REVIEW

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Is atherosclerosis an infectious disease?

ABSTRACT

Atherosclerotic coronary artery disease is multifactorial, but several lines of evidence implicate infection as a potential contributing factor. *Chlamydia pneumoniae* has the most compelling data, with *Helicobacter pylori* and cytomegalovirus also implicated. Clinical trials of antibiotics to decrease coronary events are underway. Until the results are available, however, we advise against prescribing antibiotics for this purpose.

KEY POINTS

Atherosclerosis is a specialized inflammatory response involving monocytes and T lymphocytes. Inflammatory mediators such as C-reactive protein and fibrinogen are elevated in infectious diseases and associated mechanistically with atherosclerosis.

Some studies suggest that the risk of coronary artery disease increases with the total infectious load, ie, the total number of different pathogens a person has been exposed to.

Although several small trials showed that a short course of a macrolide antibiotic could reduce coronary events, there is reason for caution; in theory, such therapy could actually increase risk.

Disclosure: This paper discusses treatment that is "off label," ie, not FDA-approved.

INTEREST IN THE ROLE OF INFECTIONS as a component of atherosclerotic cardiovascular disease is growing, fostered by gaps in current understanding of this common, devastating disease.

Atherosclerotic coronary artery disease is a contributing factor in up to 50% of US deaths, half of them due to coronary thrombosis and myocardial infarction. It is clearly a complex condition with multiple contributing factors. Yet more than one third of patients dying of atherosclerotic coronary vascular disease have none of the classic risk factors.[1]

This overview places the available data about the potential role of infections in cardiovascular disease in historical context and briefly comments on the status of current research.

ATHEROSCLEROSIS: AN INFLAMMATORY RESPONSE

Atherosclerosis in all stages of its development and progression is recognized as a specialized inflammatory response.[2–4] The process begins when the vascular endothelium, in response to a variety of insults (free radicals caused by cigarette smoking, hypertension, modified lipoproteins, glycosylation products, or elevated homocysteine), recruits monocyte-derived macrophages and T lymphocytes into the arterial intima, forming a fatty streak. Atherosclerotic lesions are characterized by significant disruption of the normal structures in association with activated macrophages (also known as foam cells) and T cells.

Infectious agents have also been proposed as triggers or mediators of this response.[5,6] Over the years, various infectious agents have been postulated to contribute to atherosclerot-

ic cardiovascular disease. Most recent studies focus on *Chlamydia pneumoniae* and cytomegalovirus (CMV), though other infectious agents have also been investigated.[6–8] Research suggests multiple possible mechanisms and associations by which infectious agents may facilitate atherosclerotic development and progression.[5]

VIRCHOW AND OSLER THOUGHT OF IT FIRST

Essential groundwork in this field was laid in the 1800s, when Virchow[9] reported on the inflammatory nature of atherosclerotic lesions in the course of detailed pathologic evaluation.

Osler's 1908 *Textbook of Medicine*[10] commented, "Experimental production of arteriosclerosis by the various bacterial toxins afford an explanation of this gradual production of sclerosis in the chronic infections." Many recent reviews have revisited this topic[7,8]; background detail and many interesting though unproven theories are found in these papers.

1/3 of patients who die of coronary disease have no traditional risk factors

COXSACKIE B VIRUS

In experiments in mice and monkeys in the 1960s, coxsackie B virus became the first infectious agent noted to produce acute coronary arteritis.[11] A few small human case series noted a striking prevalence of seroconversion to coxsackie B at the time of acute myocardial infarction (MI), but prospective studies did not support this association.[12–14]

HERPESVIRUSES

In the mid-1970s, it was noted that Marek disease herpesvirus (MDV), a herpesvirus of fowl, could induce large-vessel occlusive atherosclerotic lesions in chickens. These lesions closely resembled chronic atherosclerotic lesions in humans.

