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Autoantibodies toward pathogenic and protective molecules in systemic lupus erythematosus: anti-oxLDL/ß2GPI and anti-PTX3 antibodies.

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RIASSUNTO

Il lupus eritematoso sistemico (LES) è una malattia autoimmune caratterizzata da differenti manifestazioni cliniche e dalla produzione di una grande varietà di autoanticorpi. I più caratteristici sono gli anticorpi anti-nucleo, in particolare gli anti-DNA nativo, ma sono stati descritti anche altri autoanticorpi quali gli anti-Sm, gli anti-U1RNP e gli anti proteina P ribosomiale. Più recentemente sono stati descritti gli anticorpi anti low density lipoproteine ossidate (oxLDL), gli anti-oxLDL/β_2GPI e contro molecole protettive quali le pentrassine, cioè anti-proteina C reattiva (PCR) e anti-serum amyloid P (SAP).

Vi sono chiare evidenze cliniche e sperimentali che l'aterosclerosi è accelerate in molte malattie autoimmuni come il LES ed anche se le ragioni non sono ancora del tutto note sembra che possa giocare un ruolo importante l'immunoflogosi propria di queste malattie. L'aterosclerosi è la principale malattia a carico dei vasi ed è la principale causa di infarto miocardico e morte ai nostri giorni.

È stata descritta un'associazione tra l'aterosclerosi e alcuni autoanticorpi, come anti-HSP, anti-β_2GPI, e anti-oxLDL, mentre per altri autoanticorpi come anti-SAA anti-APO A1 e anti-oxLDL/β_2GPI, anti-insulima, anti-MBL, e anti-pentrassine è stata ipotizzata.

SCOPO

Valutare il ruolo del complesso oxLDL/β_2GPI e di autoanticorpi noti, come gli anti-oxLDL/β_2GPI, e nuovi, come gli anti-PTX3, nella patogenesi del LES e dell'aterosclerosi nel LES.

MATERIALI E METODI

Per valutare il ruolo del complesso oxLDL/β_2GPI e degli anti-oxLDL/β_2GPI si sono analizzati i sieri di 78 pazienti affetti da LES e 72 soggetti sani, confrontabili per sesso ed età con il gruppo dei LES. Nei 78 pazienti, sono stati valutati durata di malattia, fattori di rischio tradizionali e non tradizionali per l'aterosclerosi, manifestazioni cliniche, come l'impegno renale, e il titolo di alcuni autoanticorpi, come anti-cardiolipina, anti-oxLDL, anti-β_2GPI e anti-HSP 60/65. È stata inoltre studiata l'associazione tra queste variabili e le alterazioni carotidee determinate con eco Doppler.

I titoli del complesso e degli anticorpi anti-complesso sono state determinati con l'uso di kit commerciali della Corgenix.

Sono stati analizzati i sieri di 76 pazienti affetti da LES, ben caratterizzati dal punto di vista sierologico, e 76 soggetti sani, confrontabili per sesso ed età con il gruppo dei LES, per

valutare la presenza di autoanticorpi contro l'intera proteina PTX3 e contro tre peptidi appartenenti alla proteina stessa. Il primo si trova nella parte N-terminale (PTX3_1), non correlata con le altre pentrassine; il secondo (PTX3_2) nella parte centrale e il terzo (PTX3_3) nella parte C-terminale, che sono le due parti omologhe con le altre pentrassine. Queste valutazioni sono state fatte con un tecnica ELISA home-made.

RISULTATI

La prevalenza e i livelli del complesso a degli anticorpi anti-complesso sono risultati significativamente maggiori nei pazienti con LES che nei controlli sani. I titoli del complesso erano significativamente maggiori in pazienti con impegno renale e con precedenti episodi trombotici e correlavano con il numero dei fattori di rischio per l'aterosclerosi, mentre erano significativamente più bassi in pazienti con impegno neurologico. Sia le IgG che le IgM anti-complesso sono risultate associate con la sindrome da antifosfolipidi.

Rispetto ai controlli, i pazienti con LES hanno livelli più elevati di anti-PTX3, anti-PTX3_1 e anti-PTX3_2 ($p<0.001$, per tutti), ed una prevalenza maggiore di tali anticorpi ($p<0.001$, per tutti). Correlazioni sono state trovate tra gli anti-PTX3 e gli anti-PTX3_1 ($r=0.502$, $p<0.001$) e tra gli anti-PTX3 e gli anti-PTX3_2 ($r=0.714$, $p<0.001$). Una concordanza è stata trovata soltanto tra gli anti-PTX3 e gli anti-PTX3_2 ($k=0.554$). Sia l'analisi univariata che quella multivariate hanno mostrato che i livelli di anti-PTX3 e anti-PTX3_2 sono maggiori in pazienti positive anche per gli anticorpi antifosfolipidi e in quelli senza glomerulonefrite.

CONCLUSIONI

Per quanto riguarda il complesso oxLDL/β_2GPI, livelli sierci elevati sono stati trovati nai pazienti con LES, in associazione con impegno renale ed eventi trombotici. Assieme alle oxLDL, anche i titoli della β_2GPI sono elevati nei pazienti con LES, e questo spiega perché in questa malattia si possono formare i complessi oxLDL/β_2GPI.

I titoli di IgG e IgM anti-complesso sono risultati associati agli anticorpi anti-fosfolipidi. In particolare le IgG anti-complesso sono risultate correlate alle IgG anti-β_2GPI; questo risultato può dipendere dal fatto che gli anti-β_2GPI possono legare il complesso oxLDL/β_2GPI. L'accumulo di oxLDL/β_2GPI nelle arterie sembra giocare un ruolo importante nella formazione e nella rottura delle placche aterosclerotiche e gli anticorpi anti-fosfolipidi o quelli diretti contro il complesso potrebbero avere anche un'influenza sulla aterotrombosi nei pazienti con LES.

Nel LES sono stati trovati anticorpi contro molecole protettive, come la PCR e la SAP, che sono coinvolte nella rimozione di materiale apoptotico. Anche gli anti-PCR e gli anti-SAP sono stati dimostrati nei pazienti con LES, in associazione ad attività di malattia e impegno renale. Nel nostro studio abbiamo dimostrato come gli anti-PTX3 siano dosabili nel siero dei pazienti affetti da LES e come i livelli di questi anticorpi siano superiori nei pazienti con LES rispetto ai soggetti sani. Gli anticorpi diretti contro due peptidi ottenuti dall'intera molecola di PTX3, cioè PTX3_1 e PTX3_2, sono risultati altamente correlati con con gli anti-PTX3 e mostravano associazioni cliniche e sierologiche simili. Le proprietà antigeniche di PTX3_1 e, in particolare, di PTX3_2 sembrano essere molto simili a quelle della PTX3, suggerendo un loro possibile impiego come substarto per nuove analisi.

ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by different clinical manifestation and the production of a variety of autoantibodies. The most characteristic anti nuclear antibodies is anti-double stranded (ds)DNA; however other autoantibodies including anti-Sm, anti-U1RNP, anti ribosomal P protein have been reported. More recently autoantibodies anti-oxLDL, anti-oxLD/β_2GPI, and against protective molecules like anti-pentraxins, i.e. anti-C reactive protein (CRP), anti-serum amyloid P component (SAP) have been identified.

Many clinical and experimental evidences showed that atherosclerosis is accelerated in autoimmune diseases like SLE, and although the reason of such finding is unknown, immunoinflammatory process seems to play a key role. Atherosclerosis is the main disease of the vascular wall and the major cause of myocardial infarction and death in the modern world. Many studies demonstrated that atherosclerosis is a multifactorial immunological disease. An association between atherosclerosis and some autoantibodies, such as anti-HSP, anti-β_2GPI, and anti-oxLDL, has been described, whereas for other autoantibodies like anti-SAA, anti-APO A1, anti-oxLDL/β_2GPI, anti-insulin, anti-MBL, and anti-pentraxins it has been only hypothesized.

AIM OF STUDY

To evaluate the role of oxLDL/β_2GPI, anti-oxLDL/β_2GPI and anti-PTX3 in the pathogenesis and progression of SLE and in accelerated atherosclerosis observed in such disease.

MATHERIALS AND METHODS

To evaluate the role of oxLDL/β_2GPI complexes and IgG and IgM anti-oxLDL/β_2GPI we analyzed 78 sera from SLE patients and 72 matched for sex and age healthy subjects. In SLE patients, disease duration, traditional and non-traditional risk factors for atherosclerosis, clinical manifestations, such as renal involvement, levels of some autoantibodies, like anti-cardiolipin, anti-oxLDL, anti-β_2GPI, and anti-HSP 60/65 were evaluated. Moreover, we investigated the relationship between these variables and carotid alterations determined by eco Doppler.

The levels of oxLDL/β_2GPI complexes and IgG and IgM anti-oxLDL/β_2GPI were evaluated using commercial kits from Corgenix.

Seventy six SLE patients and 76 matched healthy controls were analyzed by home-made ELISA tests to evaluate the presence of the whole protein and 3 peptides obtained from the

whole protein. The first from the N-terminal portion (PTX3_1), non related with the other pentraxins; the second (PTX3_2) from the central part; and the third (PTX3_3) from the C-terminal part, the two homologous parts with the other pentraxins.

RESULTS

The prevalence and the levels of the complex and of anti-complexes antibodies were significantly higher in SLE patients than in healthy controls. The titers of oxLDL/β2GPI were significantly higher in patients with renal involvement and previous thromboembolic episodes and were correlated with the number of risk factors for atherosclerosis, whereas they were significantly lower in patients with neurological involvement. Both IgG and IgM anti-complex antibodies were associated with APL.

Compared to controls, SLE patients had higher levels of anti-PTX3, anti-PTX3_1 and anti-PTX3_2 ($p<0.001$, for all), as well as a higher prevalence ($p<0.001$, for all). Correlations were found between anti-PTX3 and anti-PTX3_1 ($r=0.502$, $p<0.001$) and between anti-PTX3 and anti-PTX3_2 ($r=0.714$, $p<0.001$). Agreement was found only between anti-PTX3 and anti-PTX3_2 ($k=0.554$). Univariate and multivariate analyses showed that anti-PTX3 and anti-PTX3_2 antibody levels were higher in patients with antiphospholipid antibodies and in those without glomerulonephritis.

CONCLUSIONS

Regarding oxLDL/β_2GPI complexes and IgG and IgM anti-oxLDL/β_2GPI complexes, our results confirm that the oxLDL/β_2GPI complexes can be found in the circulation of patients with SLE where they are associated with renal involvement and thrombotic events. Along with oxLDL also the levels of β_2GPI are elevated in SLE patients, increasing the possibility of oxLDL/β_2GPI complexes formation.

IgG and IgM anti-complexes antibodies were associated with antiphospholipid antibodies (aPL). Particularly, IgG anti-oxLDL/β_2GPI correlated with IgG anti-β_2GPI and some anti-β_2GPI can bind oxLDL/β_2GPI. The accumulation of oxLDL/β_2GPI in the arterial wall seems to play a role in formation and rupture of plaques and aPL may be related to atherothrombosis in SLE patients.

Autoantibodies against protective molecules, like CRP and SAP, which are involved in the removal of apoptotic materials, have been reported in SLE. Anti-CRP and anti-SAP have been described as significantly prevalent in SLE patients and were related to disease activity and renal involvement. In our study we demonstrated that anti-PTX3 antibodies were

significantly prevalent in SLE patients and that anti-PTX3 antibody levels were higher in SLE than in healthy subjects. Interestingly, antibodies towards two PTX3-related peptides, PTX3_1 and PTX3_2, were highly correlated with anti-PTX3 antibody and showed similar clinical and serological associations. The antigenic properties of PTX3_1 and, primarily, PTX3_2 seem to be similar to those of PTX3 suggesting their potential use, as substrate, for further analysis.

INTRODUCTION

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is a prototypical autoimmune systemic disease, characterized by different clinical manifestations and production of a variety of autoantibodies. SLE can affect different organ systems, including kidney, joint, serosal tissues, skin, and central and peripheral nervous system. The most common long-term involvements include damage to the musculoskeletal, neuropsychiatric, renal, and cardiovascular systems [1].

