

# The Host Response in Periodontitis: Anti-Phospholipid Autoantibodies as a Link between Plaque Bacteria and Extraoral Disease

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**Summary:** We have observed that autoantibodies reactive with important phospholipid antigens are present in the sera and gingival crevicular fluids of patients with periodontitis. The antibodies are likely induced by commonly occurring plaque bacteria. Phosphorylcholine (PC) is a prevalent antigen found on 30-40% of plaque bacteria, and patients with periodontal attachment loss have significantly higher concentrations of IgG anti-PC than do individuals without attachment loss. Data indicate that these antibodies bind to and opsonize oral microorganisms such as *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) and further that they react with and opsonize modified low-density lipoproteins (LDL). Anti- $\beta$ 2-glycoprotein 1-dependent anti-cardiolipin (anti-CL), another antibody of interest, is found at elevated levels in 15-20% of patients with generalized periodontitis. In patients with systemic lupus erythemathosis (SLE) or the antiphospholipid syndrome (APS) elevated levels of these antibodies are associated with prothrombotic events such as stroke and myocardial infarction, early atherosclerosis, and pregnancy loss. A cross-reactive epitope of *Porphyromas gingivalis* (*P. gingivalis*) may be responsible for the induction of such antibodies in periodontitis.

**Conclusions:** Auto-anti-phospholipids may be important in both periodontal pathogenesis and extra-oral conditions associated with periodontitis.

**Key words:** periodontitis, autoantibody, phospholipid

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## INTRODUCTION

Although many studies have been published detailing the role of specific antibody responses in protection against and in the pathogenesis of periodontal diseases, relatively few studies have explored the possible role of autoantibodies in periodontal infections. Published studies have focused on the possible role of serum, gingival crevicular fluid, salivary, and gingival tissue antibodies reactive with neutrophils, desmosomes, heat-shock proteins, collagen, and rheumatoid factors in the pathogenesis of gingivitis and periodontitis (Ftis et al, 1986; Hirsch et al, 1988, 1989; Jonsson et al, 1991; The and Ebersole, 1991; Anusaksathien et al, 1992; Sugawara et al, 1992; Govze and Herzberg, 1993; Novo and Viera, 1996; Hahn et al, 1997; Schett et al, 1997; Rohini et al, 2000; Berglundh et al, 2002; Sims et al, 2002; Rajapakse and Dolby, 2004; Yamazaki et al, 2004). Our

laboratory has been examining a class of autoantibodies reactive with phospholipids and the bacterial antigens that may be responsible for their occurrence. As detailed below, the induction of such antibodies by the oral flora may have implications for pathogenesis of both oral and systemic diseases.

## STUDIES OF ORAL BACTERIAL PHOSPHORYLCHOLINE (PC) AND ANTI-PC IN PERIODONTITIS PATIENTS

Studies of bacterial PC in our laboratories were initiated due to our interest in the IgG subclass responses to immunodominant antigens of periodontal pathogens. We initially noted that such responses were predominantly IgG2 subclass responses to carbohydrate antigens of *A. actinomycetemcomitans* and *P. gingivalis* (Lu et al, 1993; Lu et al, 1994). Thus, we included analyses of a well-

established predominantly human IgG2 response, that to PC, as a non-oral control response in these antibody studies (Brown et al, 1984). All human sera contain antibody reactive with PC and PC antigens are most notably present on *Streptococcus pneumoniae* (*S. pneumoniae*) and *Haemophilus influenzae* (*H. influenzae*). Although we considered measurement of such antibodies to be indicative of antibody responses to non-oral antigens, we discovered that patients with periodontal attachment loss had significantly higher levels of serum IgG antibody reactive with PC than did individuals without attachment loss. We consequently observed that samples of both supragingival and subgingival plaque reacted strongly with TEPC-15, a mouse myeloma protein with specificity for PC, and that 30-40% of plaque bacteria appeared to bear PC-containing antigens. These observations led us to hypothesize that a portion of the human anti-PC antibody response was due to exposure to the oral flora (Schenkein et al, 1999).

