Possiamo vaccinarci contro l'aterosclerosi?

Andrea Annoni, Giorgio Franciosi, Fabio Lionti, Silvia Tenconi, Paolo Panisi, Silvia Cirri*, Luigi Collarini**
U.O. Cardiochirurgia - Istituto Clinico Sant'Ambrogio - Milano
** U.O. Anestesia e Rianimazione - Istituto Clinico Sant'Ambrogio - Milano*
*** U.O. Cardiologia - Istituto Clinico Sant'Ambrogio - Milano*

Abstract
Atherosclerosis is the leading cause of morbidity and the most frequent reason of deaths in Western contries. Conventional risk factors including cigarette smokings, hypertension, and higt serum lipid levels do not fully explain the incidence, prevalence, and distribuition of atherosclerosis.
A number of study have found that inflammation of the vessel wall plays and essential role in both the initiation and progression of atherosclerosis, erosion, and fissure and the eventual rupture of plaques. Infectious agents that have been investigated as possible stimuli include viruses, specifically cytomegalovirus, human herpes viruses, and enteroviruses, and bacteria including H.Pylori and Chlamydia Panuemoniae. This is a review of the most important seroepidemiologic and clinical treatment intervention studies about this patology.

Introduzione

L'aterosclerosi, patologia caratterizzata dalla progressiva deposizione di lipidi e di ispessimento intimale (Fig. 1), rappresenta senza alcun dubbio la principale causa di morte e di grave invalidità del mondo occidentale.
La sua diffusione è tale, che nei paesi economicamente più sviluppati,ha raggiunto proporzioni epidemiche.
Le arterie maggiormente colpite e più precocemente interessate dalla malattia aterosclerotica sono quelle elastiche (aorta, carotidi ed arterie iliache) e le arterie muscolari di grandi e medie dimensioni (poplitee, coronarie), le lesioni di quest'ultime, causano la ben nota cardiopatia ischemica.
La patologia aterosclerotica è una malattia complessa, causata dall'interazione di innumerevoli fattori di rischio quali fumo, ipertensione, iperlidipedmia, familiarità, stress, diabete, obesità, etc. che darebbero inizio ad un processo altrettanto complesso a livello endoteliale, caratterizzato da fibrosi, deposizione di matrice lipidica, proliferazione di cellule muscolari lisce e attivazione del sistema immunitario con modalità estremamente eterogene che hanno come processo finale la formazione della placca sclerotica.

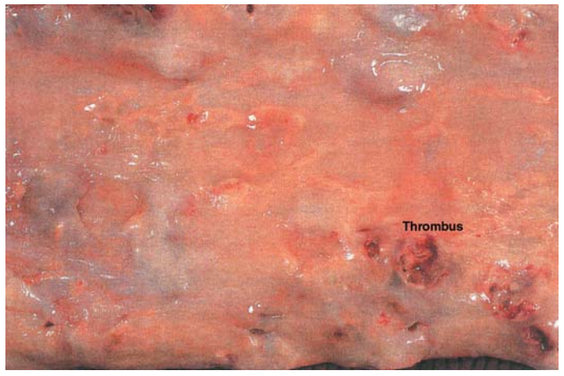

Fig. 1 - PLACCA FIBROLIPIDICA AORTICA:
iniziale formazione della placca e deposizione trombotica.

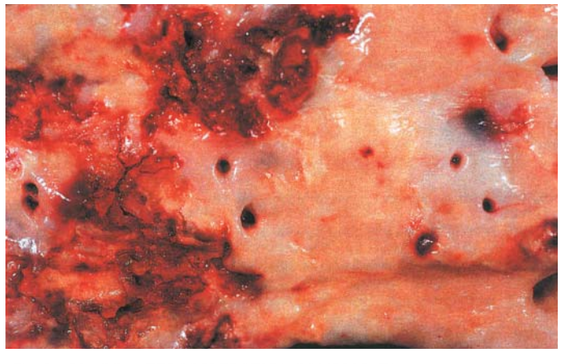
Fig. 2 - PLACCA ATEROSCLEROTICA COMPLICATA:
nelle arterie carotidi questo tipo di placca è associata alla presenza di ischemie transitorie per opera di emboli piastrinici.

Recenti ipotesi nella patogenesi nel processo aterosclerotico

Nel corso dei decenni sono state elaborate teorie per tentare di interpretare i risultati di numerosi studi epidemiologici, clinici e anatomopatologici in riferimento allo sviluppo e all'evoluzione della malattia aterosclerotica.
Tra le diverse ipotesi quella che sembra ricevere più consensi è quella che la interpreta come una reazione ad un danno tissutale (Fig. 2).
Secondo tale teoria, le cellule endoteliali sottoposte ad un continuo carico di sollecitazione a livello dell'intima perderebbero la loro capacità di barriera renderebbero alterata la permeabilità di parete.
La presenza di alcune condizioni come l'iperlipidemia e l'ipertensione renderebbero ancora più marcato il processo di alterazione delle cellule endoteliali, in particolar modo nei punti più vulnerabili come quelli di biforcazione del sistema arterioso determinando così un'esposizione del tessuto endoteliale ai costituenti plasmatici.
Queste condizioni determinerebbero l'incremento dell'adesività piastrinica e monocitica, la migrazione dei monociti stessi nell'intima e la loro attivazione a macrofagi (Fig. 3 - Fig. 4). Tali modificazioni sarebbero in grado di stimolare la proliferazione delle cellule muscolari lisce a livello della lesione localizzata dell'intima. A loro volta, la proliferazione delle cellule muscolari lisce si accompagnerebbe a deposizione di matrice del tessuto connettivo e si accumulerebbero inoltre lipidi (Fig. 5 - Fig. 6), processo che diviene ancora più marcato in caso di preesistente iperlipidemia. Secondo la teoria monoclonale, la proliferazione intimale è caratterizzata dalla moltiplicazione delle cellule muscolari lisce secondo la modalità assimilabile a quelle dei tumori benigni, sotto stimolo di diversi fattori mutageni. La teoria dell'invecchiamento clonale invece sostiene che la proliferazione delle cellule muscolari viene normalmente controllata da un feed-back mediato da inibitori della sintesi che originano e sono sintetizzati dalle cellulare muscolare lisce della media, con l'avvento della vecchiaia, le cellule di controllo normalmente preposte a tale attività muoiono e non sono più in grado di mantenere un adeguato controllo della situazione proliferativa. Infine secondo la teoria lisosomiale, con l'invecchiamento vi sarebbe un' alterazione degli enzimi lisosomiali deputati alla degradazione di prodotti cellulari e ciò determinerebbe un accumulo di esteri di colesterolo a livello delle cellule muscolari lisce della pare arteriosa. A conferma di tale ipotesi, vi è l'osservazione di una precocità e di un'accelerazione nel processo di aterosclerosi nelle persone affette da una rara patologia caratterizzata appunto da un deficit di idrolisi isosomiale che mostra, tra l'altro, accumulo di esteri di colesterolo.