SLE is classified among rheumatic diseases because of the musculoskeletal manifestations are the most common one at the disease onset as well as during disease relapses [2,3].

Epidemiology

The prevalence and the incidence of SLE are really variable among the different ethnic groups. The prevalence rate generally ranges from 14.6 to 70 per 100,000 [4-34] (Table 1). The incidence of SLE varies from 2.6 in the Scandinavian countries [18] to 4.6 per 100,000 in the Afro-Caribbean population [14].

SLE has been detected in 6 continents (Europe, North America, South America, Africa, Asia, and Australia) [34]. The disease is rare in Africa but common in African descendants in the world, in which the incidence is 5 times higher than in non-black populations [35,36].

The F/M ratio is 9:1 and the age at disease onset is between 25 and 40 years, but juvenile [37] or older occurence is not so rare. Juvenile SLE often presents with major organ system involvement including renal [38-42] and neuropsychiatric [42-45] manifestations.

The majority of studies regarding the mortality rates for specific ethnic groups [11,46-51] showed higher mortality risks for black, and Hispanic groups compared with for white populations. However, these differences were not seen in studies considering also socioeconomics variables [46,48,50,51,52]. Hence, ethnicity reflects socioeconomic variables and psychological factors that could directly affect mortality risk and that may be amenable to interventions [34].

Overall improvements in medical care have led to improve survival rate of SLE patients in the last 50 years [53].

Etiopathogenesis

The etiology of SLE is still unknown. However, it is considered that SLE has a multifactorial etiology encompassing genetic, environmental, immunological, and hormonal factors. As for as pathogenesis is concerned, SLE is considered a typical autoimmune rheumatic disease.

SLE is characterized by many alterations of the immune system that activate autoreactive T cells and B cells (Table 2) leading to the production of many autoantibodies against nuclear antigens [51-61].

It has been shown, both in human and in mice [62,63], that the total number of peripheral blood T cells is usually reduced in SLE patients, probably for the effect of anti-lymphocyte antibodies. More recently, it has been shown that T regulatory (Treg) cells are decreased in number or functionally defective in active SLE [64-68]. Valencia et al [68] demonstrated that patients with active SLE have a decreased number of $CD4^{+}CD25^{high}$ Tregs in the peripheral blood with a decreased levels of FoxP3 expression, a transcription factor for T cells. There is an inverse T cell function towards B cell help, leading to enhanced antibody production [68-70]. Although peripheral T cells are activated in lupus, both their capacity for proliferation in response to mitogenic stimulation and IL-2 production are reduced [71-73].

In contrast, the number of B cells at all stages of activation is increased in the peripheral blood of SLE patients with active disease [74]. B cells of SLE patients seem to be more prone to polyclonal activation, cytokines and other stimuli [54]. Indeed, it has been shown that B cells in SLE patients are more sensitive to the stimulation by IL-6 than B cells of normal subjects [75].

In many studies, both *in vitro* and *in vivo*, in animal models and in human patients affected with the SLE, it has been demonstrated a higher percentage of apoptotic cells, in particular mononuclear cells (limphocytes, monocytes, macrophages, etc.), associated to a reduced clearance of the apoptotic bodies by the fagocitic cells [54,76-82]. These alterations determine a secondary necrosis of the apoptotic cells not engulf with the release of their materials [79-82]. During apoptosis there is the activation of specific endonucleases that cleavage the DNA producing nucleosomes [83].

Predisposing factors

Genetic factors. It has been demonstrated that SLE has a hereditary predisposition and that the concordance is higher in homozigote than in heterozigote twins, 15-58% in homozigotes

vs. 2-8% in heterozigotes [54,84]. Moreover, the risk to develop SLE is 8-9 times higher in people with than in people without hereditary [84]. Indeed, 10-16% of papients have parents affected by SLE or other autoimmune diseases [54,84].

The hereditary for SLE was confirmed by studies demonstrating an association between the disease and genes belonging to the major histocompatibility complex (MHC), the so called human leukocyte antigen (HLA) in humans [54,84-86]; particularly the HLA II and the HLA III [85,86], that are involved in the regulation of immunocompetent cell activity and in the production of the first sequence of the complement, respectively.

The most important alleles of the HLA II are DR and DQ, in particular DR2 and DR3 [85,86]. Concerning the alleles of HLA III, the prevalence of SLE is higher in patients with a deficit of C1q, C2, and C4 [87,88] (Table 2), in which the clearance of the immunecoplexes (ICs) is reduced.

Furthermore, other genes are involved in the pathogenesis of SLE. For example, the receptors of the complement CR1 and CR2, Gm and Km that encode for some immunoglobulins (Ig), specific polimorfisms of the T cell receptor (TCR), and of the receptors of the Fc (FcR) fraction (Table 2) of IgG, playing a key role in the clearance of the ICs, in particular FcRγ2a [54,85,89-92].

Therefore, many genes can be involved in the predisposition of SLE and only in a few patients (<5%) the deficit of one gene could develop the disease [84].

Hormonal factors (Table 3). It has been thought that sexual hormones can play a role in the pathogenesis of SLE because of the prevalence of the disease in females, particularly during reproductive age [93,94]. Abnormal estrogen metabolism has been seen in SLE patients of both sexes [95]. Moreover, women with SLE also have low plasma androgens, including testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfato [96,97].

Testosterone reduces immunoglobulin production from peripheral blood mononuclear cells of both normal subjects and patients with SLE [98,99]. DHEA has been demonstrated to be associated with the enhancement of Th1 and inhibition of Th2 immune responses [100,101]. Whereas, both physiological and supraphysiological concentrations of estrogens facilitate humoral response, leading to increased B cells proliferation and antibody production [102-105]. On the other hand, high dose of estrogens inhibit T cells responses, such as proliferation and production of IL-2 [106,107]. Moreover, estrogens increase, whereas androgens decrease,

the production of both spontaneous and induced by immunization anti-DNA antibodies of SLE [99,104].

Environmental factors (Table 3). Clinical and experimental data suggest that environmental factors can induce an autoimmune disease acting in subjects genetically predisposed to SLE [108]. Viral, bacterial or parasitic infections seem to determine an aberrant immune response [109-111] (Table 3). Infections lead to the production of various autoantibodies by a non-specific activation of low-affinity autoreactive B cells, but it is still unclear the mechanisms causing the autoimmune process [112], although it has been shown that a chronic infection can activate autoreactive B cells and stimulate their differentiation into memory cells, causing autoimmunity in genetically predisposed individuals [112].

An hypothesis is that an environmental trigger acts in genetically predisposed subjects leading to a loss of tolerance to native proteins [113], by a mechanism of "molecular mimicry/cross reactivity" [111]. This phenomenon induces a normal immune response against foreing epitopes mimicring an antigenic target common to a self-antigen [111]. All types of infections, including bacterial, viral and opportunistic infections, have been reported [108,114]: Cytomegalovirus and Parvovirus B19 are frequently involved in SLE autoimmunity [113], but the most clear evidence of molecular mimicry in SLE derives from Epstein-Barr virus [115]. It is possible that pathogenetic variants of Fc receptors for IgG do not clear immune complexes adequately, disregulating the clearance of microorganisms [116]. Ultraviolet (UV) rays are also involved in the etiopathogenesis of SLE (Table 3), but it is still unclear if they cause the disease or its relapse [117]. Moreover, bacterial infections increase the apoptosis of lymphocytes and UV rays increase apoptosis of keratinocytes [117,118]. These events together with a defective clearance of apoptotic clearancecan induce SLE ingenetically predisposed individuals [118].

Finally, there are many evidences demonstrating that some drugs [119-121] are able to induce SLE.

Autoantigens and autoantibodies in SLE

Autoantibodies are the hallmark of SLE. Since they can be detected in the patients sera many years before the diagnosis of the disease; they can be cansidered as prognostic marker for future diagnosis in healthy subjects [122].

More than 100 autoantibodies have been described in SLE patients [123].

Anti-nuclear and anti-nuclear extractable antigens antibodies (ANA and ENA)

The most common are anti-nuclear antibodies (ANA), being present in 95% of SLE patients. They are directed against nuclear and cytoplasmic antigens that are present in all nucleated cells, in which play a role in transcription or translation, or as a structural protein [124]. Virtually, all SLE patients have ANA, while some patients positive for ANA do not develop SLE. Positive ANA are also common in the sick elderly population [125-127].

Among ANA, anti-double-stranded-DNA (dsDNA) are specific marker for SLE and play a key role in its pathogenesis [128]: indeed, they are detectable in 70% of SLE patients, and in less than 0.5% of healthy people or patients with other autoimmune diseases [128]. Anti-dsDNA serum levels reflect the disease activity [2,129,130], but not in all patients. In fact, it has been demonstrated that increasing titers of anti-dsDNA could cause exarcebation of lupus nephritis in mice [131,132], but this association in humans is not clear, because some patients with active nephritis are negative for anti-dsDNA and patients with persistent high titers of anti-dsDNA do not develop glomerulonephritis. An explanation could be that they may be present transiently during the disease cause [133].

Also autoantibodies against extractable nuclear antigen (ENA) have been described in SLE. Among ENA, anti-Sm have been found in 5-30% of SLE patients with a higher prevalence in Black-Americans than in Hispanic and White populations [133]. These antibodies are directed against seven proteins (B/B', D1,D2, D3, E,F,G) that are part of common *core* of U1, U2, U4 and U5 small nuclear ribonucleoprotein (snRNP) particles. They are pathognomonic for SLE [133] and high titers are an American College of Rheumatology (ACR) criterion for SLE (highly SLE specific), even though low-titer anti-Sm has been reported in other diseases [134-136].

Among the characteristic clinical manifestations of SLE, central nervous system involvement is present in up to 80% of SLE patients [137-141]. It has been shown both in humans and in mice that IgG antibodies to phosphorylated ribosomal (P ribosomal) proteins are associated with psychosis and might be associated with peripheral nervous system complications [142,143].

P ribosomal proteins are three ubiquitous highly conserved acidic phosphoproteins (P0, P1, P2), forming the 60S ribosomal subunit, where they participate in the synthesis of proteins. The P0 protein is located in an immunological accessible way on the membrane surface of neuronal, hepatic, and endothelial cells [144]. Anti-P ribosomal proteins have been related to

not only central nervous system involvement, but also to disease activity, liver, and kidney involvement in SLE patients [145].

In this group are present anti-Ro/SSA and anti-La/SSB. Anti-Ro are present in 50% of SLE patients and in children with neonatal lupus [146-148], in which are usually found with anti-LA [147-150]. Moreover, anti-Ro seem to be associated with neutropenia [150], and anti-La with amelioration of renal disease [133]. These autoantibodies are not specific for SLE, but really useful in the absence of anti-dsDNA [133]. In fact, anti-Ro and anti-La have been detected also in other disease such as systemic sclerosis [124].

Also anti-phospholipid antibodies (aPL) are important for the diagnosis of SLE. They are involved in the pathogenesis of the disease, and/or associated with cardiovascular involvement. This is a heterogeneous group of antibodies, including anticardiolipin (aCL), lupus anticoagulant (LAC), anti-phosphatidylserine, anti-phosphatidylinositol, anti-phosphatidic acid. Elevated levels of aPL have been found in patients with antiphospholipid syndrome (aPS), that could be diagnosed in patients affected by other autoimmune disorders, in particular SLE. The most common of these autoantibodies are anti-β_2 glycoprotein I (β_2GPI) antibodies, which titers were found higher in SLE patients than in healthy controls [151] (Table 4).

Another antigenic target of aPL is prothrombin [152,153], that plays a pathogenic role in SLE [153]. Like β_2GPI, prothrombin is involved in the regulation of blood coagulation. It has been shown that anti-prothrombin antibodies have a higher diagnostic accuracy for thrombosis compared to anti-β2GPI and aCL antibodies, and, along with LAC activity, are the best predictors of thromboembolic events in SLE patients [153].

Other autoantibodies

Antibodies against protective molecules, like pentraxins C-reactive protein (CRP), serum amyloid P component (SAP) and pentraxin 3 (PTX3), have been shown to play a role in the pathogenesis of SLE [154,155]. The pentraxins are a highly conserved family of proteins belonging to innate immunity which exert a key role in inflammation and apoptosis. As members of innate immunity, their major task is to recognize microbial pathogens, activate complement and stimulate the uptake of pathogens by phagocytes [156-158].