### **Anti-PC, Anti-oxLDL, and Systemic Disease**

Data have accumulated implicating anti-PC in a number of physiologic and pathologic functions. For example, serum anti-PC is capable of serving as a scavenger for apoptotic cells by opsonizing such cells and assisting with their removal by the liver (Shaw et al, 2000, 2003). In addition, high levels of anti-PC appear to protect mice from lethal infections with *S. pneumoniae* (Briles et al, 1992) though a relationship between anti-PC concentrations and similar protection in humans has not been observed (Musher et al, 1986). The ApoE<sup>-/-</sup> mouse model has also been used to examine the role of anti-PC in atherosclerosis. Shaw et al (2000) noted that such mice produce high levels of IgM antibodies reactive with oxidized low-density lipoproteins (oxLDL) and demonstrated that the specificity of some of these antibodies is towards PC. Monoclonal IgM anti-PC antibodies derived from such mice block uptake of oxLDL in macrophages *in vitro* implying that they serve a protective function. This research group further demonstrated that immunization of ApoE<sup>-/-</sup> mice with *S. pneumoniae* reduced atherogenesis in these animals due to production of IgM anti-PC (Binder et al, 2003). Our observation that humans with periodontitis have elevated levels of IgG anti-PC has led us to explore the relationships between anti-PC, anti-oxLDL, and periodontal bacterial pathogens. The experiments of Shaw et al (2000) illustrated that PC is an important antigenic epitope of oxLDL and that the antibody response to oxLDL in mice is predominantly to PC. This raised the possibility that human anti-PC is cross-reactive between oral bacteria and oxLDL. If so, then oral bacteria may induce IgG anti-PC antibodies which, in turn, bind to oxLDL. Such binding could have important biological consequences with respect to oxLDL processing in atheromatous lesions.

We were able to demonstrate that strains of *S. oralis*, *S. sanguis*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, and *H. aphrophilus* which have demonstrable PC-bearing antigens could absorb IgG anti-PC from human sera, while strains without PC did not react with human anti-PC. We also demonstrated that purified IgG anti-PC bound to a PC-bearing strain of *A. actinomycetemcomitans* (D045D-40) while little antibody binding could be demonstrated to the PC-deficient strain DB03A-42. We could further demonstrate that oxLDL, but not native LDL, was also capable of absorbing IgG anti-PC from human serum, and purified anti-PC bound directly to oxLDL but not to LDL. These experiments demonstrated that human anti-PC was cross-reactive between a variety of common oral bacterial species and oxLDL (Schenkein et al, 2001).

### **Anti-PC and Anti-oxLDL are Produced in the Periodontium**

The observation that anti-PC is elevated in periodontitis patients and binds to choline-bearing oral bacteria does not provide evidence that these antibodies may originate in the periodontium. To address this question, we examined relative IgG anti-PC and IgG anti-oxLDL concentrations in sera and gingival crevicular fluids (GCF) from 16 patients with generalized aggressive periodontitis. We found that 42% of 66 GCF samples contained higher concentrations of anti-PC than did sera from the same patients. Furthermore, 71% of the GCF samples contained elevated levels of IgG anti-oxLDL compared to serum. These results were in contrast to those obtained with anti-tetanus toxoid in which one of 62 samples contained elevated levels of GCF antibody. These results provide evidence that auto-anti-phospholipids are produced locally in periodontitis lesions and are likely to be the result of responses to cross-reactive oral bacteria (Schenkein et al, 2004).

### **Biological Consequences of Human IgG Anti-PC**

We have initiated experiments directed at testing the function of these antibodies. For example, we were interested in the consequences of binding of IgG anti-PC to oral bacteria with respect to protective interactions with leukocytes. We were able to demonstrate that anti-PC bound to PC-bearing bacterial strains of both *A. actinomycetemcomitans* and *S. pneumoniae*, and furthermore that the interaction of opsonized *A. actinomycetemcomitans* generated an oxidative burst and phagocytosis following its interaction with PMN's (Purkall et al, 2002). Thus, it is possible that anti-PC contributes to the pool of protective antibody for PC-bearing oral bacteria.

Although functional studies have been performed by other groups utilizing the mouse model of atherosclerosis

sis, the immune response to PC differs in mice as the response is predominated by IgM antibody. Thus, Shaw and coworkers have observed that anti-PC blocks uptake of LDL into macrophages and that anti-PC is protective against atheroma formation in mice. However, since human anti-PC antibodies are mainly IgG, we expected that the biological consequences of the induction of these antibodies may differ. We therefore examined the effects of purified human IgG anti-PC on the interaction of macrophages with modified LDL. We observed that, in contrast to the inhibitory effects of IgM anti-PC on LDL uptake by macrophages, IgG anti-PC promoted increased incorporation of modified LDL into cultured monocytes (Fig 1). This effect was further enhanced by complement, resulting in more than double the incorporation of LDL by monocytes. Furthermore,  $F(ab')_2$  fragments of IgG anti-PC inhibited LDL uptake by monocytes much like IgM antibody. Thus, it appears that IgG anti-PC may promote incorporation of oxidized lipids into macrophages and thus could promote, rather than inhibit, the formation of foam cells that are characteristic of atherosclerotic lesions.