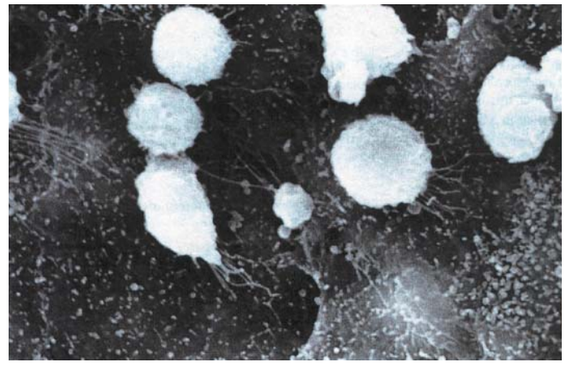
Fig. 3 - ADESIONE E MIGRAZIONE DEI MONOCITI:
espressione morfologica della adesione alle molecole di superficie leganti i monoliti e questi ultimi,fotografati al microscopio elettronico.

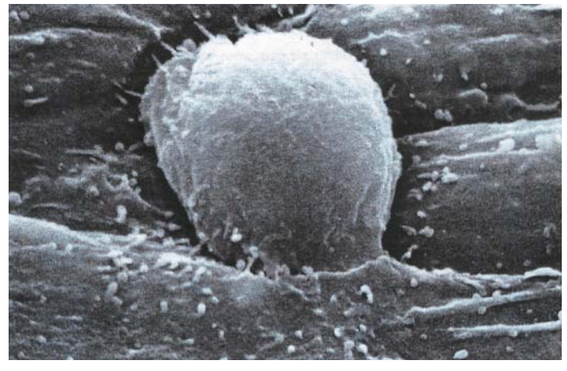
Fig. 4 - MIGRAZIONE DEL MONOCITA:
il monocita aderisce alla superficieendoteliale inserendosi tra due cellule contigue.

Ipotesi infettiva

Sempre più numerosi studi convergono nel mostrare come i meccanismi infiammatori possono essere implicati nell'iniziazione, progressione fino all'espressione clinica del processo aterosclerotico. Tale analogia tra l'aterosclerosi ed un processo infiammatorio cronico[69, 70, 85, 88, 95, 116], ha aperto la strada sull'affascinante ipotesi che tale patologia possa essere sostenuta e modulata da un'infezione acuta o cronica.
I tradizionali fattori di rischio, come fumo, diabete, obesità, ipercolesterolemia, etc., i cambiamenti dello stile di vita per correggerli ed i presidi medico-chirurgici adottati, non permettono infatti di spiegare completamente la presenza della malattia aterosclerotica nelle diverse aree geografiche.
La prospettiva che le comuni infezioni croniche possano essere annoverate tra i fattori di rischio per la malattia aterosclerotica ha aperto negli ultimi anni una nuova area di investigazione clinica. I primi studi per confermare questa affascinante ipotesi condotti su polli infettati da virus appartenenti alla famiglia degli herpesviridiae risalgono già al 1978. Tuttavia solamente nell'ultimo decennio, con il perfezionamento di metodiche efficaci quali PCR, la reverse trascriptase PCR, immunoistochimica, microscopia elettronica, la ricerca in tal senso ha avuto un notevole impulso.
Dagli albori dei primi studi ad oggi, si rilevano più di 500 pubblicazioni che mettono in correlazione la presenza di agenti infettanti, soprattutto la clamydia pnueumoniae (CP) e l'aterosclerosi.
Solo nel 2000 ne sono stati pubblicati circa 300[16, 21, 29, 37, 69, 98], la maggior parte dei quali sono studi siero-epidemiologici nei soggetti affetti da patologia aterosclerotica ed indagati con le metodiche sopracitate. Un numero più piccolo riguarda le indagine effettuate su l'indentificazione della CP su culture di tessuti vascolari (Fig. 7). Gli studi su animali comprendono indagini sia su topi che conigli, in cui si dimostra come l'infezione respiratoria da CP determina

non solo l'identificazione del patogeno nella placca, ma l'accelerazione dello sviluppo di aterosclerosi o l'aggravamento del suo quadro in questi soggetti[35, 95, 123]. Interessanti studi multicentrici sono attualmente in corso per l'analisi delle modificazioni e dell'incidenza degli eventi cardiovascolari acuti e dell'evoluzione della malattia aterosclerotica in pazienti trattati con dei trials antibiotici contro la CP versus placebo.
Tra questi di grande rilevanza sono il WIZARD (Weekli Intervention with zithromax for atherosclerosis and related disease), MARBLE (Might Azithromycin reduce by-pass-list events?) ACES (Azithromycin coronary events study)[45, 63] con trattamento di pazienti in lista d'attesa per by-pass aortocoronarico, o nei pazienti con IMA e angor (STAMINA; The south thames antibiotics in myocardial infarction and angina study).

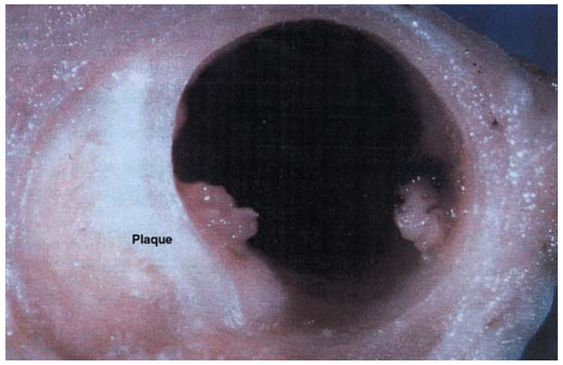

Fig. 5 - Formazione della placca aterosclerotica.

Conclusioni

Il ruolo attuale nello sviluppo ed evoluzione dell'aterosclerosi da parte dei comuni agenti infettivi, primo fra tutti la C.P, appare ancora oggi controverso e irrisolto.
Moltissimi studi mostrano una forte associazione tra presenza di questo organismo e l'aterosclerosi, tuttavia non è dimostrato che il particolare tropismo di questo agente per le placche aterosclerotiche possa essere responsabile dell'incremento ed accelerazione di tale patologia attraverso meccanismi infiammatori e immunomediati, così come il potere anti-infiammatorio che i macrolidi posseggono non permette di concludere che la riduzione dello stato infiammatorio e della riduzione della placca siano dovuti alla scomparsa del microrganismo dopo tale terapia. Inoltre la mancanza di standardizzazione delle metodiche complesse come la PCR etc. non permette di attuare risultati sempre ripetibili e verificabili. Concludendo, sebbene sia una ipotesi alquanto affascinante e diversi studi siano incoraggianti, l'ipotesi di un vaccino per l'aterosclerosi appare tuttora fantasiosa.