CRP is a pentamer, composed of five identical 23-kDa subunits [159], which is rapidly produced and released in circulation by hepatocytes in response to inflammatory stimuli like

IL-1, IL-6 [157,160], and IL-17 [161]. However, the synthesis and secretion of CRP at the inflammatory sites has also been reported [162]. The gene of CRP maps to the chromosome 1. In healthy subjects serum levels of CRP are less than 1 µg/mL and can increase 1000-fold during the acute-phase of inflammatory response, returning to normal levels with the resolution of the pathological process [160]. Baseline levels increase with age and are higher in women compared to men [160].

CRP binds various pathogens, such as bacteria fungi and yeasts [163], and cellular and nuclear ligands, such as chromatin histones and small nuclear ribonucleoprotein (snRNP) in a calcium dependent manner [164]. It has a role in the clearance of cellular and nuclear debris [160,164].

The effects of CRP on cells seems to depend on the monomeric or pentameric conformational state of the protein [165]. Indeed, it seems that the conformational shift from the pentameric to the monomeric form is necessary to exert its proinflammatory action [165]. CRP seems to increase the phagocytic activity of macrophages and the production of transforming growth factor-β (TGF-β) [166]. CRP activates neutrophils and monocytes leading to the production of proinflammatory cytokine including IL-1β, IL-6, and TNF-α [160]. CRP modulates inflammatory response also binding low-affinity Fcγ receptor IIa (FcγRIIa, CD32) and high-affinity FcγRI (CD64) on leukocytes, in which it stimulates the synthesis not only of TNF-α and IL-1, but also of IL-10 [160] (Table 5).

The reason why CRP can become an autoantigen is still unknown. In SLE patients, anti-CRP autoantibodies were found to be associated with disease activity and the occurrence of lupus nephritis [167]. These data were recently confirmed by a study [168] showing anti-CRP antibodies in SLE patients in association with lupus nephritis and clinical features of antiphospholipid antibody syndrome.

SAP is organized like a flat cyclic pentamer, formed by five non covalently associated identical subunits of 25 kDa [169,170], and its gene maps to chromosome 1 in close proximity to the CRP gene. It is produced by hepatocytes in response to IL-1 and IL-6 [157,171]. SAP is a plasma glycoprotein of systemic amyloid deposit, identified also in cerebral amyloid deposits [172]. The levels of SAP are relatively stable during early acute-phase response [173]. The plasma levels of SAP are tightly regulated and are slightly lower in women [173].

It binds pathogens including bacteria and viruses [174], in addition to lipopolisaccharyde (LPS), laminin, type IV collagen, fibronectin, chondroitin sulfate, and heparin [174]. SAP is the major DNA- and chromatin-binding protein in the plasma [157]; particularly, it binds chromatin exposed by apoptotic and necrotic cells [175], solubilizing chromatin fragments [158]. It activates complement C1q to remove cellular debris [154]. SAP has been inconstantly reported to bind FcγRI and FcγRIII on leucocytes, facilitating phagocytosis by machrophages [164,175] (Table 5).

Anti-SAP antibodies have been recently shown in patients with SLE, where they seem to be correlated to disease activity [176], anti-nuclear antibodies and anti- dsDNA antibodies [176].

PTX3 is the prototypical long pentraxin with a molecular weight of 40-50 kDa. It has homology with the short pentraxins, but differs for the presence of an unrelated N-terminal portion. Indeed, it has a sequence of 381 amino acids, of which 203 in the C-terminal pentraxin-like domain, and 178 in the N-terminal portion [177]. The protein consists predominantly of β-sheets and a minor portion of α-helical component [177]. PTX3 forms multimers of 440 kDa and disulfide bonds are required for multimer formation [178]. In humans the gene is located on chromosome 3. Its genome is organized in three exons and the region of homology between PTX3 and the other pentraxins corresponds to the third exon of PTX3 [177]. In human, the serum levels of PTX3 increase from undetectable to 200-800 ng/mL during severe infectious, autoimmune, and degenerative conditions [177,179].

PTX3 is produced and released *in situ* by many different cell types, in particular dendritic cells, macrophages, fibroblasts, activated endothelial cells, and neutrophils [178,179]. Renal cells can also produce PTX3 [179]. The production of PTX3 is stimulated by LPS, IL-1 and TNF, but not IL-6 or IFN-γ [177]. PTX3 expression in dendritic cells and monocytes is induced by IL-10, and inhibited by IFN-γ [177] (Table 5).

PTX3 plays a key role in innate resistance against selected pathogens, such as *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Aspergillus fumigatus* [180]. It seems to be involved in female fertility as well as in the regulation of inflammatory reactions and autoimmunity [180]. Although PTX3 does not recognize the ligands of the classical pentraxins, it binds C1q to inhibit or to activate the classical complement pathway [181]. The inhibitory or activating action depends on the binding of PTX3 to apoptotic cells. *In vitro* preincubation of apoptotic cells with PTX3 enhances C1q deposition and complement activation on apoptotic cells; whereas when PTX3 and C1q are simultaneously present, PTX3 sequesters C1q, decreasing

the activation of C3 on apoptotic cells [181]. In addition, it has been demonstrated that C1q enhances, instead PTX3 inhibits the phagocytosis of apoptotic cells by dendritic cells and macrophages [182], directly binding to membrane of apoptotic cells. Thus, C1q and PTX3 cooperate in the regulation of the clearance of dying cells [183] (Table 5).

No data on anti-PTX3 antibodies have been available in the literature to date. However, very recently, circulating anti-PTX3 autoantibodies have been found and it has been shown that they are significantly prevalent in SLE patients [184]. Differently from data reported for other anti-pentraxin antibodies, any relationship between these antibodies and disease activity have been observed [184].

It has been largely demonstrated that SLE is characterized by dyslipoproteinemia, for increased levels of very low-density lipoprotein (VLDL), cholesterol, triglycerides, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) [185-187]. These lipoproteins, in particular LDL, undergo an increased oxidation in SLE patients [188-190]. The titers of oxidatively modified lipoprotein correlate with the disease activity [190-193]. Oxidized LDL (oxLDL) possess mitogenic and chemotactic effects on the effector-cells of the immune system [193,194]. Antibodies against oxLDL are present in sera of healthy individuals. Moreover, it has been demonstrated that oxLDL levels are higher in SLE patients than in healthy controls [194-196] (Table 4), in particular in hypertensive SLE subjects [194].

OxLDL, but not native LDL, bind to β2GPI to form oxLDL/β2GPI complexes [196]. The major ligand of oxLDL for β2GPI is 7 ketocholesteryl-9-carboxinonanoate, the so-called oxLig-1 [197]. It has been demonstrated that ω-carboxilated 7-ketocholesteryl esters are critical for β2GPI binding [198].

The levels of circulating oxLDL/β2GPI complexes are increased in SLE patients compared to healthy controls [199]. Also IgG anti-oxLDL/β2GPI antibody levels are significantly higher in SLE patients compared to healthy controls [199,200], in particular in SLE patients with APS [194] (Table 4). The physiologic relevance of oxLDL/β2GPI complexes and of IgG antibodies has been demonstrated *in vitro* by enhanced macrophage uptake of IgG immune complexes with oxLDL/β2GPI [196].

Atherosclerosis (ATS)

Atherosclerosis (ATS) is a complex pathological process of the vascular wall which predominantly affects large and medium-sized arteries, and the most common cause of cardiovascular failure, myocardial infarction and death, in industrialized countries.

For many years ATS was considered a degenerative disease caused by a bland lipid storage in the vessels; but in the last decades several groups have demonstrated that it is a process closely related to inflammation, involving mechanisms of both innate and adaptive immunity [201-204]. Actually, ATS fullfils the four criteria proposed by Witebsky and Rose to define a condition as an autoimmune in nature [205]. Epidemiological and cohort studies demonstrated that not only traditional risk factors for ATS, but also chronic infections, inflammatory and immune factors, including cytokines, chemokines, T and B cells, and even autoantibodies are involved in atherogenesis [201-204,206]. Interestingly, atherosclerosis is accelerated in many autoimmune conditions such as SLE [207-209]. Indeed, early autopsy and angiographic studies demonstrated a high prevalence of atherosclerotic lesions in SLE [210,211]. Subclinical ATS, represented by intima/media thickness (IMT), was also more frequent in SLE than in healthy controls [195].

Immune-inflammatory mechanisms involved in atherogenesis

There is clear evidence that both innate and adaptive immunity play a role in atherogenesis [202-204,206-209,212,213]. The first step of this process is the activation of endothelial cells leading to a proinflammatory phenotype characterized by an increased expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), E- and P-selectins which, in turn, promote the rolling and sticking of monocytes to the endothelial cell membrane [201]. The endothelial dysfunction leads to an increase of endothelial wall permeability allowing the passage of LDL from the circulation to the subendothelial space. Here, LDL are modified by various processes including oxidation [214]. OxLDL are thought to be responsible for triggering inflammatory responses in macrophages and vascular wall cells. They increase the expression of monocyte chemoattractant protein-1 (MCP-1) on endothelial cells leading to migration of monocytes through the endothelial junctions into the subendothelial space [201]. Therefore, oxLDL induce the differentiation of monocytes to macrophages [201,202] and stimulate the macrophages to produce cytokines including IL-1, IL-6, TNF-α and adhesion molecules, thus amplifying the inflammatory loop [201]. OxLDL are also taken up by

macrophages transforming them into foam cells which produce growth factors and metalloproteinases. Growth factors, in turn, induce proliferation of smooth muscle cells and collagen-matrix formation whereas metalloproteinases cause matrix degradation [215]. Plaque stability and the break down of fibrous cup substantially depend on the balance between these two opposed processes [216].

Other molecules belonging to innate immunity including pattern recognition receptors (PRRs), and pentraxins have been shown to play an important role in atherogenesis promoting the inflammatory activation of endothelial cell, smooth muscle cells and macrophages [203,216]. PRRs, such as Toll-like receptors, respond to pathogen-associated molecular patterns activating intracellular signaling pathways, which lead to the activation of nuclear factor kB (NF-kB) and, in turn, the production of inflammatory cytokines and chemokines [203,216].

Complement components have been detected in early atherosclerotic lesions in humans. Indeed, the formation of atherosclerotic lesions induced by cholesterol is reduced in complement-deficient animals, suggesting that complement activation occurs at a very early stage of atherogenesis [217-219].

In atherosclerotic lesions we can find not only macrophages, the classic effector cells of innate immunity, but also lymphocytes which are the effector cells of the adaptive immune system [220].

Approximately, 20% of lymphocytes which infiltrate atherosclerotic lesions are activated lymphocytes [206]. Th1 cells are abundant in atherosclerotic lesions and can play a role in the formation of plaques, activating the cascade of cytokines. The differentiation of Th1 cells is induced by IFN-γ [221,222]. IL-12 is abundant in atherosclerotic plaques and its production is upregulated in monocytes exposed to oxLDL [220-222]. Macrophages, smooth muscle cells and endothelial cells synthesize IL-12, which can induce a Th1 response. More recently a new lineage of T cells has been found, Th17, induced by IL-17, which may be important in plaque destabilization [223].

An unusual subset of T cells, CD4+/CD28- T cells, seems to be particularly important in atherogenesis since it is clonally expanded in the peripheral blood and infiltrates coronary plaque in patients with unstable angina [224]. CD4+/CD28- T cells increase IFN-γ production leading to the activation of monocytes and macrophages and exert cytotoxic activity on endothelial cells [225]. Interestingly, this subset of T cell is also expanded in the peripheral

blood of patients with rheumatoid arthritis in association with subclinical atherosclerosis [226].

Major autoantigen–autoantibody systems involved in atherosclerosis

Natural antibodies exhibit a remarkably conserved repertoire that includes a broad specificity for self-antigens. The self-antigens do not cause an immune reaction, because immunological tolerance plays a key role to discriminate self from nonself-antigens. The tolerance renders mature lymphocytes in the peripheral lymphoid tissues to be non-functional or hyporesponsive to an antigen [227].

