger studies using different combinations of endothelial injury and experimental infection. In contrast to Gilbert and Lion’s results, Crocq (34) reported in 1894 that both injury (“locus minoris resistentiae”) and experimental infection were necessary to induce athero-

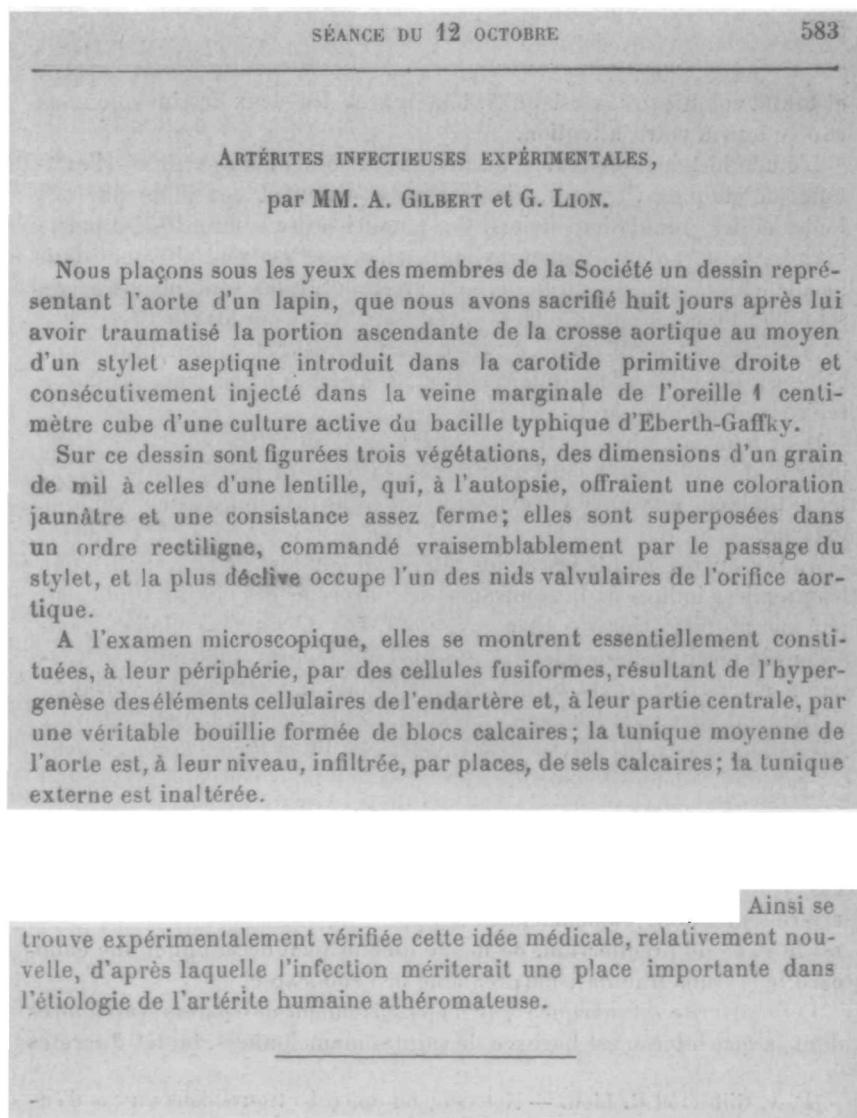


FIGURE 1. Facsimile reproductions of the initial paragraphs and conclusion of the 1889 article by Gilbert and Lion (33) (see text).

sclerosis, and Boinet and Romary (30) compared these results with the atherogenic effect of experimentally induced diabetes mellitus in rabbits.

In North America, prominent clinicians and pathologists at the turn of the century also discussed the possible causative role of infections in *chronic arteriosclerosis* (31, 32, 35, 36). Osler, who has been occasionally cited as having been the "first" to postulate this hypothesis (5, 10, 13), listed in his 1908 *Modern Medicine* (36) the existence of

four great factors in the causation of arteriosclerosis—the normal wear and tear of life, the acute infections, the intoxications [including smoking, diabetes mellitus, obesity], and those combinations of circumstances which keep the blood tension high. (36, p. 430).