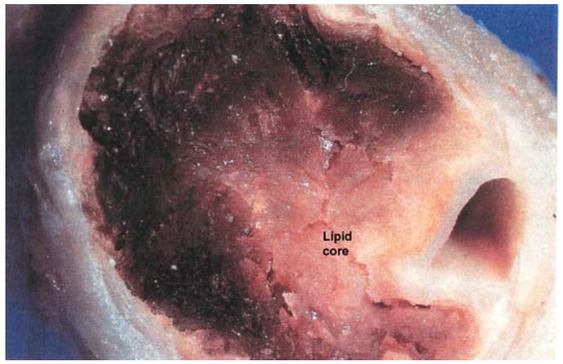

Fig. 6 - Evoluzione fino ad occlusione del vaso per opera della placca aterosclerotica.

Bibliografia

1. *Altman, R., J. Rouvier, A. Scazziota, R. S. Absi, and C. Gonzalez. 1999. Lack of association between prior infection with Chlamydia pneumoniae and acute or chronic coronary artery disease. Clin. Cardiol. 22:85-90.*
2. *Anderson, J. L., J. F. Carlquist, J. B. Muhlestein, B. D. Horne, and S. P. Elmer. 1998. Evaluation of C-reactive protein, and inflammatory marker, and infectious serology as risk factors for coronary artery disease and myocardial infarction. J. Am. Coll. Cardiol. 32:35-41.*
3. *Andreasen, J. J., S. Farholt, and J. S. Jensen. 1998. Failure to detect Chlamydia pneumoniae in calcific and degenerative arteriosclerotic aortic valves excised during open heart surgery. APMIS 106:717-720.*
4. *Apfalter, P., M. Loidl, R. Nadrchal, A. Makristathis, M. Rotter, M. Bergmann, P. Polterauer, and A. M. Hirschl. 2000. Isolation and continuous growth of Chlamydia pneumoniae from arterectomy specimens. Eur. J. Clin. Microbiol. Infect. Dis. 19:305-308.*
5. *Apfalter, P., F. Blasi, J. Boman, C. A. Gaydos, M. Kundi, M. Maass, A. Makristathis, A. Meijer, R. Nadrchal, K. Persson, M. L. Rotter. C. Y. W. Tong, G. Stanek, and A. M. Hirschl. 2001. Multicenter comparison trial of DNA extraction methods and PCR assays for detection of Chlamydia pneumoniae in endarterectomy specimens. J. Clin. Microbiol. 39:519-524.*
6. *Bartels, C., M. Maass, G. Bein, R. Malisius, N. Brill, J. F. Bechtel, F. Sayk, A. C. Feller, and H. H. Sievers. 1999. Detection of Chlamydia pneumoniae but not cytomegalovirus in occluded saphenous vein coronary artery bypass grafts. Circulation 99:879-882.*
7. *Bartels, C., M. Maass, G. Bein, N. Brill, J. F. Bechtel, R. Leyh, and H. H. Sievers. 2000. Association of serology with the endovascular presence of Chlamydia pneumoniae and cytomegalovirus in coronary artery and vein graft disease. Circulation 101:137-141.*
8. *Berger, M., B. Schroder, G. Daeschlein, W. Schneider, A. Busjahn, I. Buchwalow, F. C. Luft, and H. Haller. 2000. Chlamydia pneumoniae DNA in non-coronary atherosclerotic plaques and circulating leukocytes. J. Lab. Clin. Med. 136:194-200.*
9. *Blasi, F., F. Denti, M. Erba, R. Cosentini, R. Raccanelli, A. Rinaldi, L. Fagetti, G. Esposito, U. Ruberti, and L. Allegra. 1996. Detection of Chlamydia pneumoniae but not Helicobacter pylori in atherosclerotic plaques of aortic aneurysms. J. Clin. Microbiol. 34:2766-2769.*
10. *Blasi, F., J. Boman, G. Esposito, G. Melissano, R. Chiesa, R. Cosentini, P. Tarsia, Y. Tshomba, M. Betti, M. Alessi, N. Morelli, and L. Allegra. 1999. Chlamydia pneumoniae DNA detection in peripheral blood mononuclear cells is predictive of vascular infection. J. Infect. Dis. 180:2074-2076.*
11. *Block, S., J. Hedrick, M. R. Hammerschlag, G. H. Cassell, and C. Craft. 1995. Mycoplasma pneumoniae and Chlamydia pneumoniae in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. Pediatr. Infect. Dis. J. 14:471-477.*
12. *Bodetti, T. J., and P. Timms. 2000. Detection of Chlamydia pneumoniae DNA and antigen in the circulating mononuclear cell fractions of humans and koalas. Infect. Immun. 68:2744-2747.*
13. *Boman, J., S. Soderberg, J. Forsberg, L. S. Birgander, A. Allard, K. Persson, E. Jidell, U. Kumlin, P. Juto, A. Waldenstrom, and G. Wadell. 1998. High prevalence of Chlamydia pneumoniae DNA in peripheral blood mononuclear cells in patients with cardiovascular disease and in middleaged blood donors. J. Infect. Dis. 178:274-277.*
14. *Boman, J., C. A. Gaydos, and T. C. Quinn. 1999. Molecular diagnosis of Chlamydia pneumoniae infection. J. Clin. Microbiol. 37:3791-3799.*
15. *Boman, J., and C. A. Gaydos. 2000. Polymerase chain reaction detection of Chlamydia pneumoniae in circulating white blood cells. J. Infect. Dis. 181(Suppl. 3):S452-S454.*
16. *Camm, A. J., and K. M. Fox. 2000. Chlamydia pneumoniae (and other infective agents) in atherosclerosis and acute coronary syndromes. How good is the evidence? Eur. Heart J. 21:1046-1051.*
17. *Campbell, L. A., E. R. O'Brien, A. L. Cappuccio, C. C, Kuo, S. P. Wang, D. Stewart, D. L. Patton, P. K. Cummings, and J. T. Grayston. 1995. Detection of Chlamydia pneumoniae TWAR in human coronary atherectomy tissues. J. Infect. Dis. 172:585-588.*
18. *Carter M. W., S. A. H. Al-Mahdawi, I. G. Giles, J. D. Treharne, M. E. Ward, and I. N. Clark. 1991. Nucelotide sequence and taxonomic value of the major outer membrane protein gene of Chlamydia pneumoniae IOL-207. J. Gen. Bacteriol. 137:465-475.*