Natural antibodies can be found within atherosclerotic lesions and have been postulated to contribute to the elimination of autoantigens exposed during stress, tissue damage, or even conventional cell turnover. When they bind to autoantigens, they can play a protective role, masking the antigenic determinants by a nonspecific and low-affinity binding [228].

Under certain pathological conditions that involve increased accumulation of stress-induced self-structures, antibody-mediated clearance may become increasingly relevant [228]. However, in some particular conditions such as in the case of increased oxidation due to smoke, absence of antioxidants, etc., autoantibodies to different epitopes might be generated. These autoantibodies are able to accelerate the formation of lipid loaded foam cells stimulating atherogenesis.

Many autoantibodies and their cognate antigens may be involved in atherogenesis [229,212,213], including pentraxins and autoantibodies against pentraxins [123,156]. Some of them have been reported to associate with ATS; some other could be potentially involved but no clear data have been published yet. Therefore, on the basis of the strength of association with atherosclerosis, autoantibodies may be subdivided into 3 groups (Table 6). The autoantibodies for which the association with atherosclerosis could be considered “defined” are: anti-oxLDL, anti-β2GPI and anti-HSP60/65. For other autoantibodies, including anti-oxLDL/β2GPI complex, anti-CRP, anti-Sap, the association with ATS could be considered “probable” and for others still “possible”.

OxLDL is one of the major antigens in ATS. OxLDL is taken up by macrophages in the atherosclerotic lesions, transforming these cells into foam cells. Plasma levels of native LDL are regulated by LDL receptors located on endothelial cells and monocytederived macrophages. These LDL receptors are downregulated to prevent excessive intracellular lipid

accumulation. In contrast, subendothelial oxLDL is removed by intima macrophages via scavenger receptors, thus causing an excessive intracellular accumulation of oxLDL and foam cell formation [230]. Oxidative stress is one of the normal host responses to many stimuli, and may be self-limiting. However, chronic vascular inflammation from different types of pathologic injury may result in chronic oxidative stress and the generation of excessive amounts of oxLDL. OxLDL has been shown to be present in atherosclerotic lesions in animal models and humans [193]. IgG anti oxLDL antibodies are widely detected in patients with cardiovascular diseases (CVD) [231]. Moreover, it has been demonstrated that there is a good correlation between anti-oxLDL antibody levels and maximum IMT in SLE patients [8]. IgG antibodies seem to be pathogenic for subclinical ATS in SLE patients; while IgM anti-oxLDL antibodies found in atherosclerosis-prone $ApoE^{-/-}$ and $LDL\text{-}R^{-/-}$ mice, are thought to provide protection against proinflammatory oxidized moieties [232]. Indeed, it has been shown that high levels of IgM antibodies against oxLDL predict a favourable outcome in the development of carotid ATS in hypertensive subjects [233]. Moreover, it has been demonstrated that immunization of $LDL^{-/-}$ mice with oxLDL, protect from inflammation and plaque formation [228], inhibiting the uptake of oxLDL by macrophages [234] and hyperimmunization of $ApoE^{-/-}$ mice suppresses early atherogenesis [235]. The explanation could be that these antibodies are heterogeneous both in Ig subclass and in their epitope specificity and affinity [236]. It has been also demonstrated that IL-5 plays an important protective role causing the expansion of natural Ig antibodies specific for oxLDL [237].

β_2GPI possesses natural anticoagulant properties. It is a highly glycosylated plasma protein containing 5 short consensus repeated domains with an approximate molecular weight of 50 kDa that avidly binds negatively charged surfaces and substances, such as heparin, anionic phospholipids, and apoptotic cells [229,238].

Its partial association with various lipoproteins results in its synonymous designation as apolipoprotein H. It binds platelets and apoptotic cells; it inhibits intrinsic blood coagulation pathways and ADPdependent platelet aggregation; it has a role in the activation of endothelial cells induced by aPL; and it may assist in mediating clearance of senescent cells and foreing particles from circulation [229,238].

β_2GPI possesses several properties that may bear relevance to progression of human atherosclerotic plaque [193]. β_2GPI is abundantly present in human atherosclerotic plaques from carotid arteries [238]. Although randomly expressed in the different layers of the plaque,

it was found to be most prominent in subendothelial regions and in the intimal–medial border of the lesions. It was also shown that anti-β_2GPI antibodies could be a marker for arterial thrombosis in SLE [238]. These antibodies have been shown in vitro to activate cultured endothelial cells, leading to enhanced monocyte adherence [151]. Moreover the induction of these antibodies in transgenic mice was associated with accelerated ATS [151].

β_2GPI and anti-β_2GPI antibodies seem to be proatherogenic, but their pathogenic effect for ATS can be secondary in SLE patients. Moreover it has been shown that induced oral tolerance to β_2GPI suppresses the early atherosclerosis in LDL-$R^{-/-}$ mice [227].

HSPs are a group of evolutionarily conserved proteins, which show high sequence homology between different species, from bacteria to humans, and are involved in maintaining various cellular proteins in their correctly folded functional forms [239]. But these proteins can become autoantigens, leading to autoantigens production. Circulating anti-HSP antibodies may be induced and maintained by different mechanisms [240]: infection with microbes containing homologous HSP proteins; the protein itself could become immunogenic because of structural alteration or post translational modification; other foreign or self-antigens could interact with HSP to form immunogenic complexes; soluble HSP might not be recognized as a self-protein. It has been shown that levels of serum soluble HSP60 were significantly elevated in subjects with prevalent/incident carotid atherosclerosis and that these levels were correlated with common carotid artery IMT [240]. High levels of circulating anti- HSP autoantibodies have been associated with increasing severity of ATS in patients [240]. Moreover HSPs and their autoantibodies have been shown to elicit production of proinflammatory cytokines. These autoimmune reactions to HSPs expressed in the vascular tissue can contribute to both initiation and perpetuation of ATS. Many independent [233,241] groups subsequently confirmed that anti-HSP60 antibodies were also elevated in patients with atherosclerotic plaque and seropositive individuals not only showed a higher prevalence of coronary artery disease (CAD) but also their disease severity was correlated with antibody titres.

Also serum antibodies against HSP65 levels have been seen significantly higher in subjects with carotid ATS than in those without lesions [239], and that they remain elevated in subjects with progressive carotid ATS [242]. And other evidence derived from *in vivo* studies by immunization of mice with HSP65 mice, confirming its role in the development of fatty streaks and early ATS [243].

But it has been demonstrated that oral tolerization to HSP65 in $LDL^{-/-}$ mice determined a reduction in fatty streaks and a suppression of plaque formation, by the involvement of clonal anergy/deletion [244]. Moreover, an association between anti-HSP60/65 levels and carotid abnormalities was not found in SLE patients [195].

It has been demonstrated that HSP proteins and their antibodies play a key role in pathogenesis ATS, but probably their effect is masked in SLE patients.

It can be hypothesized that the formation of these oxLDL/β_2GPI complexes might be related to chronic inflammation of the vasculature and oxidative stress that occurs in autoimmune patients.

IgG anti-oxLDL/β_2GPI antibodies appear to be a serological marker for atherothrombotic risk in autoimmune patients and seem to be highly specific for APS and, probably are proatherogenic.

It has been suggested that while IgG antibodies are proatherogenic, IgM antibodies are protective, but the role of these autoantibodies in atherogenesis is still controversial [233,236].

CRP has been described as a prognostic factor of cardiovascular risk in both healthy subjects and patients with CAD [244,245], and it represents a good marker for monitoring responses to therapy in these patients [246]. CRP has been also shown to be a potential marker of unfavourable outcome in patients with CAD [247,248].

CRP can contribute to monocyte recruitment into the plaque, leading to foam cell formation [160]. CRP has been found in atherosclerotic lesions [175,249,250] where it is co-localized with complement deposits [47]. Whether CRP is a passive bystander or active player in atherosclerotic lesions has been largely debated up to now [250], since the studies carried out in humans and in animal models gave opposite results. Indeed, infusion of CRP in $Apob^{100/100}$ $LdlR^{-/-}$ mice or in $ApoE^{-/-}$ mice was protective [251] or had no effects [252] on atherosclerosis, respectively.

It has been demonstrated both *in vitro* and *in vivo* that CRP stimulates superoxide production in endothelial and smooth muscle cells, increasing atherosclerosis [253-256].

Whether CRP binds lipoproteins, is controversial. CRP does not seem to bind native LDL, but it binds enzymatically modified or oxLDL and very low density lipoprotein particles [255]. It has been recently demonstrated that the phosphorilcholine-binding site of CRP interacts with the 3β-OH group of cholesterol [257], playing a relevant role in foam cell formation. CRP

seems to promote release of metalloproteinases [255] and tissue factor activity [258], potentially contributing to plaque instability and atherothrombosis. Finally, it has been suggested that CRP-dependent activation of the complement system by enzymatically modified LDL might have a protective role in atherogenesis [257].

It has also been suggested that anti-CRP antibodies could be involved in atherogenesis, but this finding is still controversial [154,156,168].

In general population, SAP has been associated to CAD [259].

SAP has been detected in human atherosclerotic lesions by immunohistochemical analysis [260], where it colocalizes with apolipoproteins and seems to have a concentration about 50 times higher than plasma [260,261]. SAP binds tissue amyloid fibrils formed by amyloid-β (Aβ) [261], serum amyloid component A (SAA) [262], β_2-microglobulin [262], and apoC-II [262]. Interestingly, amyloid deposits are associated with CVD, and are present in up to 60% of aortic atherosclerotic lesions [262]. By binding Aβ and SAA, SAP inhibits their proteolytic degradation [261]; moreover, it promotes the self-association and tangling of apoC-II inhibiting the phagocytosis of primary macrophages and macrophage cell lines, modulating inflammatory response to amyloid fibrils in ATS [261]. It has been demonstrated both *in vitro* and *in vivo*, that SAP binds to FcγR for aggregated IgG and can inhibit fibrocyte differentiation [263]. Furthermore, the daily amministration of SAP to mice undergoing closed-chest ischemia virtually eliminated cardiac fibrosis, maintaining cardiac structure and function [261]. SAP can also bind oxLDL preventing the uptake of oxLDL by peritoneal macrophages thus suggesting a protective role in foam cell formation during early atherogenesis [264].

Although anti-SAP antibodies might potentially affect atherosclerotic process, no association between these antibodies and ATS has been reported to date.

By binding Fibroblast Growth Factor 2 (FGF2), PTX3 might interfere with plaque stability. In fact, FGF2 plays a key role in the induction, proliferation, migration, and survival of vascular smooth muscle cells as well as in inducing an excessive growth of SMC [158].

PTX3 increases tissue factor expression in mononuclear cells and endothelial cells [265,266] which can potentially enhance atherothrombosis [267]. Indeed, high levels of PTX3 are associated with acute myocardial infarction [267,268] and with an unfavourable outcome in patients with heart failure [269]. Moreover, immunohistochemical analysis of advanced atherosclerotic lesions revealed a high expression of PTX3 [270,271] where it is produced by

neutrophils as well as macrophages [271]. Finally, foam cells could induce the expression of PTX3 in atherosclerotic lesions by the generation of an acute inflammation [272].

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Table 1: Prevalence of systemic lupus erythematosus in adults, by location, in studies spanning 1975 to 2000.

Country (Area), study period, reference	Total rate per 100,000 (n)	Female rate per 100,000 (n)
United States, 1992, [23]	Total 42 (454)	Total 74 (401)
		Non-whites 78 (315)
		Whites 71 (86)
Curacao, 1989, [14]	Afro-Caribbean 48 (69)	84 (63)
United States, 1991, [25]	112 (9)	166 (8)
United States, 2000, [24]	California 108(-)	Total 184 (-)
		African American 406 (-)
		Hispanic 139 (-)
		Asian 93 (-)
		Whites 164 (-)
	Pennsylvania 150 (-)	Total 253 (-)
		African American 694
		Hispanic 245 (-)
		Asian 103 (-)
		Whites 203 (-)
United Kingdom, 1991, [26]	7 (20)	13 (20)
United Kingdom, 1990, [8,9]	Total 25 (147)	Total 45 (136)
Sweden, 1986, 1991, [15]	1986 42 (44)	
	1991 68 (41)	
Denmark, 1994, [17]	22 (104)	38 (93)
Norway, 1995, [14]	50 (89)	89 (79)
Saudi-Arabia, 1992, [30]	19 (2)	37 (2)
Australia, 1996 to 1998, [31]	Total 45 (108)	-

Table 2: Summary of abnormal immune responses and immunoregulation in patients with SLE.