Despite questions regarding whether these pathologists clearly distinguished atherosclerosis from other forms of arteriosclerosis (37), it is evident from their writings that atherosclerosis was well recognized and clearly distinguished from other forms of "aortitis" such as that seen in syphilis (36, 38). Osler described the early fatty streaks of atheroma as well as their high prevalence, "even in children" (36, p. 434). In 1906, Klotz (39) described the aortic lesions induced by experimental inoculations of rabbits with *B. typhosus* and streptococci as follows:

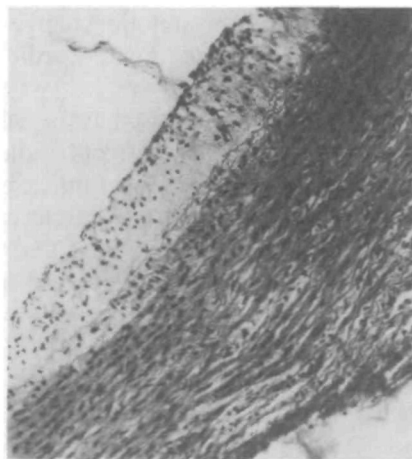
[lesions characterized by] warty thickening of the intima. . . . Microscopically there was a fatty degeneration of the subendothelial tissue, while there was,

however, much connective tissue advancing into the degenerated area. (39, p. 1770).

### The shift in paradigm

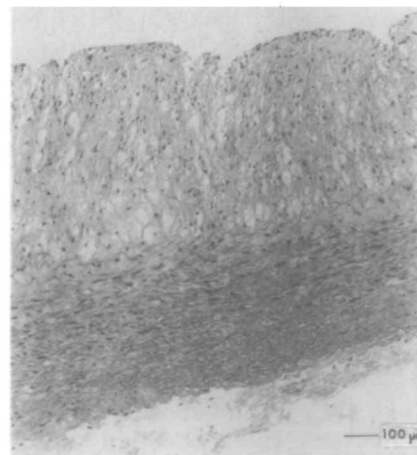
In the first few decades of this century, and for reasons that are not entirely defined and that are briefly discussed below, the focus of pathophysiologic theories of atherogenesis shifted away from the "germ theory" and into the "multifactorial" (17, 18) or "black box" (40) paradigm. As a result of this change in focus, little attention was devoted to the inflammation and infectious hypotheses in subsequent decades, with only a few exceptions (41–44). Notable among these was the 1931 article by Benson et al. (42), which described the experimental induction of atherosclerosis-like lesions in rabbits by inoculation with streptococci (see figure 2, panel A). In these experiments, control animals were included, and, interestingly, atherosclerosis was most frequent when the infection was combined with a high cholesterol diet (42). Subsequently, Jones and Rogers (43, 44) observed that bacteria experimentally introduced into the perinasal lymph nodules could be later recovered by culture from the walls of the coronary arteries. In a 1948 article, Jones and Rogers argued that:

[It might be] justifiable to look on certain chronic infections with a possible "elective" affinity for vascular tissues as atheromatogenic agents which, operating through the years, may, by disturbing the nutritive and oxidative metabolism of the vascular walls,



**A**

(Benson et al., 1931)



**B**

(Muhlestein et al., 1998)

**FIGURE 2.** Photomicrographs of the aorta in rabbits experimentally inoculated with infectious agents included in reports published 67 years apart. Panel A: rabbit intravenously inoculated with streptococcus and subject to cholesterol feedings (Benson et al., 1931 (42)). Panel B: rabbit intranasally inoculated with *C. pneumoniae* and subject to cholesterol feedings (Muhlestein et al., 1998 (53)) (see text). Panel A is reproduced with the permission of the *Archives of Pathology and Laboratory Medicine*, successor to the *Archives of Pathology*. Panel B is reproduced with permission of *Circulation*.

be an important factor in the genesis of the disease; or on the other hand, [it may be] due to the pathogenic action of specific blood vessel autoantibodies. . . . (44, p. 277).

For the most part, however, mainstream medicine became widely skeptical (37) and the possible role of infections in atherogenesis all but disappeared from the discussion until the last quarter of the century.

## THE RE-EMERGENCE OF THE INFECTIOUS HYPOTHESIS

In a landmark study in 1973, Benditt and Benditt (45) reported that cells in atherosclerotic plaques had a monoclonal origin, and thus they concluded that factors that transform cells (e.g., viruses) ought to be considered as possibly atherogenic. Initially not exempt of controversy (46), these findings were soon replicated by other investigators (47, 48), and prompted the modern revival of interest in the infectious hypothesis (49), first manifested in the experimental field.