19. *Chiu, B., E. Viira, W. Tucker, and I. W. Fong. 1997. Chlamydia pneumoniae, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. Circulation 96:2144-2148.*
20. *Choussat, R., G. Montalescot, J. Collet, C. Jardel, A. Ankri, A. Fillet, D. Thomas, J. Raymond, J. Bastard, G. Drobinski, J. Orfila, H. Agut, and D. Thomas. 2000. Effect of prior exposure to Chlamydia pneumoniae, Helicobacter pylori, or cytomegalovirus on the degree of inflammation and oneyear prognosis of patients with unstable angina pectoris or non-q-wave acute myocardial infarction. Am. J. Cardiol. 86:379-384.*
21. *Danesh, J., R. Collins, and R. Peto. 1997. Chronic infections and coronary heart disease: is there a link? Lancet 350:430-436.*
22. *Danesh, J., Y. Wong, M. Ward, and J. Muir. 1999. Chronic infection with Helicobacter pylori, Chlamydia pneumoniae, or cytomegalovirus: population based study of coronary heart disease. Heart 81:245-247.*
23. *Danesh, J., P. Whincup, M. Walker, L. Lennon, A. Thomson, P. Appleby, Y. Wong, M. Bernardes-Silva, and M. Ward. 2000. Chlamydia pneumoniae IgG titres and coronary heart disease: prospective study and meta-analysis. Br. Med. J. 321:208-213.*
24. *Danesh, J., P. Whincup, M. Walker, L. Lennon, A. Thomson, P. Appleby, J. R. Gallimore, and M. B. Pepys. 2000. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Br. Med. J. 321:199-204.*
25. *Davidson, M., C. C. Kuo, J. P. Middaugh, L. A. Campbell, S. P. Wang, W. P. Newman III, J. C. Finley, and J. T. Grayston. 1998. Confirmed previous infection with Chlamydia pneumoniae (TWAR) and its presence in early coronary atherosclerosis. Circulation 98:628-633.*
26. *Dean, D., P. Roblin, L. Mandel, J. Schachter and M. Hammerschlag. 1998. Molecular evaluation of serial isolates from patients with persistent Chlamydia pneumoniae infections, p. 219-222. In R. S. Stephens, G. I. Byrne, G. Christiansen, I. N. Clarke, J. T. Grayston, R. G. Rank, G. L. Ridgway, P. Saikku, J. Schachter, and W. E. Stamm (ed). Chlamydial infections. Proceedings of the Ninth International Symposium on Human Chlamydia Infections. International Chlamydia Symposium, San Francisco, Calif.*
27. *Dowell, S. F., J. Boman, G. M. Carlone, B. S. Fields, J. Guarner, M. R. Hammerschlag, L. A. Jackson, C. C. Kuo, M. Maass, T. O. Messmer, R. W. Peeling, D. Talkington, M. L. Tondella, S. R. Zaki, and the C. pneumoniae Workshop participants. 2001. Standardizing Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA), and the Laboratory Centre for Disease Control (Canada). Clin. Infect. Dis. 33:492-503.*
28. *Dunne, M. W. 2000. Rationale and design of a secondary prevention trial of antibiotic use in patients after myocardial infarction: the WIZARD (weekly intervention with zithromax [azithromycin] for atherosclerosis and its related disorders) trial. J. Infect. Dis. 181(Suppl. 3):S572-S578.*
29. *Ellis, R. W. 1997. Infection and coronary heart disease. J. Med. Microbiol. 46:535-539.*
30. *Ericson, K., T. G. Saldeen, O. Lindquist, C. Pahlson, and J. L. Mehta. 2000. Relationship of Chlamydia pneumoniae infection to severity of human coronary atherosclerosis. Circulation 101:2568-2571.*
31. *Espinola-Klein, C., H. J. Rupprecht, S. Blankenberg, C. Bickel, H. Kopp, G. Rippin, G. Hafner, U. Pfeifer, and J. Meyer. 2000. Are morphological or functional changes in the carotid artery wall associated with Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, or herpes simplex virus infection? Stroke 31:2127-2133.*
32. *Esposito, G., F. Blasi, L. Allegra, R. Chiesa, G. Melissano, R. Cosentini, P. Tarsia, L. Dordoni, C. Cantoni, C. Arosio, and L. Fagetti. 1999. Demonstration of viable Chlamydia pneumoniae in atherosclerotic plaques of carotid arteries by reverse transcriptase polymerase chain reaction. Ann. Vasc. Surg. 13:421-425.*
33. *Farsak, B., A. Yildirir, Y. Akyon, A. Pinar, M. Oc, E. Boke, S. Kes, and L. Tokgozoglu. 2000. Detection of Chlamydia pneumoniae and Helicobacter pylori DNA in human atherosclerotic plaques by PCR. J. Clin. Microbiol. 38:4408-4411.*
34. *File, T. M., J. Segreti, L. Dunbar, R. Player, R. Kohler, R. R. Williams, C. Kojak, and A. Rubin. 1997. A multicenter, randomized study comparing the efficacy and safety of intravenous and/or oral levofloxacin versus ceftriaxone and/or cefuroxime axetil in the treatment of adults with community-acquired pneumonia. Antimicrob. Agents Chemother. 41:1965-1972.*
35. *Fong, I. W., B. Chiu, E. Viira, D. Jang, M. W. Fong, R. Peeling, and J. B.*