Hyperactivated B cells

- Number of activated B cells producing Ig increased in peripheral blood
- Lupus B cells are more prone to polyclonal activation by specific antigens
- Raised IL-6 and IL-10 concentrations may promote B cell hyperactivity
- B cells responses to activatins signals are abnormal

Hyperactivated T cells

- Number of activated T cells increased in peripheral blood
- Abnormal early events of T cell activation
- Lupus T cells produce little IL-2 on stimulation

Abnormal phagocytic functions

- Phagocytic cells cannot bind or process immune complexes efficiently
- Phagocytosis of apoptotic cells impaired

Ig, immunoglobulin; IL, interleukin; SLE, systemic lupus erythematosus.

Table 3: Environmental factors that may be relevant in the pathogenesis of SLE

Chemical/physical factors

- Drugs
- Tobacco smoke
- Ultraviolet light

Infectious agents

- Bacteria
- Viruses

Hormones and environmental estrogens

- ?prenatal exposure to estrogens

Table 4: Titers of autoantibodies in general population and in SLE patients.

Autoantibodies	General population	Patients	p<	References
Anti-oxLDL	(O.D.) 0.6±0.1	(O.D.) 1.3±0.1	0.05	144
Anti-β2GPI	(mg/dl) 2±8	(mg/dl) 12±7.5	0.0001	213
Anti-HSP60/65	(U.I.) 260±276	(U.I.) 325±601	0.04	214
Anti-oxLDL/β2GPI	(U.I.) 19.18±7.68	(U.I.) 43.57±34.62	0.05	150

Table 5: Potential immunomodulatory functions and effects of pentraxins on atherogenesis

Pentraxin	Immunomodulatory functions	Effects on atherogenesis
CRP	Stimulates ROS production in EC and SMC leading to inhibition of NO production [253,254,256]	Endothelial dysfunction
	Contributes to monocyte recruitment [160]	Amplification of inflammatory loop
	Activates neutrophils and monocytes [160]	
	Increases the production of pro-inflammatory cytokines (IL-1, IL-6, TNFα) [161]	
	Activates complement cascade increasing adhesion molecule expression on EC [217]	
	Binds enzymatically modified or oxidized LDL [160,255]	Increase of foam cell formation
	Increases phagocyte activity of macrophages [166]	
	Promotes the release of metalloproteinases [255]	Plaque instability
	Increases the production of TGF-β [166]	Plaque stability
	Induces tissue factor activity [258]	Atherothrombosis
	Activates complement cascade inducing platelet granular release and upregulation of TF [218]	
SAP	Binds amyloid fibrils (Aβ, SAA, β2-microglobulin, apoC-II) inhibiting their proteolytic degradation [261,262]	Modulation of inflammation
	Increases phagocyte activity of macrophages [164,213]	Increase of foam cell formation
	Binds oxLDL preventing the uptake of oxLDL by macrophages [264]	Prevention of foam cell formation
	Aggregates with IgG inhibiting fibrocyte differentiation [263]	Plaque instability
PTX3	Regulates, along with C1q, phagocytosis by macrophages and dendritic cells [181–183]	Inhibition/stimulation of foam cell formation
	Binds FGF2 inhibiting the induction, proliferation, migration, and survival of SMC and excessive growth of SMC [158]	Plaque instability
	Increases TF expression in mononuclear and endothelial cells [265,266]	Atherothrombosis

CRP C-reactive protein, SAP serum amyloid P, PTX3 pentraxin 3, ROS reactive oxygen species, EC endothelial cell, SMC smooth muscle cells, NO nitric oxide, IL Interleukin, TNF-α tumor necrosis factor-α; LDL low density lipoprotein, TGF-β transforming growth factor-β; TF tissue factor, Aβ A-β fibrils, SAA serum amyloid A component; apoC-II apolipoprotein C-II, IgG G immunoglobulin, oxLDL oxidized low density lipoprotein, FGF2 fibroblast growth factor 2.

Table 6: Groups of autoantibodies involved in atherogenesis.

Strength of association	Autoantibodies
Defined	Anti-β2GPI
	Anti-oxLDL
	Anti-HSP (60/65)
Probable	Anti-HDL
	Anti-APO A-1
	Anti-ECA
	Anti-SAA
	Anti-oxLDL/β2GPI complex
	Anti-LPL
	Anti-cardiolipin
	Anti-phosphorylcholine
Possible	Anti-SAP
	Anti-insulin
	Anti-MBL
	Anti-CRP

EXPERIMENTAL STUDIES

1) OxLDL/β_2GPI COMPLEX AND ANTI-oxLDL/β_2GPI IN SLE: PREVALENCE AND CORRELATES

Manuscript accepted by J Autoimmun

2) IgG ANTI-PENTRAXIN 3 IN SYSTEMIC LUPUS ERYTHEMATOSUS

Manuscript in preparation

1) OxLDL/β_2GPI COMPLEX AND ANTI-oxLDL/β_2GPI IN SLE: PREVALENCE AND CORRELATES

ABSTRACT

High levels of oxLDL/β2GPI complexes and anti-complex IgG as well as IgM have been reported in SLE. We analyzed this complexes and antibodies against the complexes in SLE patients and evaluated their relationship with clinical and serological findings, traditional risk factors for atherosclerosis, and subclinical atherosclerosis. The prevalence and the levels of the complex and of anti-complexes autoantibodies were significantly higher in SLE patients than in healthy controls. The titers of oxLDL/β2GPI were significantly higher in patients with renal involvement and previous thromboembolic episodes and were correlated with the number of risk factors for atherosclerosis, whereas they were significantly lower in patients with neurological involvement. Both IgG and IgM anti-complexes antibodies were associated with APL. In conclusion, the oxLDL/β2GPI complexes as well as antibodies against the complexes are prevalent in SLE where they seem to be involved in organ damage.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypical autoimmune rheumatic disease involving a plethora of organ systems. Although the etiology is not clear, immunological, hormonal and viral factors have been shown to contribute to tissue injury in SLE. One of the major mechanisms of the damage is the generation of reactive oxygen species (ROS), leading to the formation of oxidized low density lipoprotein (oxLDL). Elevated levels of anti-oxLDL antibodies have been found in SLE patients where they correlated with anti-β_2 glycoprotein I (anti-β_2GPI) antibodies [1]. It has also been shown that oxLDL forms complexes with β_2GPI [1]. The first step in the formation of oxLDL/β2GPI complexes is an electrostatic interaction between ω-carboxyl functions and lysine residues of β_2GPI leading to an intermediate reversible complex. This interaction later progresses into a much more stable bond by an intramolecular formation of a Schiff base [1].

Serum oxLDL/β_2GPI complexes and antibodies directed to the complexes have been detected in patients with SLE, antifosfolipid syndrome (APS), Sjogren's syndrome (SS) and, in addition, in diabetes and infections - all together in patients with chronic vascular damage [1]. However, a clear association between oxLDL/β_2GPI complexes or antibodies and clinical manifestations or activity in SLE has not yet been reported. It has also been shown that both IgG and IgM anti-oxLDL/β_2GPI play a role in arterial thrombotic events and atherosclerosis in humans and animal models [1]. Concerning the Ig subclasses IgG have been described as proatherogenic, whereas IgM was reported to be protective [1]. Interestingly, atherosclerosis is accelerated in patients with SLE [2-5].

The aim of our study was to evaluate the prevalence and correlates of oxLDL/β_2GPI complexes and IgG and IgM anti-oxLDL/β_2GPI in SLE patients, including their potential effect on atherosclerosis predictors like carotid abnormalities.

MATHERIALS AND METHODS

Patients

The study group included 78 SLE patients classified according to the ACR criteria (65 female and 11 male; mean age 37±9 yrs (20-63 yrs), mean disease duration 10±4 yrs (4-23 yrs) (Table 1). Disease activity was measured by the ECLAM score. All patients signed an informed consensus.

SLE clinical data, serological abnormalities, including anti-dsDNA, anti-ENA, anti-CL, LAC, anti-HSP 65, anti-β2GPI, anti-oxLDL, traditional risk factors for atherosclerosis and carotid abnormalities by means of ultrasonography were evaluated, as previously described [3]. We also considered the number of risk factors for atherosclerosis in each patient.

At the time of sample collection, the mean (SD) cumulative prednisone equivalent dose previously taken by the patients was 32 (18) g; moreover, 22 patients (29%) were taking low dose aspirin, 50 (66%) hydroxychloroquine, and 37 (47%) immunosuppressants, including 28 (36%) azathioprine. Age and sex matched healthy subjects (n=72) served as controls (Table 1).

ELISA tests

Circulating levels of oxLDL/β_2GPI complexes and IgG or IgM anti-oxLDL/β_2GPI were detected with commercially available ELISA assays (Corgenix). For oxLDL/β_2GPI complexes, wells were coated with anti-β_2GPI specific for complexed β_2GPI. A HRP-labeled monoclonal antibody to human APOB100 was used for detection. For anti-oxLDL/β_2GPI antibodies, wells were coated with purified oxLDL/β_2GPI complexes. After the addition of the sera a HRP-labeled anti-human IgG or IgM antibodies were employed for detection. The cut-off levels for a positive reaction were determined from sera of healthy controls (61.9 IU/ml for oxLDL/β_2GPI complex; 34.7 units/ml for IgG anti-oxLDL/β_2GPI, and 11.0 units/ml for IgM anti-oxLDL/β_2GPI.

The sensitivity and specificity of these kits declared by the producer were determined in SLE and the values were: a sensitivity of 95.5% and a specificity of 99.4% for the complexes, a sensitivity of 53% and a specificity of 85% for IgG, and a sensitivity of 40% and a specificity of 94% for IgM.

Statistical analyses

The differences between groups were analyzed using the two-tailed *t* test and Fisher's exact test. The correlations between oxLDL/β_2GPI complexes or IgG or IgM anti-complexes levels and other continuous variables were evaluated by Pearson's correlation. SPSS 15.0 software was used for calculations.

RESULTS

The prevalence of oxLDL/β_2GPI complexes, IgG and IgM anti-complexes antibodies were higher in SLE than in healthy controls: 30 (38.5%) vs. 12 (17.1%), p=0.006; 64 (82.1%) vs. 16 (22.9%), p<0.001; 66 (84.6%) vs. 23 (32.9%), p<0.001; respectively (Figure 1). The levels of the complexes, IgG or IgM anti-oxLDL/β2GPI were significantly higher in SLE patients than in healthy subjects: 31.85±3.14 vs. 19.87±4.02, p=0.019; 43.09±3.93 vs. 17.53±1.05 p<0.001; 17.29±1.93 vs. 5.28±0.38 p<0.001, respectively (Figure 2). No relationship was found between complexes and IgG or IgM anti-complexes.

The titers of oxLDL/β_2GPI complexes were significantly higher in patients with renal involvement (p=0.042), and those with thrombotic events (72.1±0.4 vs. 30.8±3.1, p=0.036), and lower in patients with neurological involvement (p=0.023) (Table 2). IgG anti-oxLDL/β_2GPI levels were higher in patients positive for IgG anti-CL (p=0.041), or LAC (p=0.028) (Table 2). A correlation between IgG anti-oxLDL/β_2GPI and IgG anti-β_2GPI (R^2=0.211, p<0.001) was found (Figure 3). IgM anti-complexes antibodies levels were higher in patients positive for IgM-CL (p=0.007) (Table 2). No correlations exists between complexes and IgG or IgM anti-complexes antibodies and SLE activity or other clinical or serological disease factors. We observed a correlation between oxLDL/β_2GPI complexes and the number of risk factors for atherosclerosis (R^2=0.202, p<0.001) (Figure 4) and an inverse relationship between IgM anti-complexes and BMI (12.1±1.5 vs. 19.7±2.7, p=0.016). No relationship between oxLDL/β_2GPI complexes or anti-complexes antibodies and other risk factors for atherosclerosis, including carotid abnormalities, was observed.