Minick et al[15] and Fabricant et al[16] conducted an experiment in chickens in which some were deliberately infected with MDV, some were fed a diet high in cholesterol, some received both interventions, and some received neither. The MDV-infected chickens had dramatically more atherosclerotic lesions than did the untreated chickens or those fed the high-cholesterol diet but not infected. In addition, cholesterol feeding had a significant enhancing effect on the prevalence of large coronary artery lesions, consistent with the hypothesis that atherosclerotic cardiovascular disease is multifactorial.

Yet studies of herpesvirus type 1 and type 2 in human atherosclerosis have been unrevealing,[17] even though both can infect and grow within human endothelial cells in vitro.[18,19]

CYTOMEGALOVIRUS

CMV is a member of the herpesvirus family that can grow in endothelial and smooth muscle cells. Studies evaluating the potential role of CMV in atherosclerosis were conducted as early as 1983.[20] In a more recent study, Zhou et al[21] noted a dramatically higher incidence of restenosis after atherectomy and angioplasty in patients who were CMV-seropositive than in those who were CMV-seronegative, 43% vs 8% ($P = .002$).

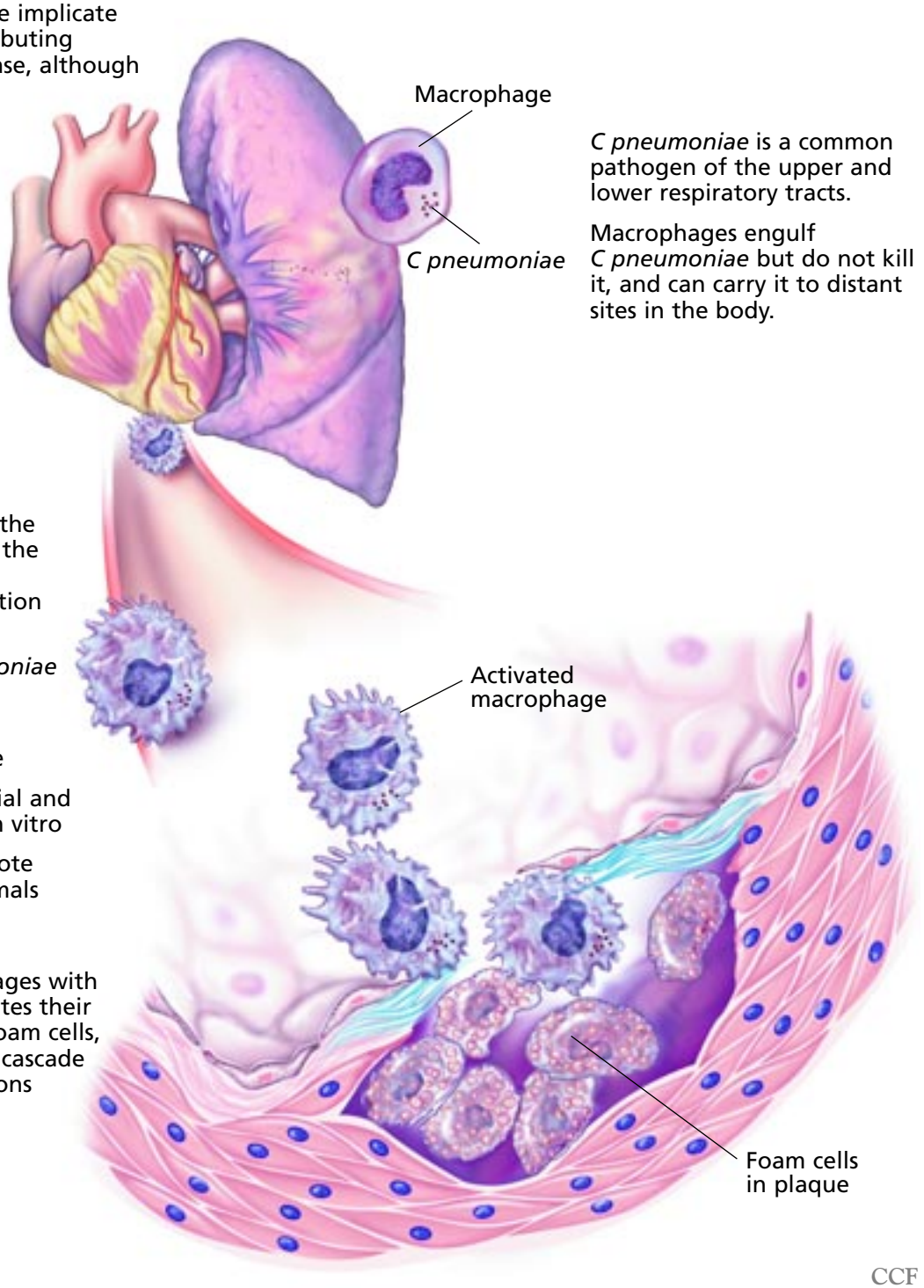
Restenosis appears to be largely mediated by smooth muscle proliferation, which is part of the process of atherosclerosis. When CMV infects vascular smooth muscle cells it inhibits p53, a cellular protein that normally regulates smooth muscle proliferation.[22] By inhibiting the inhibitor, CMV is believed to promote clonal smooth muscle cell proliferation in coronary restenosis lesions.[23]