### Modern animal models

In 1978, Fabricant et al. (50) reported that arterial lesions resembling those seen in human atherosclerosis were much more frequently observed in chickens experimentally infected with a herpesvirus (Marek disease virus) than in control chickens. Consistent with the study by Benson et al. (42) a half century earlier, the atherogenic effect of the infection was potentiated when combined with a high cholesterol diet (50). A later report by Fabricant et al. (51) showed that the atherogenic effect of virus in chickens is preventable with the use of a herpesvirus vaccine.

More recently, Laitinen et al. (52) and Muhlestein et al. (53) described the experimental induction of aortic atherosclerosis in rabbits by nasal inoculations of *C. pneumoniae*, a chlamydia strain frequently involved in acute respiratory infections in individuals of all ages (54, 55). In the study by Muhlestein et al. (53), the experimental infection was combined with cholesterol feedings. The similarity of their experiments to the controlled experiments with streptococci described in 1931 by Benson et al. (42) is remarkable (see figure 2). The study by Muhlestein et al. added a powerful component by demonstrating that the atherogenic effect of the infection was prevented with azithromycin (53). Recent studies that have demonstrated that *C. pneumoniae* can be detected in the atherosclerotic plaques in the aorta following intranasal inoculations in rabbits (52) and mice (56) are also similar to the studies a half century earlier by Jones and Rogers (43, 44) discussed above.

In another line of research, Zhou et al. (57) recently reported that infection with CMV increased neointimal proliferation after experimentally induced balloon injury of the carotid arteries in rats. Again, these studies essentially replicate some of the old observations that described the results of combining infections with experimental injury of the endothelium discussed earlier (e.g., 33, 34, 39).

### Laboratory studies: biologic plausibility of the hypothesis

In 1973, concurrently with the Benditt and Benditt study (45), Fabricant et al. (58) reported that infection of cell cultures by adenovirus induced the intracellular accumulation of cholesterol. The last two decades have seen the publication of a large number of additional in vitro studies that have explored plausible pathogenetic mechanisms (59–82). It is not within the scope of this paper to review the vast literature on possible pathogenesis of the infections-atherosclerosis hypothesis, although table 1 provides a summary of possible mechanisms and supporting evidence. As discussed elsewhere (6–10, 60), infections can directly or indirectly cause all three of the main pathologic events leading to atheromatous formation, namely, endothelial cell damage, smooth muscle cell proliferation, and foam cell formation. Moreover, recent studies suggest that infections can potentiate the adherence to the endothelium and leukocyte migration into the arterial tissue, and induce acute or chronic inflammatory changes (table 1). In addition to atherogenesis, infections may cause endothelial dysfunction and acute inflammatory or thrombotic changes, induce plaque instability, and trigger acute cardiovascular disease events (8, 83, 84).

Furthermore, infections may be athero-thrombogenic by their *systemic* effects rather than by local endothelial or vascular infection. It has been recently postulated (85, 86) that atherosclerosis may have an autoimmune component, an idea that was advanced by Jones and Rogers (44) 50 years ago, as reflected in the quote given earlier. For example, circulating antibodies against bacterial *heat shock proteins* (hsp 60/65) may cross-react against human hsp 60 expressed in endothelial cells in response to stress (e.g., free radicals, hemodynamic stress). In a recent study (87), chlamydial hsp 60 was found within macrophages in human carotid atherosclerotic arteries; both chlamydial and human hsp 60 were found to induce macrophage production of tissue necrosis factor- $\alpha$  and matrix-degrading metalloproteinase, two mediators of atherosclerosis complications (87). Systemic athero-thrombotic effects of infections may also stem from an abnormal inflammatory response of the endothelium

**TABLE 1. Summary of possible atherogenic effects of infections and available supporting evidence from experimental studies**