DISCUSSION

Our results confirm that the oxLDL/β_2GPI complexes can be found in the circulation of patients with SLE where it is associated with renal involvement and thrombotic events [1,5,6]. In human SLE and in murine lupus the oxidative stress is enhanced, especially in patients with glomerulonephritis [6-8]. The urinary excretion of isoprostanes is increased and the activity of the antioxidant enzyme paraoxonase is decreased, consistent with enhanced lipid peroxidation related to renal involvement [6,7]. Using a murine model, Njoku et al [8] demonstrated that the reduction of lipid oxidation resulted in renal improvement. Along with oxLDL also the levels of β_2GPI are elevated in SLE patients [1-4], increasing the possibility of oxLDL/β_2GPI complexes formation. IgG and IgM anti-complexes antibodies were

associated with APL. In particular, IgG anti-oxLDL/β_2GPI correlated with IgG anti-β_2GPI and some anti-β_2GPI can bind oxLDL/β_2GPI [1,5].

We found an inverse relationship between oxLDL/β_2GPI complexes and neurological involvement. This was unexpected since thromboembolisms related or not with APL are involved in the pathogenesis of neuropsychiatric manifestations of SLE [9]. However, other autoantibodies [10] and/or pathogenetic mechanisms may blur the results. The accumulation of oxLDL/β_2GPI in the arterial wall seems to play a role in formation and rupture of plaques [1,2] and APL may be related to atherothrombosis in SLE patients [1-4].

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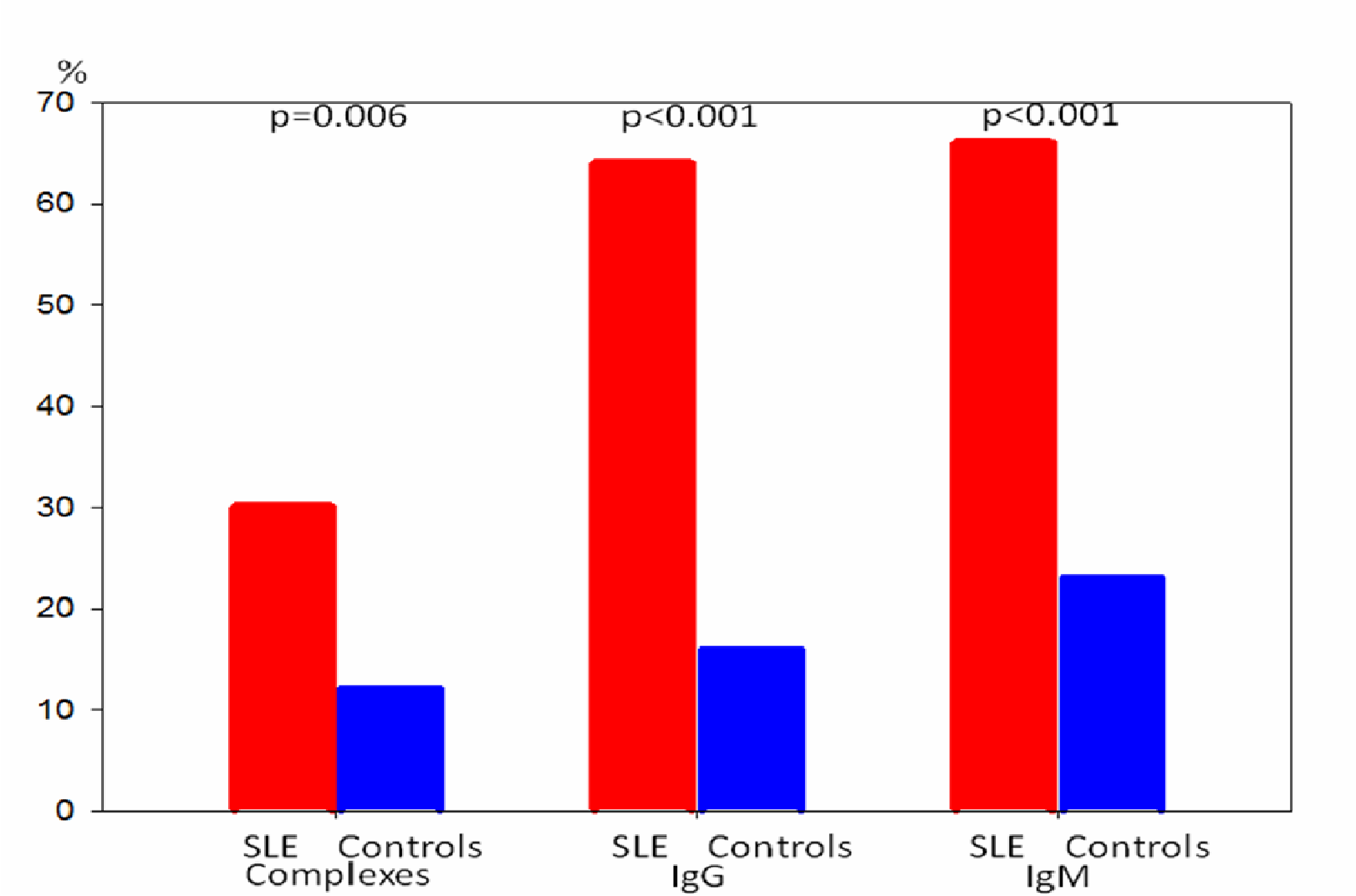

Figure 1 prevalence of oxLDL/β_2GPI complexes and IgG and IgM anti-complexes in SLE patients

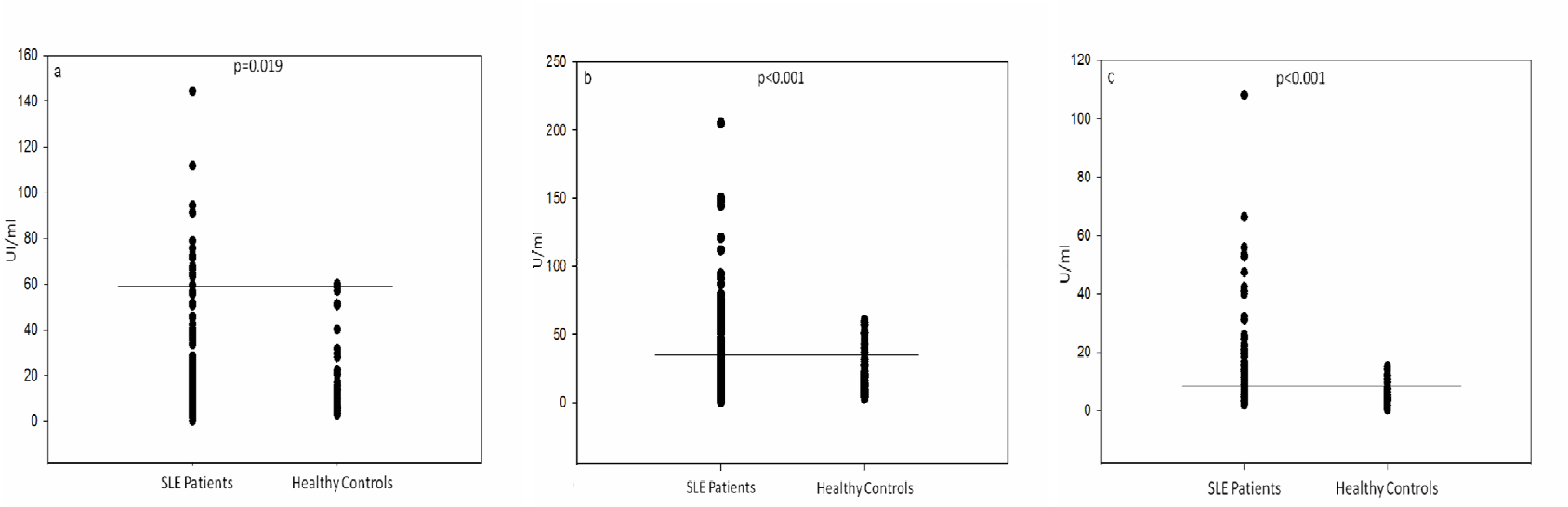

Figure 2 Titers in SLE patients and healthy controls of: a) oxLDL/β_2GPI complexes, b) IgG anti-oxLDL/β_2GPI, c) IgM anti-oxLDL/β_2GPI

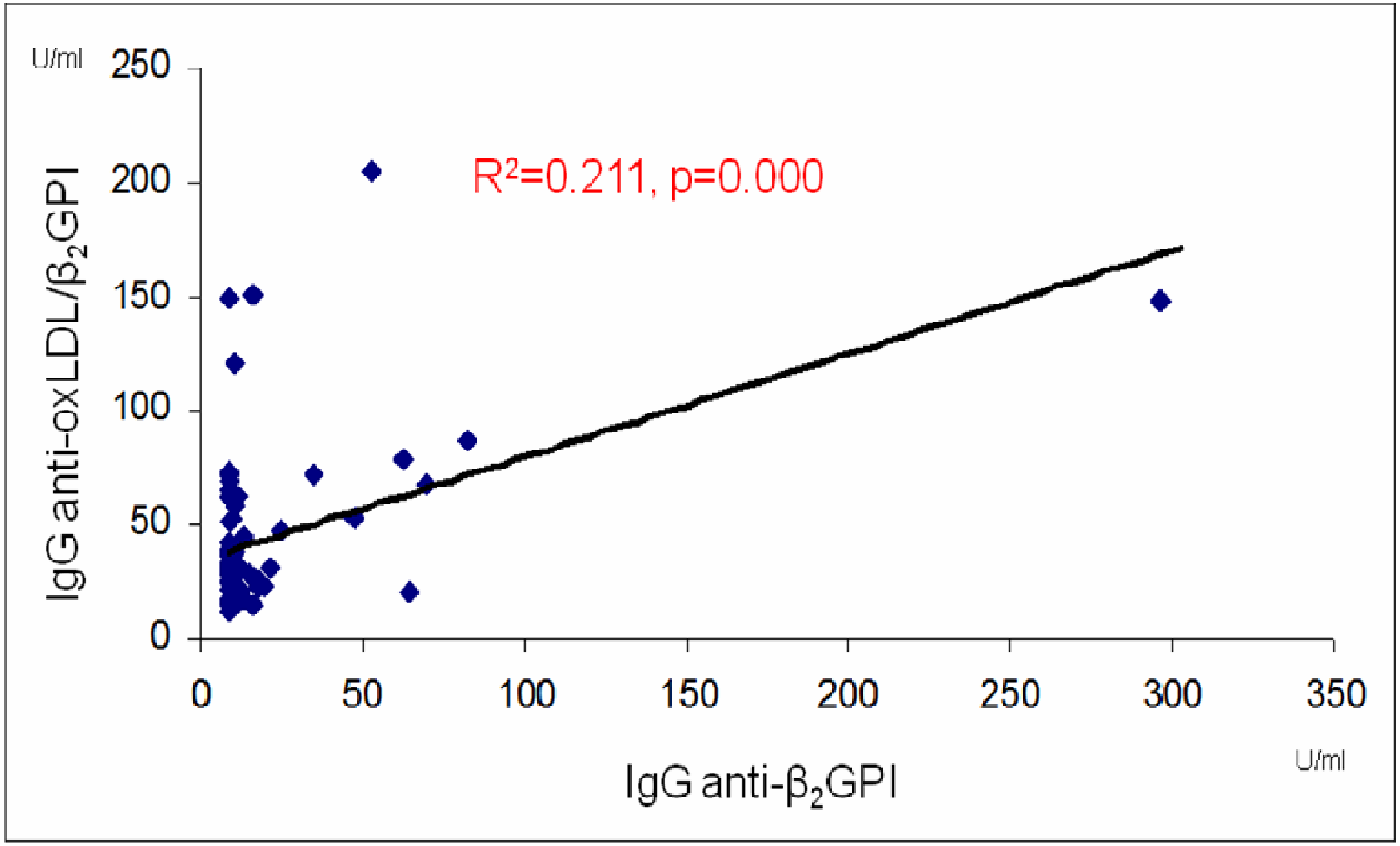

Figure 3 Correlation between titers of IgG anti-β_2GPI and titers of IgG anti-oxLDL/β_2GPI complexes in SLE patients