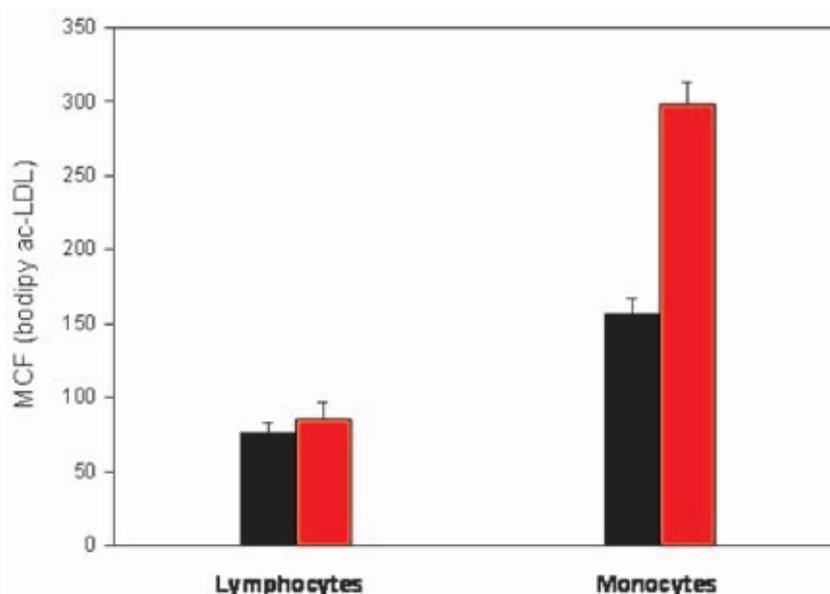
### Anti-Cardiolipin in Periodontitis

A second antiphospholipid autoantibody of considerable interest is  $\beta$ 2-glycoprotein 1-dependent anti-cardiolipin (anti-CL).  $\beta$ 2-glycoprotein 1 is an abundant serum protein that is thought to function as a natural anticoagulant (Kandiah and Krilis, 1994). This protein has been shown to bind to anionic phospholipids and prevent coagulation and thrombus formation occurring due to cell damage. It has also been shown to bind to the anionic phospholipids exposed on LDL following oxidation (Matsuura et al, 2002). A significant percentage

of patients with SLE and most patients with APS produce autoantibodies to  $\beta$ 2-glycoprotein 1 or to  $\beta$ 2-glycoprotein 1/anionic phospholipid complexes (Levine et al, 2002). Such antibodies can be measured in the laboratory in an assay that utilizes  $\beta$ 2-glycoprotein 1-coated cardiolipin as the antigen, and such antibodies are termed anti-CL. Notably, there exist anti-CL antibodies which bind only to the lipid cardiolipin, such as those detected in patients with infectious diseases such as syphilis and leprosy. Such antibodies do not have the pathogenic properties of  $\beta$ 2-glycoprotein 1-dependent anti-CL (Hojnik et al, 1994; Ordi-Ros et al, 2000; Sorice et al, 2000; Dalekos et al, 2001; Guglielmone et al, 2001; Uthman and Gharavi, 2002). Interestingly, notable clinical symptoms in antibody positive patients with APS include thrombotic events such as stroke, myocardial infarction, premature atherosclerosis, and adverse pregnancy outcomes such as fetal loss (Levine et al, 2002).

The commonality of the sequellae of APS and systemic conditions proposed by many investigators to be related to periodontal infections prompted us to examine periodontitis patients for anti-CL (Schenkein et al, 2003). To date we have examined sera from 629 periodontally characterized subjects for IgG and IgM antibody to anti-CL. The results indicated that approximately 15% of chronic periodontitis patients and 20% of generalized aggressive periodontitis patients test positive for these antibodies. In contrast, approximately 7% of subjects without periodontitis test positive for anti-CL. We have hypothesized that these antibodies may identify a subset of periodontitis patients at additional risk for thrombotic events and accelerated atherosclerosis due to the presence of this autoantibody.