Pathophysiologic process	Supporting evidence	Reference(s)
Endothelial injury	Viral or bacterial antigen-antibody complexes induce or contribute to endothelial injury. Infected endothelial cells show increased expression of membrane receptors for binding immunocomplexes.	33, 39, 59 60
Adherence-migration of leukocytes	CMV*/HSV-1*-infected endothelium shows increased adherence for PMN* leukocytes. Increased PMN adhesion associated with replication of CMV in endothelial cells is mediated by ELAM-1* and ICAM-1.*	61, 62 63
Foam cell formation	HSV* infection in cultured macrophages and SMC* induces accumulation of cholesterol crystals and altered lipid metabolism, an effect that is prevented by vaccination. CMV infection of human SMC increases class A scavenger receptor and modified LDL* uptake. Atherogenic effect of infections in animals is increased when combined with hypercholesterolemic diet.	8, 58, 64 65 42, 50
SMC proliferation	Monoclonal character of cells in atherosclerotic plaque. CMV infection is correlated with p53 accumulation in excessive proliferating SMCs associated with restenosis post-angioplasty. CMV blocks apoptosis of fibroblasts and endothelial cells, an effect that seems to be associated with abnormal cytoplasmic accumulation of p53.	45, 47, 48 66 67, 68
Procoagulation	CMV infection produces a depletion of vWF* of cultured endothelial cells. HSV- and chlamydia-infected endothelial cells have procoagulant properties that depend on plasma and tissue coagulation factors.	69 70–74
Inflammation	<i>Chlamydia pneumonia</i> increases lymphocyte proliferative response, particularly in subjects with CHD.* CMV and <i>C. pneumonia</i> replicate in human endothelial cells, macrophages, and SMC, and trigger the cytokine pathway. CMV and <i>C. pneumonia</i> infection is associated with upregulation of endothelial cytokine expression. The degree of <i>C. pneumonia</i> infection in plaque tissue is associated with the degree of inflammatory changes. Infections are associated with acute phase reactants.	75 76, 77 8, 20, 78–80 81 82

\* CMV, cytomegalovirus; HSV-1, herpes simplex virus type 1; PMN, polymorphonuclear leukocytes; ELAM-1, endothelial leukocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; HSV, herpes simplex virus; SMC, smooth muscle cells; LDL, low density lipoprotein; vWF, von Willebrand factor; CHD, coronary heart disease.

to circulating endotoxins produced by bacteria elsewhere in the body (88).

Based on the strong biologic plausibility and the available evidence at the time, a 1978 *Lancet* editorial (49) emphasized the need for well-designed epidemiologic studies aimed at the evaluation of acute and chronic infections as risk factors for atherosclerosis. It was not until the late 1980s that the first epidemiologic studies in humans were reported (89, 90).

### Epidemiologic and pathology studies

Since 1987, a number of cross-sectional and cohort studies have documented an association between clinical or subclinical cardiovascular disease and chronic infection by CMV, *C. pneumonia*, and *H. pylori* (6, 7). Clinical studies have also shown that CMV infection is a strong predictor of restenosis postangioplasty (91) and of graft atherosclerosis post-transplantation (92). Recent studies suggest that chronic respiratory infec-

tions (93) and periodontal infections (88) are associated with cardiovascular disease. The latter associations might be mediated by the putative systemic atherothrombotic effects of endotoxins alluded to above (88).

Seroepidemiologic studies are subject to the possibility of confounding (6, 7, 15), although some of the prospective studies of CMV and *C. pneumoniae* have shown that the associations remain significant after other important cardiovascular disease risk factors are taken into account (94, 95). Some negative case-control studies have recently been reported (96, 97). However, these studies are limited by the high infection prevalence in the control groups, consistent with the high prevalence of these infections in older adults (98, 99). Epidemiologic studies in older adults may have limited power to detect differences in prevalence of such common infections, differences that may have more relevance earlier in life. Furthermore, serum antibodies may be a poor measure of chronic CMV or

*C. pneumoniae* infection (100–102), thus limiting the statistical power of these studies (6).

A number of recent pathology studies using polymerase chain reaction and immunocytochemistry have identified DNA from CMV (103) and *C. pneumoniae* (81, 101, 102, 104) in human atheroma tissue obtained from necropsy or atherectomy. In contrast, pathology studies have failed to demonstrate *H. pylori* in atheroma (104), which along with negative results in recent prospective epidemiologic studies (6, 105) weaken the hypothesized atherogenic role of this pathogen.

### Clinical trials

Preliminary results from two small clinical trials suggest that treatment with macrolide antibiotics (azithromycin or roxithromycin) is associated with a reduced risk of recurrence of coronary disease events in survivors of myocardial infarction (106) and among patients with unstable angina (107). These studies, however, were based on small numbers, and it is unclear whether the observed difference is due to the anti-inflammatory effect rather than the antibiotic effect (14, 16).

