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Letter to the Editor

Antibodies against *Chlamydia pneumoniae* and their relation to lymphocyte population levels

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Abstract

Chlamydia pneumoniae infection has long been suspected as a possible cause of atherosclerosis and has been frequently detected in atheromatous plaques of the coronary arteries. Nevertheless, its distribution is not correlated to the severity or extent of the disease, but it would support the hypothesis that the organism may be an active factor in the pathogenesis of atherosclerosis. A group of patients with stable angina were examined as to whether or not the positivity of antibodies against *Chlamydia pneumoniae* modified cellular populations as mechanisms responsible for the alterations of inflammatory response. We concluded that the presence of IgG anti-*C. pneumoniae* antibodies do not participate in the activation of inflammatory mechanisms that may intervene in the genesis of atherosclerosis in patients with stable angina. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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Chlamydia pneumoniae infection has been highly suspected as a possible cause of atherosclerosis. Numerous pathological and sero-epidemiological studies have demonstrated the positive association between past or actual infection by *C. pneumoniae* and coronary arterial disease. Viable *C. pneumoniae* has even been cultivated from atherosclerotic tissue. In other studies, *C. pneumoniae* has been frequently detected in atheromatous plaques of the coronary arteries; nevertheless, its distribution is not correlated to the severity or extent of the disease [1], but it would support the hypothesis that the organism may be an active factor in the pathogenesis of atherosclerosis [2].

The object of our study was to evaluate in a group of patients with stable angina whether or not the positivity of antibodies against *Chlamydia pneumoniae* modified cellular populations as mechanisms responsible for the alterations of inflammatory response.

The study of lymphocyte populations was carried out in a random subgroup of patients with stable angina, composed of 28 males and 13 females. The patients voluntarily submitted to blood tests in order to determine the hematological parameters.

Whole blood with EDTA was obtained from each patient. The following MoAbs were used in immunofluorescence studies: CD3+/CD5/CD2+ (T-lymphocytes), CD4+ (T-helper lymphocytes), CD8+ (T-suppressor lymphocytes), CD3+/HLA-DR+ (activated T-lymphocytes), CD3+/CD8+/CD56+ (T-lymphocytes with NK activity), CD19+/HLA-

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DR+ (B-lymphocytes), CD16+/CD56+/CD2+ (NK cells). All antibodies were from Becton-Dickinson (San Jose, CA, USA). Phycoerythrin (PE)-, fluorescein isothiocyanate (FITC)-, or PerCP-conjugated MoAbs were used for fluorescence staining of whole blood samples. Cells were analyzed on a FACscan flow cytometer (Becton-Dickinson, San José, CA, USA). *Chlamydia pneumoniae* IgG antibodies were determined by the microimmunofluorescence (micro-IF) test. The samples were analyzed by the same laboratory physician and the point at which positive serology was established was 1/64. The patients were stratified in two groups according to antibody titer: group 1 in which no anti-*C. pneumoniae* antibodies (seronegative) were detected and group 2 with a titer above 1/64 (seropositive). The statistical analysis was done with an SPSS System, version 9.0 for Windows. A Pentium II 450 with 64 MB of RAM was used in the work.

As demonstrated in Table 1, no statistically significant association was found in the different lymphocyte populations with relation to serology to *Chlamydia pneumoniae*. However, the existence of higher mean values in some of the cellular populations in the group with positive serology in relation to the one with negative serology were observed.

The results revealed no significant association between positive serology to *Chlamydia pneumoniae* and lymphocyte population levels in a group of patients with stable angina.

The coronary lesions were infiltrated among other cells by macrophages and activated T-lymphocytes. The immunohistochemical analysis detected CD4+ and CD8+ lymphocytes in all stages of development of the atheromatous plaque. The CD4+ predominated in the mature plaques and the CD8+ in the precursor lesions or on the periphery of the evolving plaques.

There are few bibliographic references supporting the direct intervention of *Chlamydia pneumoniae* in the activation of lymphocytes. A study carried out by Halme et al. [3] in patients with coronary disease, confirmed by angiography, established that coronary cardiac disease is intensely associated with a humoral and cellular immune response mediated by *Chlamydia pneumoniae* in males. There are, however, numerous studies referring to the activation of T-lymphocytes in unstable angina compared with stable angina. One in particular was carried out by Gastone et al. [4], who wished to establish whether or not circulating T-lymphocytes were involved in the inflammatory reaction associated with attacks of unstable angina. They observed that the CD4+ and CD8+ in patients with unstable angina, but not in those with stable angina, had a higher expression of the HLA-DR antigen, which indicated that the T-lymphocytes were activated during the acute phase of unstable angina. The presence of activated T-lymphocytes in unstable angina implies antigenic stimulation, but the nature of such antigens is unknown.

In another related study, Liusso et al. [5] observed

Table 1
Mean values (%) of the different lymphocyte populations in patients with stable angina in relation to serology

Lymphocyte populations	Serology negative (%) (n=17)	Serology positive (%) (n=22)	Significance
T-lymphocytes (CD3/CD5/CD2)	69.29±6.51	66.76±9.56	NS
T-helper lymphocytes (CD4+)	38.82±8.42	38.18±8.63	NS
T-suppressor lymphocytes (CD8+)	24.94±9.86	22.36±10.32	NS
Quotient CD4/CD8	1.82±0.97	2.11±1.21	NS
Activated T-lymphocytes (CD3+/HLA-DR+)	2.94±1.33	3.63±4.26	NS
NK T-lymphocytes (CD3+/CD8+/weak/CD56)	3.77±4.33	4.49±4.08	NS
B-lymphocytes (CD19+/HLA-DR+)	9.88±3.28	9.00±3.87	NS
NK line (CD16+/CD56+/CD2+)	18.11±6.54	20.00±8.87	NS

that the CD8+ cells that synthesize IFN- γ were higher in patients with unstable angina than in control groups and patients with stable angina ($P<0.001$) and that the mean frequency of CD4+, producers of IL-2, was higher in patients with stable angina than in control groups and patients with unstable angina, and the CD4+ cells, producers of IL-4, were also higher in stable angina.

In our study we concluded that the presence of IgG anti-*C. pneumoniae* antibodies do not participate in the activation of inflammatory mechanisms that may intervene in the genesis of atherosclerosis in patients with stable angina.

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