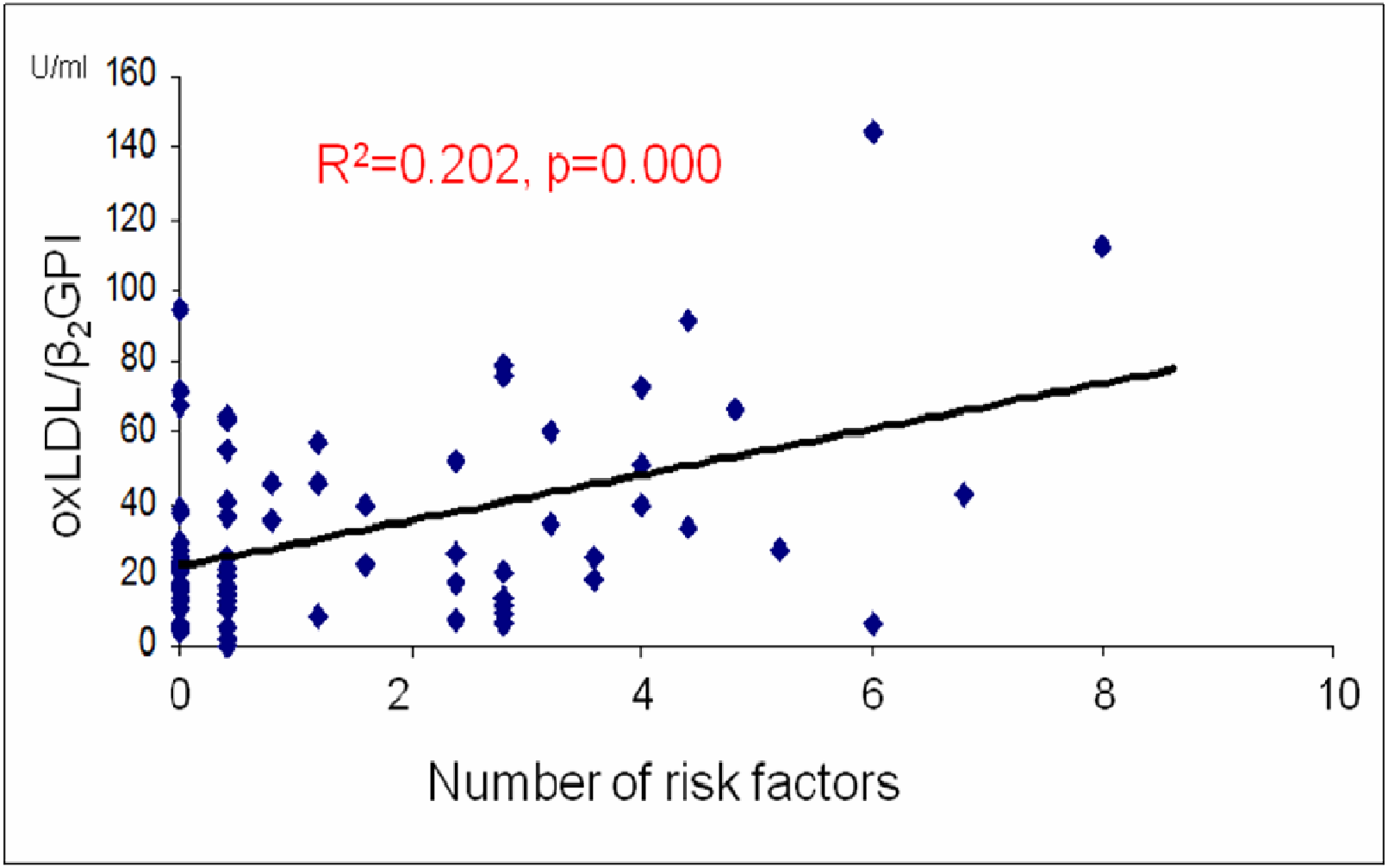

Figure 4 Correlation between titers of oxLDL/β_2GPI complexes and number of risk factors for atherosclerosis in SLE patients

Table 1 group of patients and healthy controls of the study

	SLE patients	Controls
Patients n.	78	72
Female	67	60
Male	11	12
Age Mean (±SD)	41±9	43±7
Disease duration (years)		
Mean (±SD)	39±9	

Table 2 Relationships between oxLDL/β_2GPI complexes, IgG or IgM anti-oxLDL/β_2GPI antibodies and clinical or serological data.

Clinical Factors	CNS Involvement			Renal involvement			Thrombosis		
	Yes		No	Yes		No	Yes		No
OxLDL/β2GPI complex	22±2.2	£	32±3.3	40±3.5	$	21±2.7	72±0.4	*	31±3.1
IgG anti-oxLDL/β2GPI	47±14		43±4.1	36±8.0		45±4.5	34±11		43±4.0
IgM anti-oxLDL/β2GPI	34±15		16±1.9	16±3.3		18±2.3	4.9±2.0		18±2.0

Serological data	LAC			aCL		
	Yes		No	Yes		No
OxLDL/β2GPI complex	44±12		30±2.9	34±5.7		31±3.5
gG anti-oxLDL/β2GPI	73±16	£	37±3.1	52±6.9	$	37±4.4
IgM anti-oxLDL/β2GPI	29±8.3		15±1.5	33±6.2	^	13±1.4

CNS Central Nervous System; LAC Lupus Anticoagulant; aCL anticardiolipin antibodies;
£= $p<0.03$; $= p<0.05$; *= $p<0.04$; ^= $p=0.01$.

2) IgG ANTI-PENTRAXIN 3 IN SYSTEMIC LUPUS ERYTHEMATOSUS

ABSTRACT

Objective: To evaluate the presence and clinical relevance of antibodies directed to pentraxin 3 (PTX3) in systemic lupus erythematosus (SLE) patients.

Methods: IgG anti-PTX3 were analyzed in the sera of 76 SLE patients (ACR criteria) and 76 matched healthy controls by home-made ELISA tests; the whole protein and 3 peptides obtained from the whole protein and identified as potential antigenic sites using Lasergene DNA program (DNA Star) (PTX3_1 from the N-terminal part; PTX3_2 from the central part; and PTX3_3 from the C-terminal part) were used as substrate.

Results: Compared to controls, SLE patients had higher levels of anti-PTX3, anti-PTX3_1 and anti-PTX3_2 ($p<0.001$, for all), as well as a higher prevalence of anti-PTX3, anti-PTX3_1 and anti-PTX3_2 ($p<0.001$, for all). Correlations were found between anti-PTX3 and anti-PTX3_1 ($r=0.502$, $p<0.001$) and between anti-PTX3 and anti-PTX3_2 ($r=0.714$, $p<0.001$). Agreement was found only between anti-PTX3 and anti-PTX3_2 ($k=0.554$). Univariate and multivariate analyses showed that anti-PTX3 and anti-PTX3_2 antibody levels were higher in patients with antiphospholipid antibodies and in those without glomerulonephritis.

Conclusions: Anti-PTX3 are significantly prevalent in SLE patients where they might provide protection from renal involvement.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease affecting different organ systems. To date 156 different autoantibodies in SLE patients have been reported,[1] but it is still unclear which are the principal autoantigens and how these autoantibodies are pathogenic.

A disturbed apoptosis and impaired clearance play a relevant role in the pathogenesis of SLE.[2]

The highly conserved family of pentraxins, C-reactive protein (CRP), serum amyloid-P (SAP), and the long pentraxin 3 (PTX3), are proteins, belonging to the humoral arm of innate immunity, which contribute to the removal of damaged and apoptotic cells.[3,4] PTX3 is the prototypical long pentraxin formed by 203-amino acid pentraxin-like domain coupled with an N-terminal 178-amino acid unrelated portion.[3,5]

It has been demonstrated that antibodies against CRP and SAP are characteristic of SLE, where they seem to contribute to clearance impairment,[4-8] and are related to the disease activity and renal involvement.[9-11] The occurrence of anti-PTX3 antibodies has been hypothesized, albeit never demonstrated. The aim of our study was to evaluate the presence of IgG anti-PTX3 antibodies in SLE patients.

PATIENTS AND METHODS

Study Group

The study group included 76 SLE, patients classified according to the American College of Rheumatology (65 female and 11 male; mean age 37±9 years, range 20-63 years, mean disease duration 10±4 years, range 4-23 years). SLE disease activity was measured by the European Consensus Lupus Activity Measurement score.

SLE clinical data, including traditional risk factors for atherosclerosis,[12] and serological abnormalities, including anti-double stranded DNA, anti-cardiolipin, lupus anticoagulant, anti-heat shock protein 65 (HSP65), anti-β_2 glycoprotein 1 (β_2GPI), anti-oxidized low density lipoproteins (oxLDL), oxLDL/β_2GPI and anti-oxLDL/β_2GPI complex,, as previously described, [12,13] were evaluated.

At the time of sample collection, the mean (standard deviation, SD) cumulative prednisone equivalent dose previously taken by the patients was 31.7 (18.3) g; moreover, 22 patients

(29%) were taking low dose aspirin, 50 (66%) hydroxychloroquine, and 37 (47%) immunosuppressant drugs, including 28 (36%) azathioprine.
Seventy two healthy subjects, matched for age and sex with SLE patients, were evaluated as controls.

Protein and peptides

Autoantibodies against the whole PTX3 and antibodies towards 3 different peptides, identified from the whole protein as possible antigenic sites using the Lasergene DNA program (DNA Star) were tested. The first peptide (PTX3_1:ENSDDYD**LM**YVNLDN) derives from the N-terminal portion (from aa 18 to aa 32), the second (PTX3_2:LFSYGTK**RNP**YEIQLYL) from the central part (from aa 224 to aa 240) and the third (PTX3_3:SVLSNEE**IRE**TGGAESC) from the C-terminal portion (from aa 341 to aa 357). The underlined sequences are the hypothetical antigenic sites.

ELISA tests

To evaluate the presence of anti-PTX3 and antibodies to the 3 peptides, we performed home-made ELISA tests using the same protocol. Briefly, Maxisorp immunoplates (Nalge Nunc) were coated with 50 µl/well of the antigen diluted in PBS at the concentration of 5 µg/ml and incubated overnight at 4°C. The wells were blocked with 3% BSA in PBS and incubated at room temperature for 2 hours. After washes, the sera were added at the concentration of 1:200 in 1% BSA/PBS and incubated in double at room temperature for 4 hours. After washing, alkaline phosphatase-conjugated anti-human IgG (H+L) (Jackson) was added and incubated for 1 hour at 37°C. After washes, *p*-nitrophenyl phosphate (Sigma) was added. Optical density (OD) was measured at 405 nm.

Inhibition tests with BSA

We performed an inhibition test with BSA to evaluate if it can interfere with the autoantibodies against PTX3 or to the other 3 peptides. Sera of 3 patients positive for all autoantibodies were incubated overnight with different concentrations of BSA, from 400 µg/ml to 0 µg/ml in PBS, at the final diluition of 1:300. All ELISA tests were performed as described before, apart from blocking (0.5% gelatin/PBS).

Statistical analysis

The values were expressed as mean OD of the doubles of each serum. The optimal cut-off levels were determined by Receiving Operating Characteristics (ROC) curve analyses, in which sensitivity was calculated in 76 SLE patients and specificity in 76 healthy subjects. We used 0.278 as cut-off for anti-PTX3 (48.7% sensitivity and 94.3% specificity), 0.287 for anti-PTX3_1 (60.5% sensitivity and 96.1% specificity), 0.332 for anti-PTX3_2 (67.1% sensitivity and 92.1% specificity) (Figure 1). Since ROC curve analysis for anti-PTX3_3 antibodies was not significant, we chose 0.136 as the cut-off level, i.e. the mean OD+2 SD of the value in controls.

The differences between groups were analyzed using the two-tailed *t* test and Fisher's exact test. The correlations between anti-PTX3 or anti-peptide levels and other continuous variables were evaluated by Pearson's correlation, the agreement between anti-PTX3 and the other anti-peptide by kappa statistics. SPSS 15.0 software was used for calculations.

RESULTS

Anti-PTX3 autoantibodies

The inhibition test with BSA was negative for anti-PTX3 and for antibodies against the 3 peptides.