Most studies relating CMV to atherosclerosis evaluated restenosis after angioplasty, but other recent studies did not find an association between angioplasty, CMV, and restenosis.[24–27] Angioplasty alone without atherectomy may create a different local effect, which might account for the varied results.

CMV is also thought to play a role in accelerated atherosclerotic coronary disease after heart transplantation,[28,29] a leading cause of death in this patient population.[28] A recent post-hoc analysis of a study of ganciclovir to control CMV after transplantation found that ganciclovir recipients had a somewhat lower incidence of post-transplantation atherosclerotic disease than did placebo recipients (43% vs 60%, $P < .1$).[29]

Chlamydia pneumoniae: Perpetrator or innocent bystander in coronary artery disease?

Several lines of evidence implicate *C pneumoniae* as contributing to coronary artery disease, although formal proof is difficult to establish.



C pneumoniae is a common pathogen of the upper and lower respiratory tracts.

Macrophages engulf *C pneumoniae* but do not kill it, and can carry it to distant sites in the body.

Macrophages infiltrate the subendothelial layer of the coronary arteries and contribute to inflammation and plaque formation.

Evidence that *C pneumoniae* plays a role:

- It can be detected in atherosclerotic plaque
- It can infect endothelial and smooth muscle cells in vitro
- It can induce or promote atherosclerosis in animals after respiratory tract inoculation
- Infection of macrophages with *C pneumoniae* facilitates their transformation into foam cells, which are part of the cascade of atherosclerotic lesions

FIGURE 1

CHLAMYDIA PNEUMONIAE

C *pneumoniae* is an obligate intracellular gram-negative organism capable of chronic or persistent infection. It is a recognized cause of acute upper and lower respiratory infection, and its seroprevalence increases with age.[30,31]

Of the various organisms studied as possible causes of coronary artery disease, C *pneumoniae* has the most evidence in its favor.

Evidence that C *pneumoniae* contributes to atherosclerosis

Koch's postulate states that to prove that a given organism causes a given disease, one has to do four things:

- Observe the organism in every case of the disease
- Isolate the organism from a subject with the disease and grow it in culture
- Inoculate the culture into a susceptible animal and observe that this reproduces the disease
- Observe and recover the organism from the experimentally diseased animal.

Although we cannot fully meet Koch's postulate in a multifactorial disease such as atherosclerosis, a number of lines of evidence support the hypothesis that C *pneumoniae* contributes to the disease (**FIGURE 1**).