### INFECTIONS AND ATHEROGENESIS: IMPLICATIONS OF THE HYPOTHESIS

Assuming that there is some epidemiologic and biologic basis to accept the possibility that certain infections (namely CMV or other herpesvirus and *C. pneumoniae*) may be involved in atherogenesis, I propose that the evidence presented above suggests that other infectious diseases may also have atherogenic potential. Experimental and animal studies with *B. typhosus*, streptococci, and other agents conducted five to ten decades ago had strikingly similar results to those of modern experiments with CMV and *C. pneumoniae*. Contrary to the fallacy of the “specificity criterion of causality” (108), different infectious agents can cause inflammatory syndromes in other organs and tissues; consequently, it is conceivable that a number of chronic (or even some acute) infections could cause inflammation and initiate or potentiate atherogenesis by one or several of the mechanisms summarized in table 1. Rather than directly infecting the endothelium, some of these infections may be atherogenic because of their systemic effects (endotoxins, heat shock proteins), as discussed above.

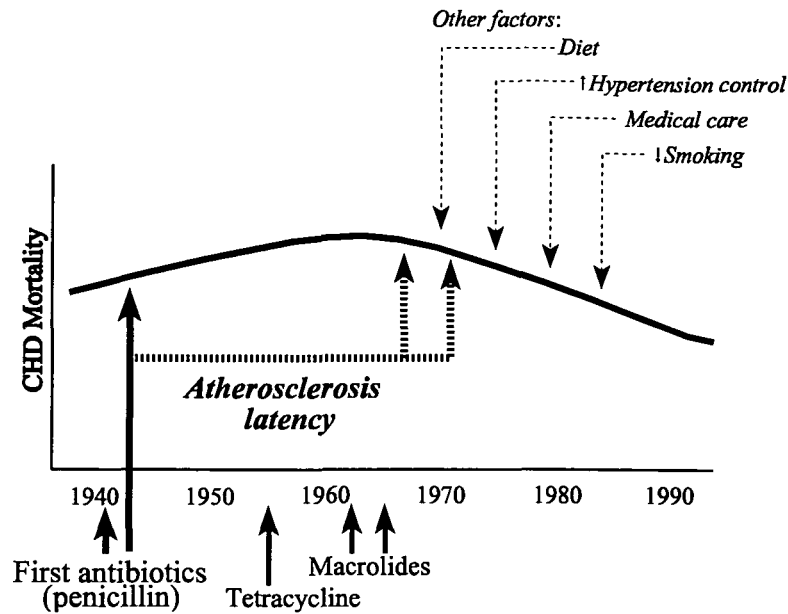
Investigators began studying herpesvirus and CMV as potentially atherogenic in humans inspired by studies showing atherogenic effects of herpesvirus in chickens (3). The initial studies by Saikku et al. involving *C. pneumoniae* (90) were prompted by the “quite unexpected” finding of an unusually high anti-

body seroprevalence in a coronary disease case series (98, p. 31). How many other pathogens could also be potentially atherogenic but have not been studied? I would suggest that the early studies by Boinet and Romary (30), Gilbert and Lion (33), Crocq (34), Klotz (39), Benson et al. (42), Jones and Rogers (43, 44), and others (31, 32, 35, 36, 38, 39, 40, 41) indicate that other infectious agents might also be involved.

### Infections, antibiotics, and coronary disease trends

The hypothesis that many bacteria or viruses (including but not limited to *C. pneumoniae* and CMV) may be involved in atherogenesis would be consistent with the early observations reviewed in this paper and could also help to explain the downturn in coronary disease incidence and mortality observed in much of the Western world since the 1960s (109–112). The introduction of penicillin in 1940 and other antibiotics (e.g., tetracycline and chloramphenicol) in the late 1940s and 1950s allowed the primary and secondary prevention of many common infections (113, 114). If some of these infectious diseases (including streptococcal infections, typhus, and possibly other diseases) were involved in atherogenesis, as suggested above, it would take two to three decades to translate the consequent improvement in atherogenic profile in the population—and particularly among young adults—into observable declines in clinical coronary disease incidence or mortality among older adults, due to the necessary latency period for clinical atherosclerosis manifestations (figure 3). Given that most bacterial infections with alleged atherogenic potential that were common in the beginning of the century (e.g., *B. typhosus* and streptococcus) are now prevented and treated with antibiotics, what is left are all other atherogenic infections that either produce apparently banal or subclinical disease or that are resistant to most antibiotics (e.g., CMV and *C. pneumoniae*).