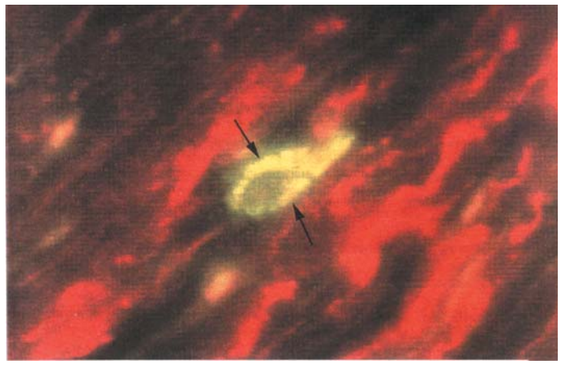
Fig. 7 - CHLAMYDIA PNEUMONIAE IN UNA PLACCA ATEROSCLEROTICA UMANA: visualizzazione mediante anticorpi fluorescenti contro la capsula polisaccaridica della chlamydia.

Mahony. 1999. Can an antibiotic (macrolide) prevent Chlamydia pneumoniae-induced atherosclerosis in a rabbit model? Clin. Diagn. Lab Immunol. 6:891-894.

36. *Gay, K., W. Baughman, Y. Miller, D. Jackson, C. G. Whitney, A. Schuchat, M. M. Farley, F. Tenover, and D. S. Stephens. 2000. The emergence of Streptococcus pneumoniae resistant to macrolide antimicrobial agents: a 6-year population-based assessment. J. Infect. Dis. 182:1417-1424.*
37. *Gaydos, C. A., and T. C. Quinn. 2000. The role of Chlamydia pneumoniae in cardiovascular disease. Adv. Intern. Med. 45:139-173.*
38. *Gibbs, R. G., M. Sian, A. W. Mitchell, R. M. Greenhalgh, A. H. Davies, and N. Carey. 2000. Chlamydia pneumoniae does not influence atherosclerotic plaque behavior in patients with established carotid artery stenosis. Stroke 31:2930-2935.*
39. *Gieffers, J., W. Solbach, and M. Maass. 1998. In vitro susceptibilities of Chlamydia pneumoniae strains recovered from atherosclerotic coronary arteries. Antimicrob. Agents Chemother. 42:2762-2764.*
40. *Gieffers, J., H. Fu¨llgraf, J. Jahn, M. Klinger, K. Dalhoff, H. A. Katus, W. Solbach, and M. Maass. 2001. Chlamydia pneumoniae infection in circulating human monocytes is refractory to antibiotic treatment. Circulation 103:351-356.*
41. *Glader, C. A., J. Boman, P. Saikku, H. Stenlund, L. Weinehall, G. Hallmanns, and G. H. Dahlen. 2000. The proatherogenic properties of lipoprotein (a) may be enhanced through the formation of circulating immune complexes containing Chlamydia pneumoniae-specific IgG antibodies. Eur. Heart J. 21:639-646.*
42. *Grayston J. T., L. A. Campbell, C. C. Kuo, C. H. Mordhorst, P. Saikku, D. H. Thom, and S. P. Wang. 1990. A new respiratory tract pathogen: Chlamydia pneumoniae strain, TWAR. J. Infect. Dis. 161:618-625.*
43. *Grayston, J. T., C. C. Kuo, A. S. Coulson, L. A. Campbell, R. D. Lawrence, M. J. Lee, E. D. Strandness, and S. P. Wang. 1995. Chlamydia pneumoniae (TWAR) in atherosclerosis of the carotid artery. Circulation 92:3397-3400.*
44. *Gupta, S., E. W. Leatham, D. Carrington, M. A. Mendall, J. C. Kaski, and A. J. Camm. 1997. Elevated Chlamydia pneumoniae antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. Circulation 96:404-407.*
45. *Gupta, S. 1999. Chlamydia pneumoniae, monocyte activation, and azithromycin in coronary heart disease. Am. Heart J. 138:S539-S541.*
46. *Gurfinkel, E., G. Bozovich, A. Daroca, E. Beck, and B. Mautner. 1997. Randomised trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS Pilot Study. ROXIS Study Group. Lancet 350:404-407.*
47. *Gurfinkel, E., G. Bozovich, E. Beck, E. Testa, B. Livellara, and B. Mautner. 1999. Treatment with the antibiotic roxithromycin in patients with acute non-Q-wave coronary syndromes. The final report of the ROXIS Study. Eur. Heart J. 20:121-127.*
48. *Hahn, D. L., R. W. Dodge, and R. Golubjatnikov. 1991. Association of Chlamydia pneumoniae (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA 266:225-230.*
49. *Hahn, D. L., and R. McDonald. 1998. Can acute Chlamydia pneumoniae respiratory tract infection initiate chronic asthma? Ann. Allergy Asthma Immunol. 81:339-344.*
50. *Hammerschlag, M. R., and A. Gleyzer. 1983. The in vitro activity of a group of broad spectrum cephalosporins and other beta-lactam antibiotics against Chlamydia trachomatis. Antimicrob. Agents Chemother. 23:492-493.*
51. *Hammerschlag M. R., K. Chirgwin, P. M. Roblin, M. Gelling, W. Dumornay, L. Mandel, P. Smith, and J. Schachter. 1992. Persistent infection with Chlamydia pneumoniae following acute respiratory illness. Clin. Infect. Dis. 14:178-182.*
52. *Hammerschlag, M. R., and P. M. Roblin. 2000. Microbiologic efficacy of moxifloxacin for the treatment of community-acquired pneumonia due to Chlamydia pneumoniae. Int. J. Antimicrob. Agents 15:149-152.*
53. *Hammerschlag, M. R., and P. M. Roblin. 2000. Microbiological efficacy of levofloxacin for treatment of community-acquired pneumonia due to Chlamydia pneumoniae. Antimicrob. Agents Chemother. 44:1409.*
54. *Hammerschlag. M. R. 2000 Activity of gemifloxacin and other new quinolones against Chlamydia pneumoniae: a review. J. Antimicrob. Chemother. 45(Suppl. 1):35-39.*
55. *Harkonen, T., M. Puolakkainen, M. Sarvas, U. Airaksinen, T. Hovi, and M. Roivainen. 2000. Picornavirus proteins share antigenic determinants with heat shock proteins 60/65. J. Med. Virol. 62:383-391.*
56. *Harris, J.-A., A. Kolokathis, M. Campbell, G. H. Cassell, and M. R. Hammerschlag. 1998. Safety and efficacy of azithromycin in the treatment of community acquired pneumonia in children. Pediatr. Infect. Dis. J. 17:865-871.*
57. *Hyman, C. L., P. M. Roblin, C. A. Gaydos, T. C. Quinn, J. Schachter, and M. R. Hammerschlag. 1995. Prevalence of asymptomatic nasopharyngeal carriage of Chlamydia pneumoniae in subjectively healthy adults: assessment by polymerase chain reaction-enzyme immunoassay and culture. Clin. Infect. Dis. 20:1174-1178.*
58. *Jackson, L. A., L. A. Campbell, R. A. Schmidt, C. C. Kuo, A. L. Cappuccio, M. J. Lee, and J. T. Grayston. 1997. Specificity of detection of Chlamydia pneumoniae in cardiovascular atheroma: evaluation of the innocent bystander hypothesis. Am. J. Pathol. 150:1785-1790.*
59. *Jackson, L. A., L. A. Campbell, C. C. Kuo, D. I. Rodriguez, A. Lee, and J. T. Grayston. 1997. Isolation of Chlamydia pneumoniae from a carotid endarterectomy specimen. J. Infect. Dis. 176:292-295.*
60. *Jackson, L. A., N. L. Smith, S. R. Heckbert, J. T. Grayston, D. S. Siscovick, and B. M. Psaty. 1999. Lack of association between first myocardial infarction and past use of erythromycin, tetracycline, or doxycycline. Emerg. Infect. Dis. 5:281-284.*
61. *Jackson, L. A., D. K. Stewart, S. P. Wang, D. B. Cooke, T. Cantrell, and J. T. Grayston. 1999. Safety and effect on anti-Chlamydia pneumoniae antibody titres of a 1 month course of daily azithromycin in adults with coronary artery disease. J. Antimicrob. Chemother. 44:411-414.*
62. *Jackson, L. A., J. D. Cherry, S. P. Wang, and J. T. Grayston. 2000. Frequency of serological evidence of Bordetella infections and mixed infections with other respiratory pathogens in university students with cough illnesses. Clin. Infect. Dis. 31:3-6.*
63. *Jackson, L. A. 2000. Description and status of the azithromycin and coronary events study (ACES). J. Infect. Dis. 181(Suppl. 3):S579-S581.*
64. *Jantos, C. A., A. Nesseler, W. Waas, W. Baumgartner, H. Tillmanns, and W. Haberbosch. 1999. Low prevalence of Chlamydia pneumoniae in atherectomy specimens from patients with coronary heart disease. Clin. Infect. Dis. 28:988-992.*