The total number of infections may increase the risk

MI patients have a high prevalence of infection. In 1988, while evaluating a serologic assay for C *pneumoniae*, Saikku et al[32] in Finland noted a highly statistically significant association between the presence of C *pneumoniae* antibodies and prior MI: 27 (68%) of 40 MI patients had elevated chlamydial IgG and IgA titers, compared with only 1 (2%) of 41 control subjects ($P < .00001$). In a similar study in the United States, Kuo et al[33] confirmed this seroepidemiologic association, especially among smokers.

C *pneumoniae* can be detected in atherosclerotic plaque. Multiple studies detected C *pneumoniae* in atherosclerotic plaque specimens by a variety of techniques, including immunohistochemistry, polymerase chain reaction (PCR), and electron microscopy.[34–36] A recent review summarized 17 studies of cardiovascular arterial specimens and noted that C *pneumoniae* was identified in 303 (50.8%) of 597 specimens with atherosclerotic involvement.[37] In contrast, the organism was found in only 5 (3.8%) of 131 coronary artery specimens without atherosclerotic disease.

Laboratory detection of the organism in atherosclerotic plaque does not prove a causal relationship. Nevertheless, Jackson et al[38] and Maass et al[39] recently recovered C *pneumoniae* organisms, viable and capable of reproducing, in both carotid and coronary artery specimens. Moreover, the paucity of organisms in nonatherosclerotic tissue and other types of macrophage-rich inflammatory lesions suggests an active role for C *pneumoniae* in the complex atherosclerotic process.[40]

C *pneumoniae* and CMV can infect endothelial and smooth muscle cells in vitro, adding further support to their possible role in atherosclerotic cardiovascular disease. Cholesterol loading makes smooth muscle cells more vulnerable to infection with *Chlamydia* organisms.[41]

C *pneumoniae* is carried through the body by macrophages. C *pneumoniae*-infected macrophages are capable of infecting endothelial cells,[41,42] and PCR testing of peripheral blood monocytes shows C *pneumoniae* in up to 25% of people.[43,44] These data suggest how an intracellular pulmonary infection may migrate to a distant vascular site, using circulating macrophages as a kind of Trojan horse.[45,46]

Experimental C *pneumoniae* infection can induce or promote atherosclerosis in animals.[39,47–51] In mice and rabbits, inoculation of C *pneumoniae* into the upper respiratory tract can produce atherosclerotic lesions in the aorta of these animals. In apoE-deficient mice, C *pneumoniae* infection accelerates the progression of atherosclerosis in the aortic arch.[52] Normocholesterolemic rabbits develop intimal alterations when infected with C *pneumoniae*.[49,53] Muhlestein et al[50] showed that rabbits fed a modestly cholesterol-enhanced diet developed accelerated aortic atherosclerosis, which was dramatically reduced by treatment with the macrolide antibiotic azithromycin. C *pneumoniae* antigen could be detected in the aortic atheroma in this model, and while treatment ameliorated the accelerated atherosclerosis, C *pneumoniae* antigen was still detected in the atheroma after treatment. It is unclear whether this persistence represented viable quiescent organism or merely antigenic rem-

TABLE 1

Ongoing clinical trials of antibiotics in atherosclerotic cardiovascular disease

TRIAL	POPULATION	REGIMEN*	SIZE	ENROLLMENT	END POINTS
Preliminary trials					
ACADEMIC	Coronary disease†	Azithromycin	300	Closed	Composite‡
ROXIS	Peri-MI	Roxithromycin	202	Closed	Composite
Ongoing trials					
WIZARD	Post-MI†	Azithromycin	7,000	Closed	Composite‡
ACES	Coronary disease	Azithromycin	4,000	Closed	Composite‡
MARBLE	Awaiting CABG	Azithromycin	1,200	Open	Composite‡
ANTIBIOS	Post-MI†	Roxithromycin	4,000	Closed	Composite‡
PROVE-IT	Coronary disease	Gatifloxacin ± statin	4,000	Open	Composite‡
STAMINA	Peri-MI	Anti-*H pylori*	600	Open	Inflammatory markers
APRES	Angioplasty	Roxithromycin	1,000	Open	Restenosis
MUNICH	Angioplasty	Roxithromycin	1,000	Closed	Restenosis