This hypothesis is a generalization of that recently proposed by Anestad et al. (115), who showed that cardiovascular disease mortality started its decline in Norway right after the introduction of tetracyclines in the country (in the late 1950s), while dietary fat intake and smoking was still high. Saikku has made similar observations relating the decline of coronary disease mortality in Finland to the introduction of erythromycin and other macrolide antibiotics (Dr. Pekka Saikku, National Public Health Institute, Oulu, Finland, personal communication, 1998). Tetracyclines and macrolides are effective against *C. pneumoniae* and their introduction may have helped reduce the prevalence of this (then unidentified) infection as well as its alleged atherogenic/thrombogenic consequences.



**FIGURE 3.** The hypothesized relation between the introduction of antibiotics and the decline in coronary heart disease (CHD) mortality in the last third of the twentieth century. The introduction of the first antibiotics (penicillin) reduced the prevalence of common infections with putative atherogenic effects, which, due to the latency period of the clinical manifestations of atherosclerosis, had a noticeable impact in coronary disease mortality 2–3 decades later (see text). The introduction of tetracycline in the 1950s and of macrolide antibiotics in the 1960s may have contributed to these trends because of their effectiveness against *C. pneumoniae* infection (115; Dr. Pekka Saikku, National Public Health Institute, Oulu, Finland, personal communication, 1998).

The preceding conjecture does not exclude the influence on these trends of changes in other risk factors (e.g., smoking, diet, medical care, and hypertension control) (109, 110). It is offered as a working hypothesis that may help to decipher some of the remaining questions related to these trends, such as their inconsistency with changes in prevalence of major risk factors (109) and their variations across different nations (116). This hypothesis, however, does not intend to explain entirely the puzzling diversity of cardiovascular disease trends across populations. For example, it could be argued that this hypothesis is in contradiction with the low coronary disease incidence observed in developing countries with a large population infection burden. However, this could be explained by low levels in these countries of other key risk factors (e.g., high cholesterol diet and sedentary life-style) that may act synergistically with infections in atherogenesis (see below).

#### Infections: mediators of the atherogenic effect of low SES?

It is well established that SES is an important determinant of coronary disease trends, both at the population level (111) and at the individual level (117). However, controlling for the standard coronary disease risk factors only explains a fraction of the observed SES-coronary disease associations (118, 119).

Because infections are more frequent in populations that have lower SES (120), they could constitute one of the missing links in explaining these associations.

#### Can infections explain the association between fetal/childhood growth and adult coronary disease?

Infections may also be one of the confounding mechanisms responsible for the intriguing associations between early childhood growth and adult cardiovascular morbidity. A number of studies have consistently shown that low birth weight or weight in infancy are associated with cardiovascular disease risk later in life (121, 122), leading to the so-called “programming hypothesis” of coronary disease and other chronic diseases (123). However, the possible confounding role of SES and other environmental factors in explaining these associations has been emphasized (124). Poor growth in children is more frequent in unhealthy environments with higher risk of infections, which in turn could initiate or enhance the development of early atherosclerosis and heighten coronary disease risk later in adult life. This hypothesis is consistent with the study by Buck and Simpson (125), which showed a correlation between infant mortality from diarrhea and enteritis in the 1917–1921 US birth cohorts and arteriosclerosis heart disease at ages 40–44 and 50–54 years.



## COMMENT AND CONCLUSION

More than one hundred years of research has produced a large number of clinical observations, epidemiologic studies, as well as pathology, *in vitro*, and animal studies, which all suggest a potential role of infections in atherosclerosis disease. Although this hypothesis is far from proven (6, 7, 15), the consistency of all these different types of observations with current pathogenic theories of atherogenesis supports its biologic plausibility, one of the primary causal criteria in epidemiology (126).

As with the recent case linking *H. pylori* with peptic ulcer (127), the infectious hypothesis of atherogenesis challenges old paradigms of *chronic* versus *infectious* diseases. In my view, this explains the highly prevalent skepticism among many cardiovascular disease epidemiologists even today.

French pathologist Crocq opened his 1894 article with the following statement:

The question of infectious arteritis is relatively recent, and although it is currently of great interest to clinicians and experimenters, its history is still far from being positively established. (34, p. 583).