64a. *Jauhiainen, T., T. Tuomi, M. Leinonen, J. D. Kark, and P. Saikku. 1994. Interference of immunoglobulin G (IgG) antibodies in IgA determinations of Chlamydia pneumoniae by microimmunofluorescence test. J. Clin. Microbiol. 32:839-840.*

65. *Juvonen, J., T. Juvonen, A. Laurila, H. Alakarppa, K. Lounatmaa, H. M. Surcel, M. Leinonen, M. I. Kairaluoma, and P. Saikku. 1997. Demonstration of Chlamydia pneumoniae in the walls of abdominal aortic aneurysms. J. Vasc. Surg. 25:499-505.*
66. *Juvonen, J., A. Laurila, T. Juvonen, H. Alakarppa, H. M. Surcel, K. Lounatmaa, J. Kuusisto, and P. Saikku. 1997. Detection of Chlamydia pneumoniae in human nonrheumatic stenotic aortic valves. J. Am. Coll. Cardiol. 29:1054-1059.*
67. *Kaul, R., J. Uphoff, J. Wiedeman, S. Yadlapalli, and W. M. Wenman. 2000. Detection of Chlamydia pneumoniae DNA in CD3 lymphocytes from healthy blood donors and patients with coronary artery disease. Circulation 102:2341-2346.*
68. *Kern, D. G, M. A. Neill, and J. Schachter. 1993. A seroepidemiologic study of Chlamydia pneumoniae in Rhode Island. Evidence of serologic crossreactivity. Chest 104:208-213.*
69. *Kol, A., and P. Libby. 1998. The mechanisms by which infectious agents may contribute to atherosclerosis and its clinical manifestations. Trends Cardiovasc. Med. 8:191-199.*
70. *Kuller, L. H., R. P. Tracy, J. Sjaten, and E. N. Meilahn. 1996. Relation of C-reactive protein and coronary heart disease in the MRFIT nested casecontrol study. Am. J. Epidemiol. 144:537-547.*
71. *Kuo, C. C., A. Shor, L. A. Campbell, H. Fukushi, D. L. Patton, and J. T. Grayston. 1993. Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries. J. Infect. Dis. 167:841-849.*
72. *Kuo, C. C., A. M. Gown, E. P. Benditt, and J. T. Grayston. 1993. Detection of Chlamydia pneumoniae in aortic lesions of atherosclerosis by immunocytochemical stain. Arterioscler. Thromb. 13:1501-1504.*
73. *Kuo, C. C., J. T. Grayston, L. A. Campbell, Y. A. Goo, R. W. Wissler, and E. P. Benditt. 1995. Chlamydia pneumoniae (TWAR) in coronary arteries of young adults (15-34 years old). Proc. Natl. Acad. Sci. USA 92:6911-6914.*
74. *Kuo, C. C., A. S. Coulson, L. A. Campbell, A. L. Cappuccio, R. D. Lawrence, S. P. Wang, and J. T. Grayston. 1997. Detection of Chlamydia pneumoniae in atherosclerotic plaques in the walls of arteries of lower extremities from patients undergoing bypass operation for arterial obstruction. J. Vasc. Surg. 26:29-31.*
75. *Kutlin, A., N. Tsumura, U. Emre, P. M. Roblin, and M. R. Hammerschlag. 1997. Evaluation of Chlamydia immunoglobulin M (IgM), IgG, and IgA rELISAs Medac for diagnosis of Chlamydia pneumoniae infection. Clin. Diagn. Lab. Immunol. 4:213-216.*
76. *Kutlin, A., P. M. Roblin, and M. R. Hammerschlag. 1998. Antibody response to Chlamydia pneumoniae infection in children with respiratory illness. J. Infect. Dis. 177:720-724.*
77. *Kutlin, A., P. M. Roblin, and M. R. Hammerschlag. 1999. In vitro activities of azithromycin and ofloxacin against Chlamydia pneumoniae in a continuous-infection model. Antimicrob. Agents Chemother. 43:2268-2272.*
78. *Kutlin, A., C. Flegg, D. Stenzel, T. Reznik, P. M. Roblin, S. Mathews, P. Timms, and M. R. Hammerschlag. 2001. Ultrastructural study of Chlamydia pneumoniae in a continuous infection model. J. Clin. Microbiol. 39:3721-3723.*
79. *Kutlin, A., P. M. Roblin, and M. R. Hammerschlag. 2000. Effect of Azithromycin and clarithromycin on Chlamydia pneumoniae in a continuous infection model, p. 180. In P. Saikku (ed.), Proceedings of the Fourth Meeting of the European Society for Chlamydia Research. Universitas Helsingiensis, Helsinki, Finland.*
80. *Lindholt, J. S., L. Ostergard, E. W. Henneberg, H. Fasting, and P. Andersen. 1998. Failure to demonstrate Chlamydia pneumoniae in symptomatic abdominal aortic aneurysms by a nested polymerase chain reaction (PCR). Eur. J. Vasc. Endovasc. Surg. 15:161-164.*
81. *Lipsky B. A., K. J. Tack, C. C. Kuo, S. P. Wang, and J. T. Grayston. 1990. Ofloxacin treatment of Chlamydia pneumoniae (strain TWAR) lower respiratory tract infections. Am. J. Med. 89:722-724.*
82. *Maass, M., E. Krause, P. M. Engel, and S. Kruger. 1997. Endovascular presence of Chlamydia pneumoniae in patients with hemodynamically effective carotid artery stenosis. Angiology 48:699-706.*
83. *Maass, M., C. Bartels, P. M. Engel, U. Mamat, and H. H. Sievers. 1998. Endovascular presence of viable Chlamydia pneumoniae is a common phenomenon in coronary artery disease. J. Am. Coll. Cardiol. 31:827-832.*
84. *Maass, M., J. Gieffers, E. Krause, P. M. Engel, C. Bartels, and W. Solbach. 1998. Poor correlation between microimmunofluorescence serology and polymerase chain reaction for detection of vascular Chlamydia pneumoniae infection in coronary artery disease patients. Med. Microbiol. Immunol. 187:103-106.*
85. *Maass, M., C. Bartels, S. Kruger, E. Krause, P. M. Engel, and K. Dalhoff. 1998. Endovascular presence of Chlamydia pneumoniae DNA is a generalized phenomenon in atherosclerotic vascular disease. Atherosclerosis 140(Suppl 1):S25-S30.*
86. *Maass, M., J. Jahn, J. Gieffers, K. Dalhoff, H. A. Katus, and W. Solbach. 2000. Detection of Chlamydia pneumoniae within peripheral blood monocytes of patients with unstable angina or myocardial infarction. J. Infect. Dis. 181(Suppl. 3):S449-S451.*
87. *Maurin, M., F. Eb, J. Etienne, and D. Raoult. 1997. Serologic cross-reactions between Bartonella and Chlamydia species: implications for diagnosis. J. Clin. Microbiol. 35:2283-2287.*
88. *Mehta, J. L., T. G. Saldeen, and K. Rand. 1998. Interactive role of infection, inflammation and traditional risk factors in atherosclerosis and coronary artery disease. J. Am. Coll. Cardiol. 31:1217-1225.*
89. *Meier, C. R., L. E. Derby, S. S. Jick, C. Vasilakis, and H. Jick. 1999. Antibiotics and risk of subsequent first-time acute myocardial infarction. JAMA 281:427-431.*
90. *Meier, C. R. 2000. Antibiotics in the prevention and treatment of coronary heart disease. J. Infect. Dis. 181(Suppl. 3):S558-S562.*
91. *Melissano, G., F. Blasi, G. Esposito, P. Tarsia, L. Dordoni, C. Arosio, Y. Tshomba, L. Fagetti, L. Allegra, and R. Chiesa. 1999. Chlamydia pneumoniae eradication from carotid plaques. Results of an open, randomised treatment study. Eur. J. Vasc. Endovasc. Surg. 18:355-359.*
92. *Melnick, S. L., E. Shahar, A. R. Folsom, J. T. Grayston, P. D. Sorlie, S. P. Wang, and M. Szklo. 1993. Past infection by Chlamydia pneumoniae strain TWAR and asymptomatic carotid atherosclerosis. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am. J. Med. 95:499-504.*
93. *Miettinen, H., S. Lehto, P. Saikku, S. M. Haffner, T. Ronnemaa, K. Pyorala, and M. Laakso. 1996. Association of Chlamydia pneumoniae and acute coronary heart disease events in non-insulin dependent diabetic and nondiabetic subjects in Finland. Eur. Heart J. 17:682-688.*
94. *Muhlestein, J. B., E. H. Hammond, J. F. Carlquist, E. Radicke, M. J. Thomson, L. A. Karagounis, M. L. Woods, and J. L. Anderson. 1996. Increased incidence of Chlamydia species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. J. Am. Coll. Cardiol. 27:1555-1561.*
95. *Muhlestein, J. B., J. L. Anderson, E. H. Hammond, L. Zhao, S. Trehan, E. P. Schwobe, and J. F. Carlquist. 1998. Infection with Chlamydia pneumoniae accelerates the development of atherosclerosis and treatment with azithromycin prevents it in a rabbit model. Circulation 97:633-636.*
96. *Muhlestein, J. B., J. L. Anderson, J. F. Carlquist, K. Salunkhe, B. D. Horne, R. R. Pearson, T. J. Bunch, A. Allen, S. Trehan, and C. Nielson. 2000. Randomized secondary prevention trial of azithromycin in patients with coronary artery disease: primary clinical results of the ACADEMIC study. Circulation 102:1755-1760.*
97. *Nadrchal, R., A. Makristathis, P. Apfalter, M. Rotter, W. Trubel, I. Huk, P. Polterauer, and A. M. Hirschl. 1999. Detection of Chlamydia pneumoniae DNA in atheromatous tissues by polymerase chain reaction. Wien Klin. Wochenschr. 111:153-156.*
98. *Nieto, F. J. 1998. Infections and atherosclerosis: new clues from an old hypothesis. Am. J. Epidemiol. 148:937-948.*
99. *Nieto, F. J., A. R. Folsom, P. D. Sorlie, J. T. Grayston, S. P. Wang, and L. E. Chambless. 1999. Chlamydia pneumoniae infection and incident coronary heart disease: the Atherosclerosis Risk in Communities Study. Am. J. Epidemiol. 150:149-156.*