*All studies have a placebo group
Azithromycin dosage is 600 mg once weekly for 3 months, except in ACES, in which the dosage is weekly for 1 year
STAMINA compares two anti-*H pylori* regimens: omeprazole + azithromycin + metronidazole vs omeprazole + amoxicillin + metronidazole
Roxithromycin dosage is 300 mg daily for 4 (MUNICH) to 6 (ANTIBIOS, APRES) weeks
†Patients must be seropositive for *C pneumoniae* antibodies
‡Death, MI, revascularization, angina, or other cardiovascular event

nants of killed organism.

Infection of monocytes with C *pneumoniae* facilitates their transformation into foam cells, which are clearly part of the initiation cascade of atherosclerotic lesions.[54]

Together, these data are consistent with the view of atherosclerosis as a complex disease process with a strong inflammatory component. The idea that inflammation, in addition to being a response to injury, is the overall pathologic process in atherosclerotic lesions would certainly fit with C *pneumoniae* or other chronic infectious agents playing *part* of the role in this disease process. It remains unproven whether C *pneumoniae* is actively involved in driving this inflammatory process or whether it is merely an "innocent bystander."

HELICOBACTER PYLORI

A possible association between the chronic infectious agent *Helicobacter pylori* and atherosclerotic coronary vascular disease was first evaluated in 1994.[55] Reasons for investigating this agent included a known association between low childhood socioeconomic status (a risk factor for *H pylori* acquisition) and coronary artery disease as an adult.[56] There also is evidence of an association between gastric cancer, peptic ulcer disease, and atherosclerotic cardiovascular disease.[57] Patients who have *H pylori* antibodies have higher levels of fibrinogen and C-reactive protein, which are both markers associated with clinically relevant atherosclerotic cardiovascular disease. Treatment of *H pylori* (and C *pneumoniae*) reduces fibrinogen levels.[58]

By itself, seropositivity for *H pylori* seems to have a much weaker association with atherosclerotic coronary artery disease than does seropositivity for C *pneumoniae*, and this avenue of research is waning.

People typically acquire more than one infection in their lifetimes, with more than one organism. Recent work noted an increased odds ratio for the development of atherosclerotic cardiovascular disease in peo-

ple who were seropositive for multiple infectious agents.[59] Coinfection with all three agents—C *pneumoniae*, CMV, and *H pylori*—outpaced any single infectious agent.

PERIODONTAL DISEASE

A potential association between periodontal disease and atherosclerosis was first proposed in 1963 by Mackenzie and Millard.[60] Recently, interest in this topic has been renewed.

Some studies found periodontal disease to be an independent risk factor for atherosclerotic cardiovascular disease, but others did not.[61] Even if periodontal disease is not an independent risk factor, it may work synergistically with other risk factors such as smoking, or may be more important in men younger than 50 who are less likely to have other traditional risk factors. There is a significant increase in fatal and nonfatal coronary heart disease among patients with progressive alveolar bone loss on mandibular radiographs, a marker for severity of periodontal and gingival disease.[61]

If there is an association, a plausible mechanism exists, as the gingivitis-associated bacterium *Porphyromonas gingivalis* is capable of infecting endothelial cells.[62]

If infections contribute to coronary disease, antibiotics should reduce events

TRIALS OF ANTIBIOTIC THERAPY

If infections contribute to coronary artery disease progression, then treating these infections ought to reduce coronary events. To evaluate this hypothesis, preliminary trials of antibiotic therapy in humans have been conducted, and several larger trials are underway (TABLE 1).