Crocq probably did not know how far the medical community really was from even considering this then "relatively recent" hypothesis. At the turn of the century, research on infections and atherosclerosis suddenly ceased. The reason does not seem to be that the hypothesis was proven wrong, but that there was a change of paradigms in atherogenesis research. This was the triumphant time of the "germ theory" (40), when the microorganisms that cause many infectious diseases were being identified. Paradoxically, these advances resulted in a somewhat arbitrary, mutually exclusive, acute-chronic disease taxonomy. Infectious diseases were often acute, often with an identifiable incubation period, and frequently accompanied by episodic febrile symptoms. In contrast, cardiovascular and other *chronic* diseases (e.g., cancer, peptic ulcer) were increasingly perceived as multifactorial, degenerative, aging-related and, *by definition*, non-infectious (128). The complexity of such chronic diseases required a shift in emphasis, from the concept of "cause" to the concept of "risk factor" and the "web of causation" (17, 18). Among the postulated risk factors, cardiovascular disease research focused primarily on the emergent cholesterol hypothesis (129, 130). With the subsequent publication of the first results from the Framingham Study (131) and other studies (132), the role of cholesterol and the other major cardiovascular disease risk factors (hypertension, smoking, diabetes mellitus, male gender, and age) was confirmed. The multifactorial-degenerative model of atherogenesis

was further strengthened, at the expense of an almost total abandonment of research on the putative atherogenic role of infections.

It should be noted, however, that the infectious hypothesis does not contradict the multifactorial model for the etiology of atherosclerosis and the role of traditional risk factors. On the contrary, the infectious hypothesis is quite consistent with the cholesterol hypothesis. Animal studies have repeatedly shown a synergistic atherogenic effect of experimental infection and a high cholesterol diet (42, 50, 53), consistent with experimental work showing lipid metabolism abnormalities in infected macrophages (8, 37, 58, 64, 65), and some epidemiologic evidence (94). Furthermore, epidemiologic studies have found evidence which suggests a synergistic effect of infections with smoking (133), fibrinogen (134), and lipoprotein(a) levels (134, 135).

The past and recent reluctance to admit the possibility of the infectious theory of atherosclerosis is more dramatic given the extraordinary preventive potential that it could offer. Fortunately, after decades of neglect, in recent years there have been signs of renewed interest in this hypothesis (3–16). Many investigators still call for caution and emphasize the need for prospective observational studies that adequately control for confounding factors (6, 15). Others call for large and long-term clinical trials to confirm preliminary findings (105, 106) and to prove the potential benefits of antibiotics for the secondary prevention of coronary disease (6, 7, 14–16). Such trials, however, will not definitely resolve the question of a putative causal association of infections and atherosclerosis. A positive result could potentially be explained by anti-inflammatory (or other) effects of these antibiotics (7, 14). Because these trials are being conducted using antibiotics in patients with significant atherosclerosis disease, a negative result will not rule out the role of viruses, and/or the possibility that infections are involved in earlier phases of the natural history of atherosclerosis. Conversely, a positive result will have little relevance regarding coronary disease *primary* prevention, particularly considering the lifespan of exposure to many chronic infectious diseases. Furthermore, even if antibiotics prove to be effective in the *secondary* prevention of coronary disease, the potential problems of resistances and long-term secondary effects of these therapies need to be carefully evaluated before they become routine medical practice.

If specific infectious agents are singled out in prospective epidemiologic studies, the development of vaccines could create previously unthinkable primary prevention opportunities. For example, experimental CMV vaccines are available and have been success-



fully used in renal transplant patients (136, 137). On the other hand, if many infectious agents are involved, as hypothesized above, prevention will require more complex efforts including the continuing improvement of environmental and hygienic conditions, particularly among the populations in the lower social class.

This review also illustrates how going back beyond the 1966 "magic" Medline cut-off can provide new perspectives regarding the infectious hypothesis of atherogenesis, possibly increasing the understanding of cardiovascular disease trends and prevention measures. A potentially important conclusion of this review is the need to expand the quest for potentially atherogenic infections beyond the limited number of agents that are currently being investigated. At least, replication of the early experiments involving *B. typhosus*, streptococci, and other microbes (30–36, 38–44) needs to be attempted. Furthermore, examining the role of infections as possible mediators of the association between SES and childhood growth with cardiovascular disease deserves further investigation.

Adoption of a broad epidemiologic view of atherosclerosis disease could again bring together the infectious and the chronic disease paradigms. A synthesis of past and current research may assist in the search for new clues that could provide the answers to old questions.

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