100. *Nystro¨m-Rosander, C., S. Thelin, E. Hjelm, O. Lindquist, C. Pahlson, and G. Friman. 1997. High incidence of Chlamydia pneumoniae in sclerotic heart valves of patients undergoing aortic valve replacement. Scand. J. Infect. Dis. 29:361-365.*

101. *Ochiai, Y., H. Fukushi, C. Yan, T. Yamaguchi, and K. Hirai. 2000. Comparative analysis of the putative amino acid sequences of chlamydial heat shock protein 60 and Escherichia coli GroEL. J. Vet. Med. Sci. 62:941-945.*

102. *Ong, G., B. J. Thomas, A. O. Mansfield, B. R. Davidson, and D. Taylor-Robinson. 1996. Detection and widespread distribution of Chlamydia pneumoniae in the vascular system and its possible implications. J. Clin. Pathol. 49:102-106.*

102a. *Osler, W. 1908. Diseases of the arteries, p. 426-447. In W. Osler and T. MacCrae (ed.), Modern medicine: its theory and practice in original contributions by American and foreign authors. Lea & Febiger, Philadelphia, Pa.*

103. *Ossewaarde, J. M., E. J. Feskens, A. de Vries, C. E. Vallinga, and D. Kromhout. 1998. Chlamydia pneumoniae is a risk factor for coronary heart disease in symptom-free elderly men, but Helicobacter pylori and cytomegalovirus are not Epidemiol. Infect. 120:93-99.*

104. *Ouchi, K., B. Fujii, Y. Kanamoto, M. Karita, M. Shirai, and T. Nakazawa. 1998. Chlamydia pneumoniae in coronary and iliac arteries of Japanese patients with atherosclerotic cardiovascular diseases. J. Med. Microbiol. 47:907-913.*

105. *Ouchi, K., B. Fujii, S. Kudo, M. Shirai, K. Yamashita, T. Gondo, T. Ishihara, H. Ito, and T. Nakazawa. 2000. Chlamydia pneumoniae in atherosclerotic and nonatherosclerotic tissue. J. Infect. Dis. 181(Suppl. 3):S441-S443.*

106. *Ozanne, G., and J. Lefebvre. 1992. Specificity of the microimmunofluorescence assay for the serodiagnosis of Chlamydia pneumoniae infections. Can. J. Microbiol. 38:1185-1189.*