Preliminary trials

Gupta et al,[63] in a trial in the United Kingdom, screened 220 men who had suffered an MI at least 6 months previously and stratified them by their antibody titers for C *pneumoniae*. Of the 80 men with the highest titers, 40 were randomized to receive azithromycin 500 mg by mouth every day for 3 days, 20 received placebo, and the other 20, who elected not to take medication, were labeled a "control" group. Over the next 18 months, significant coronary events (nonfatal MI, unstable angina, or cardiovascular death), occurred in 8% of the azithromycin-treated high-titer group, compared with 25% in the placebo-treated high-titer group and 30% in the high-titer control group. The 8% rate was similar to the rate of coronary events in a seronegative group who did not receive treatment.

The ACADEMIC trial,[64] in 302 patients seropositive for C *pneumoniae*, showed no difference in clinical outcome at 6 months after treatment with azithromycin compared with placebo, but did show a significant decrease in C-reactive protein and the inflammatory cytokine markers interleukin 1 and interleukin 6.

The ROXIS trial,[65] in contrast, did not use seropositivity as an entry criterion. Two hundred and two patients entered the study within 8 days of experiencing unstable angina or non-Q-wave MI and were randomized to receive either roxithromycin 150 mg by mouth twice a day or placebo for 30 days. At 1 month the treated patients had a lower incidence of the primary composite end point (coronary ischemic death, MI, or severe recurrent ischemia), but there was a loss of effect over time.

Comment. The differences in study design and outcome reflect the complexity of potential associations between infection and atherosclerotic cardiovascular disease, as well as uncertainty about the best way to treat chronic chlamydial infection. There is also uncertainty as to whether the antiatherosclerotic effects of macrolide antibiotics in animal studies and preliminary clinical trials[60,62] are due to an antimicrobial effect, the weak but measurable anti-inflammatory properties of this class of antibiotics, or a combination of the two.[66–69]

Ongoing randomized trials of antibiotics as secondary prevention

In view of the fairly strong seroepidemiologic evidence, positive animal studies, and results of the preliminary clinical trials, several large multicenter placebo-controlled trials of antibiotic use for the prevention of coronary events have been undertaken.

The WIZARD trial is a randomized, double-blind, placebo-controlled trial of the effi-

cacy of weekly azithromycin on the incidence of coronary artery disease in patients with a remote history of MI (> 6 months) and positive IgG serology of C *pneumoniae*. Patients in the active-treatment group received azithromycin 600 mg daily for 3 days followed by maintenance therapy of 12 weekly doses. Starting in the fall of 1998, 7,000 patients were enrolled in only 3 months. The study should have the statistical power to detect a 15% reduction in clinical end points (death, MI, need for coronary revascularization, or hospital admission for angina). However, because the estimated annual event rate in this very stable patient population is only 8%, follow-up must continue for 36 months to reach statistical significance. Results are expected in early 2003 (Personal communication; Michael Dunne, MD).

The ACES trial. Perhaps a short course (12 weeks) of antibiotic therapy is insufficient to effectively treat a potentially chronic chlamydial infection.[70] This concept could help explain the loss of effect over time in the ROXIS trial. This potential shortcoming will be addressed in the Azithromycin and Coronary Events Study (ACES). Funded by the National Heart, Lung, and Blood Institute, this study is currently enrolling patients with a history of MI regardless of *Chlamydia* serology. Patients are being randomly assigned to receive either placebo or a weekly dose of azithromycin for 1 year.

A group of patients is also being enrolled in a substudy using serial blood collection to examine the potential association between serology, immune markers, and response to therapy. Recruitment is complete, and results are expected in late 2003.

Comment. While the WIZARD and ACES trials should help determine whether a short or long course of azithromycin therapy reduces the incidence of coronary events, both trials are targeting similar patients at a very stable stage of atherosclerotic progression. Both studies require patients to be at least 6 months out from the most recent MI or coronary revascularization.