**Fig 1** IgG anti-PC increases incorporation of modified LDL in to human monocytes/macrophages. 25 $\mu$ g/ml of human IgG anti-PC was added to cultures of highly purified lymphocytes or monocytes for 24h in the presence of acetylated LDL. Cells were collected and incorporation of the modified LDL (shown on the ordinate) was assessed. Lymphocytes incorporated modest levels of ac-LDL, and anti-PC failed to increase incorporation. However, anti-PC significantly enhanced incorporation of ac-LDL in monocyte cultures. Black bars indicate cultures without anti-PC, red bars indicate that cultures contained anti-PC.





**Table 1 Absorption of anti-CL from GAgP sera by oral bacterial strains**

Bacterial strain	% residual anti-CL
<i>P. gingivalis</i> W83	4
<i>P. gingivalis</i> W83 (TLCK-treated <sup>1</sup> )	7
<i>P. gingivalis</i> D40C-4 (clinical isolate)	6
<i>P. gingivalis</i> D40C-4 (TLCK-treated)	7
<i>P. gingivalis</i> 33277	2
<i>P. gingivalis</i> 33277 (TLCK-treated)	6
<i>P. gingivalis</i> HF 18 (RGP-deficient mutant)	85
<i>A. actinomycetemcomitans</i> Y4	89
<i>A. actinomycetemcomitans</i> DB03A-42	96
<i>A. naeslundii</i> 49340	100
<i>H. aphrophilus</i> 13252	100
<i>S. mutans</i> 25175	99
<i>S. oralis</i> 35037	100
<i>S. sanguis</i> 10556	100
<i>S. pneumoniae</i> 39937	96

Bacteria at approximately  $5 \times 10^8$ /ml were incubated with high-anti-CL titer serum from a patient with generalized aggressive periodontitis at 4°C overnight. Anti-CL titers were determined by ELISA. <sup>1</sup>Bacteria were treated with 10mM TLCK to inhibit proteolytic activity.

### Possible Origin of Anti-CL in Periodontitis

It has been observed that certain infectious agents, including bacteria and viruses, may induce pathogenic anti-CL due to the presence of bacterial antigens that cross-react with  $\beta$ 2-glycoprotein 1. For example, Blank and co-workers observed that immunization of mice with certain bacterial pathogens such as *H. influenzae* or *Neisseria gonorrhoea* resulted in production of anti- $\beta$ 2-glycoprotein 1 antibodies which, when infused into naïve mice, produce symptoms of APS. Such bacterial pathogens are cross-reactive with  $\beta$ 2-glycoprotein 1 because they bear peptide sequences homologous to a similar immunodominant peptide sequence of  $\beta$ 2-glycoprotein 1 (TLRVYK). We have noted that similar sequences are present in the arg-gingipain (RGP) protease of *P. gingivalis*. In experiments assessing the ability of strains of *P. gingivalis*, a *P. gingivalis* W83 RGP-deficient mutant HF18, and other oral bacteria strains to absorb anti-CL from periodontitis sera we have noted that only the wild type strains of *P. gingivalis* but not the RGP-deficient strain or other oral bacteria reacts with this antibody (Table 1). This implies that *P. gingivalis* may bear an epitope that is cross-reactive with  $\beta$ 2-glycopro-

tein 1 and could induce this autoantibody in some periodontitis patients.

### CONCLUSIONS

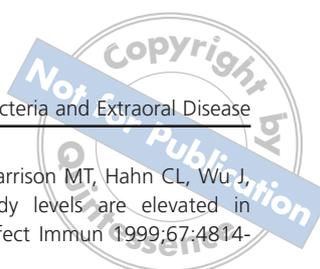
The studies described in this review establish the groundwork for consideration of auto-anti-phospholipid antibodies as possible pathogenic links between the oral flora and systemic sequelae such as atherosclerosis and stroke. Although anti-PC is a ubiquitous antibody that likely has important physiologic functions, it is significantly elevated in patients with periodontal attachment loss and likely to be induced in part by the oral flora. High levels of these antibodies can be shown to induce increased uptake of LDL into monocytes especially during early phases of monocyte differentiation to macrophages and could be important in the generation of foam cells during the early stages of atheroma formation. Similarly, elevated levels of anti-CL antibodies are more prevalent in periodontitis patients than in healthy subjects and may also be induced by the oral flora. Pathogenic levels of anti-CL antibodies are known to be associated with early atherosclerosis in patients with SLE and APS and are thought to be prothrombotic and to induce vascular inflammation. It remains to be shown that anti-CL antibodies in periodontitis patients are pathogenic in the sense that they can induce vascular inflammation *in vivo* and *in vitro* and simulate symptoms typical of patients with APS such as adverse pregnancy outcomes and accelerated atherosclerosis.

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