107. *Paterson, D. L., J. Hall, S. J. Rasmussen, and P. Timms. 1998. Failure to detect Chlamydia pneumoniae in atherosclerotic plaques of Australian patients. Pathology 30:169-172.*

108. *Peeling, R. W., S. P. Wang, J. T. Grayston, F. Blasi, J. Boman, A. Clad, H. Freidank, C. A. Gaydos, J. Gnarpe, T. Hagiwara, R. B. Jones, J. Orfila, K. Persson, M. Puolakkainen, P. Saikku, and J. Schachter. 2000. Chlamydia pneumoniae serology: interlaboratory variation in microimmunofluorescence assay results. J. Infect. Dis. 181(Suppl. 3):S426-S429.*

109. *Peeling R. W., S. P. Wang, J. T. Grayston, B. Blasi, J. Boman, J. Deak, H. Freidank, C. Gaydos, T. Hagiwara, T. Harrison, R. B. Jones, A. Kutlin, T. Messmer, J. Orfila, K. Persson, E. Peterson, M. Poulakkainen, G. Ridgway, P. Saikku, J. Schachter, and W. E. Stamm. 2000. Inter-laboratory comparison of microimmuno-fluorescence results, p. 130. In P. Saikku (ed.), Proceedings of the Fourth Meeting of the European Society for Chlamydia Research. Universitas Helsingiensis, Helsinki, Finland.*

110. *Persson, K., and S. Haidl. 2000. Evaluation of a commercial test for antibodies to the chlamydial lipopolysaccharide (Medac) for serodiagnosis of acute infections by Chlamydia pneumoniae (TWAR) and Chlamydia psittaci. APMIS 108:131-138.*

111. *Persson, K., and J. Boman. 2000. Comparison of five serologic tests for diagnosis of acute infections by Chlamydia pneumoniae. Clin. Diagn. Lab. Immunol. 7:739-744.*

112. *Petersen, E., J. Boman, K. Persson, C. Arnerlov, G. Wadell, P. Juto, A. Eriksson, G. Dahlen, and K. A. A¨ngquist. 1998. Chlamydia pneumoniae in human abdominal aortic aneurysms. Eur. J. Vasc. Endovasc. Surg. 15:138-142.*

113. *Ramirez, J. A., and the Chlamydia pneumoniae/Atherosclerosis Study Group. 1996. Isolation of Chlamydia pneumoniae from the coronary artery of a patient with coronary atherosclerosis. Ann. Intern. Med. 125:979-982.*

114. *Ridgway, G. L. 1993. Quinolones in sexually transmitted diseases. Drugs 45(Suppl. 3):135-138.*

115. *Ridker, P. M., M. Cushman, M. J. Stampfer, R. P. Tracy, and C. H. Hennekens. 1997. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N. Engl. J. Med. 336:973-979.*

116. *Ridker, P. M. 1998. Inflammation, infection, and cardiovascular risk. How good is the clinical evidence? Circulation 97:1671-1674.*

117. *Ridker, P. M., R. B. Kundsin, M. J. Stampfer, S. Poulin, and C. H. Hennekens. 1999. Prospective study of Chlamydia pneumoniae IgG seropositivity and risks of future myocardial infarction. Circulation 99:1161-1164.*

118. *Ridker, P. M., C. H. Hennekens, J. E. Buring, R. Kundsin, and J. Shih. 1999. Baseline IgG antibody titers to Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus, and cytomegalovirus and the risk for cardiovascular disease in women. Ann. Intern. Med. 131:573-577.*

119. *Roblin, P. M., G. Montalban, and M. R. Hammerschlag. 1994. Susceptibility to clarithromycin and erythromycin of isolates of Chlamydia pneumoniae from children with pneumonia. Antimicrob. Agents Chemother. 38:1588-1589.*

120. *Roblin, P. M., and M. R. Hammerschlag. 1998. Microbiologic efficacy of azithromycin and susceptibilities to azithromycin of isolates of Chlamydia pneumoniae from adults and children with community-acquired pneumonia. Antimicrob. Agents Chemother. 42:194-196.*

121. *Roblin, P. M., and M. R. Hammerschlag. 1998. In vitro activity of a new ketolide antibiotic, HMR 3647, against Chlamydia pneumoniae. Antimicrob. Agents Chemother. 42:1515-1516.*

122. *Roivainen, M., G. Alfthan, P. Jousilahti, M. Kimpima¨ki, T. Hovi, J. Tuomilehto. 1998. Enterovirus infections as a possible risk factor for myocardial infection. Circulation 98:2534-2537.*

123. *Rothstein, N. M., T. C. Quinn, G. Madico, C. A. Gaydos, and C. J. Lowenstein. 2001. Effect of azithromycin on murine arteriosclerosis exacerbated by Chlamydia pneumoniae. J. Infect. Dis. 183:232-238.*

124. *Saikku, P., M. Leinonen, K. Mattila, M. R. Ekman, M. S. Nieminen, P. H. Makela, J. K. Huttunen, and V. Valtonen. 1998. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet ii:983-986.*

125. *Saikku, P., M. Leinonen, L. Tenkanen, E. Linnanmaki, M. R. Ekman, V. Manninen, M. Manttari, M. H. Frick, and J. K. Huttunen. 1992. Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann. Intern. Med. 116:273-278.*

126. *Scaglione, F., and G. Rossoni. 1998. Comparative anti-inflammatory effects of roxithromycin, azithromycin and clarithromycin. J. Antimicrob. Chemother. 41(Suppl. B):47-50*

127. *Semaan, H. B., P. A. Gurbel, J. L. Anderson, J. B. Muhlestein, J. F. Carlquist, B. D. Horne, and V. L. Serebruany. 2000. The effect of chronic azithromycin therapy on soluble endothelium-derived adhesion molecules in patients with coronary artery disease. J. Cardiovasc. Pharmacol. 36:533-537.*

128. *Shor, A., C. C. Kuo, and D. L. Patton. 1992. Detection of Chlamydia pneumoniae in coronary arterial fatty streaks and atheromatous plaques. S. Afr. Med. J. 82:158-161.*

129. *Shor, A., J. I. Phillips, G. Ong, B. J. Thomas, and D. Taylor-Robinson. 1998. Chlamydia pneumoniae in atheroma: consideration of criteria for causality. J. Clin. Pathol. 51:812-817.*

130. *Sinisalo, J., K. Mattila, M. S. Nieminen, V. Valtonen, M. Syrjala, S. Sundberg, and P. Saikku. 1998. The effect of prolonged doxycycline therapy on Chlamydia pneumoniae serological markers, coronary heart disease risk factors and forearm basal nitric oxide production. J. Antimicrob. Chemother. 41:85-92.*