Opinion differs as to whether the association between C *pneumoniae* and atherosclerosis is due to an effect on the chronic progression of atherosclerosis, activation and destabilization of a fairly advanced plaque, or nonhealing of a recently destabilized plaque.[5]

If the role of C *pneumoniae* is in early lesion progression, treating patients with established but stable atherosclerosis may be too late.

On the other hand, if the primary role of treatment is in healing a ruptured plaque, the trials starting 6 months after an acute coronary event may be outside the window in which lesion stabilization can be maximally influenced. Possibly the appropriate time to treat patients with antibiotics is when they present with an acute coronary syndrome of unstable angina or non-Q wave MI caused by a recently inflamed and ruptured plaque. It was this patient population that was targeted in the pilot ROXIS trial.

ANTIBIOS, a larger post-MI placebo-controlled roxithromycin trial, is enrolling 4,000 patients to gain greater statistical significance.

STAMINA, another peri-MI trial underway, will enroll 600 patients, and compare regimens with anti-*H pylori* activity (a proton pump inhibitor plus azithromycin vs amoxicillin plus metronidazole).

PROVE-IT. Quinolones, like macrolides, also have in vitro activity against chlamydial organisms. At least one placebo-controlled trial underway (PROVE-IT) is evaluating the quinolone antibiotic gatifloxacin. This trial will also randomize patients to receive lipid-lowering ("statin") drugs or placebo.

The best time to intervene may be at presentation

The MARBLE study in the United Kingdom is taking advantage of the delay in that country between the time coronary artery bypass grafting is found to be needed and the time it is actually performed. Researchers want to see if azithromycin (vs placebo) has any impact on disease progression preoperatively. At 1,200 patients, and with planned coronary catheterization before surgery, this study should address unique issues not evaluated in the other trials.

The APRES and MUNICH studies are each enrolling approximately 1,000 patients who have undergone angioplasty, randomizing them to receive roxithromycin or placebo. Enrollment in APRES is still open, but MUNICH is already closed. These studies should help address any

potential role for antichlamydial therapy to reduce restenosis.

Smooth muscle proliferation seems to play more of a role in restenosis after angioplasty than in other types of coronary disease, and CMV seems to promote smooth muscle proliferation. However, we are not aware of any anti-CMV drug trials in angioplasty patients.

Can antibiotics prevent first-time acute MI?

A large case-control study in the United Kingdom[71] enrolled more than 3,000 patients with a first MI who did not have classic risk factors (hyperlipidemia, hypertension, or diabetes) and compared them with 13,139 matched healthy controls. In the 3 years before the MI, the MI patients were less likely to have been prescribed tetracycline or fluoroquinolone antibiotics for various infections ($P < .05$ for both comparisons). These agents are active against *C pneumoniae*. Though macrolide antibiotics also have antichlamydial activity, the MI patients were not less likely to have received macrolides. A possible explanation is that the macrolides probably were used at doses ineffective in treating *C pneumoniae* infection.

Subsequent studies in the United States did not find an association between a first MI and use of erythromycin, tetracycline, or doxycycline.[72]

Do not start giving antibiotics for coronary disease until the results are in

LOOKING BACK, LOOKING FORWARD

The trials performed so far support a possible association between infectious pathogens and atherosclerotic cardiovascular disease, but they are far from establishing a cause-effect relationship. Placebo-controlled treatment trials may provide more direct evidence of an association. Overall, the data reinforce the concept that atherosclerotic cardiovascular disease is multifactorial and that inflammatory changes contribute to its progression.

The epidemiologic studies and the preliminary treatment trials do not clearly settle the issue about *C pneumoniae*'s role in atherosclerotic cardiovascular disease, nor should they encourage widespread application of antibiotic therapy in patients with atherosclerotic cardiovascular disease. Treatment of chronic bacterial pathogens may result in transient immune activation, which may even worsen coronary artery disease.

It is hoped that as ongoing study data emerge, better understanding of pathophysiologic associations and the role of anti-infective therapy will guide future treatment of atherosclerosis.

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