The levels of anti-PTX3, anti-PTX3_1 and anti-PTX3_2 peptides were significantly higher in SLE patients than in healthy controls: 0.33±0.21 vs. 0.13±0.09, $p<0.001$; 0.37±0.22 vs. 0.14±0.08 $p<0.001$; 0.45±0.28 vs. 0.17±0.11 $p<0.001$, respectively (Figure 2). The prevalence of anti-PTX3, anti-PTX3_1 and anti-PTX3_2 was higher in SLE than in healthy controls: 37 (48.7%) vs. 4 (5.7%), $p<0.001$; 46 (60.5%) vs. 3 (3.9%), $p<0.001$; 51 (67.1%) vs. 6 (7.9%), $p<0.001$; respectively. No difference in the concentration or in the prevalence of anti-PTX3_3 between patients and controls was observed.

Correlations were found between anti-PTX3 and anti-PTX3_1 ($r=0.502$, $p<0.001$) and between anti-PTX3 and anti-PTX3_2 ($r=0.714$, $p<0.001$). Agreement was found only between anti-PTX3 and anti-PTX3_2 (kappa=0.554).

Relationship with clinical and serological data

No association or correlation between anti-PTX3 or anti-PTX3 related peptides and disease activity was observed. Anti-PTX3 and anti-PTX3_2 antibody levels were significantly lower in patients with renal involvement than in those without: 0.17±0.07 vs. 0.35±0.21, $p<0.001$ and 0.21±0.15 vs. 0.48±0.28, $p=0.013$, respectively.

Anti-PTX3 antibodies were observed in 25 (32.9%) patients with and in 12 (15.8%) without antiphospholipid antibodies (aPL, i.e IgG and/or IgM anticardiolipin and/or lupus anticoagulant) ($p=0.036$); anti-PTX3_2 antibodies in 34 (44.7%) aPL positive and in 17 (22.4%) aPL negative patients ($p=0.004$).

The relationships between anti-PTX3 or PTX3_2 antibodies and glomerulonephritis or aPL were confirmed by multivariate analysis (Table 1).

No other significant relationships between anti PTX3 and anti-PTX3 related peptides and clinical or serological abnormalities were observed.

DISCUSSION

SLE is a prototypical autoimmune disease characterized by a disturbed apoptosis and impaired clearance.[2] Autoantibodies against protective molecules, like CRP and SAP, which are involved in the removal of apoptotic materials, have been reported in SLE.[2] Anti-CRP and anti-SAP have been described as significantly prevalent in SLE patients and were related to disease activity and renal involvement.[9-11]

PTX3 is a molecule belonging to innate immunity. To date, autoantibodies against this protein have never been reported. In our study we demonstrated that anti-PTX3 antibodies were significantly prevalent in SLE patients and that anti-PTX3 antibody levels were higher in SLE than in healthy subjects. Interestingly, antibodies towards two PTX3-related peptides, PTX3_1 and PTX3_2, were highly correlated with anti-PTX3 antibody and showed similar clinical and serological associations.

Like other pentraxins, PTX3 is considered a protective molecule;[2,3,5] however, it is still debated whether or not autoantibodies against molecules belonging to innate immunity are protective[2,4] or pathogenic.[3,5]

Differently from previous studies on anti-CRP and anti-SAP,[9-11] we found that anti-PTX3 or anti-PTX3 derived peptides were associated neither with disease activity nor with glomerulonephritis, which is considered the immune-complex prototypic feature in SLE.[12]

It has been demonstrated that CRP and SAP may facilitate the phagocytosis of apoptotic and necrotic material or immune-complexes by macrophages through the binding of Fcγ receptor, [2,6,7] and a defect of these elements, caused by autoantibodies, may impact the development of SLE leading to chronic inflammation in organ tissues.[7]

Differently from CRP and SAP which are mainly produced in the liver in response to IL-6, the main source of PTX3 is mononuclear phagocytes and myeloid DCs.[3] The *in situ* release of PTX3 at the inflammation site[2,3] is a relevant protective event since it promotes the removal of pathogens by phagocytes, but, unfortunately, it inhibits the removal of apoptotic materials leading to the impairment of autoantigen clearance[11,13] and, in turn, potentially contributes to SLE immunopathology.

It has also been shown that PTX3 may activate complement and coagulation cascades.[3] Data collected so far in different diseases indicate a correlation between PTX3 plasma levels and disease severity suggesting a possible role as marker of pathology.[3] Thus, anti-PTX3 may interfere with PTX3 functions exerting a protective effect on tissue from damage, including kidneys in SLE patients.

Differently from anti-CRP antibodies which were associated with aPL and aPL syndrome,[11] anti-PTX3 and anti-PTX3_2 were associated with aPL, but not with thrombotic events or foetal losses. Since PTX3 may increase the expression of tissue factor,[3] we might speculate that anti-PTX3 could exert a protective effect even in thrombotic episodes or foetal complications.

Autoantibody levels might have been reduced due to the treatment taken by our patients; however, no relationship between anti-PTX3 antibodies and drugs, particularly immunosuppressants was found.

In conclusion, anti-PTX3 antibodies are significantly prevalent in SLE patients where they might provide protection from renal involvement. The antigenic properties of PTX3_1 and, primarily, PTX3_2 seem to be similar to those of PTX3 suggesting their potential use, as substrate, for further analysis.

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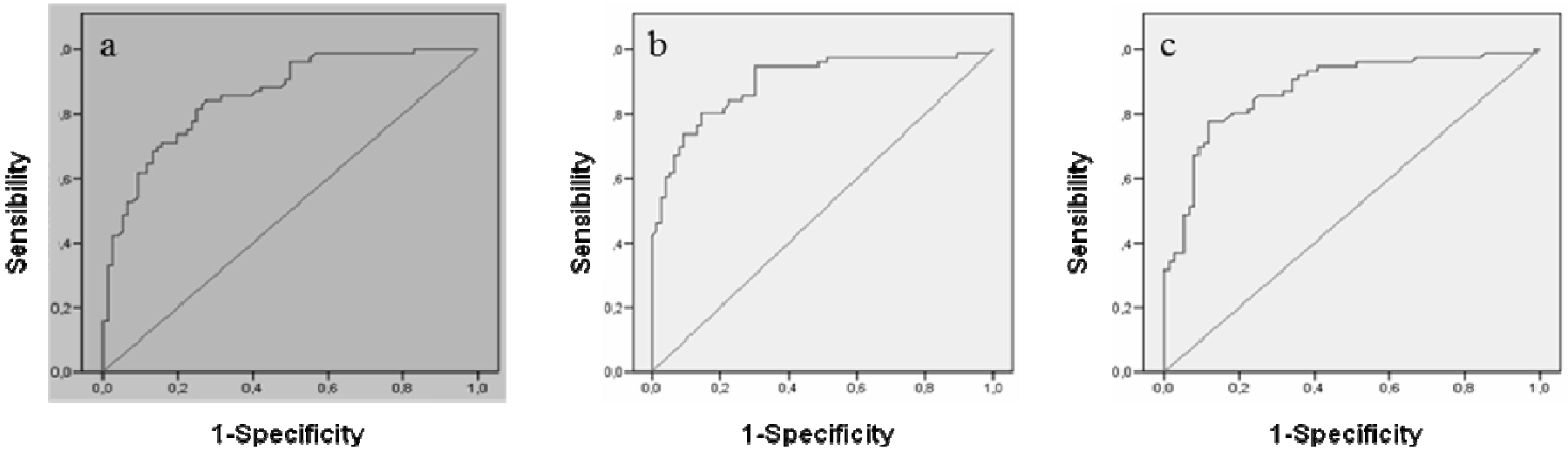

Figure 1. ROC curve analyses between healthy controls and SLE patients to detect the cut-off levels of: **a)** IgG anti-PTX3; **b)** IgG anti-PTX3_1; **c)** IgG anti-PTX3_2.

Footnotes:

Anti-PTX3= antibodies against the long pentraxin 3;

Anti-PTX3_1= antibodies against the peptide derived from the N-terminal portion of the long pentraxin 3;

Anti-PTX3_2= antibodies against the peptide derived from the central part of the long pentraxin 3.

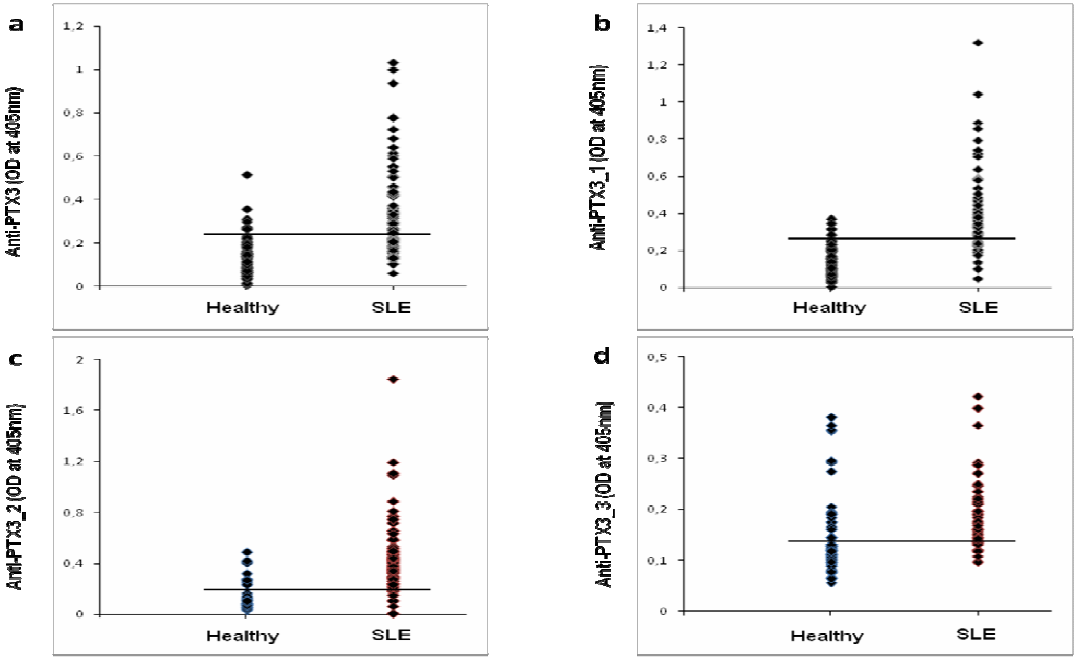

Figure 2. Titers of anti-PTX3 and anti-PTX3 related antibodies in healthy controls and SLE patients: **a)** IgG anti-PTX3; **b)** IgG anti-PTX3_1; **c)** IgG anti-PTX3_2; **d)** IgG anti-PTX3_3.
The lines represent the cut-off level of each antibody.

Footnotes:

Anti-PTX3= antibodies against the long pentraxin 3;
Anti-PTX3_1= antibodies against the peptide derived from the N-terminal portion of the long pentraxin 3;
Anti-PTX3_2= antibodies against the peptide derived from the central part of the long pentraxin 3;
Anti-PTX3_3= antibodies against the peptide derived from the C-terminal portion of the long pentraxin 3.

Table 1. Multiple linear regression analysis (best model) of factors associated with anti-PTX3 and anti-PTX3-2 antibodies in the 76 SLE patients.

Dependent variable: anti-PTX3 antibodies			
Indipendent Variables	**Coefficient**	**F**	**p**
Anti-phospholipid antibodies	0.320	8.435	0.005
Renal involvement	-0.225	6.571	0.002
R^2=0.153			
Dependent variable: anti-PTX3_2 antibodies			
Independent Variables	**Coefficient**	**F**	**p**
Anti-phospholipid antibodies	0.339	9.625	0.003
Renal involvement	-0.257	8.059	0.001
R^2=0.181			

PTX3: pentraxin 3

PTX3_2: peptide 2 belonging to the whole molecule of pentraxin 3

DISCUSSION AND CONCLUSIONS

ATS is considered, at least in part, a chronic immunoinflammatory disease, because it involves the components of both innate and adaptive immunity including some autoantibodies. On the other hand, atherosclerosis is accelerated in many autoimmune diseases, such as SLE, due to traditional and non-traditional risk factors.

SLE is a prototypic autoimmune disease, involving many organ systems, whose etiology is still unclear. Many factors can contribute to SLE development and evolution, and some of them might mask the pathogenic role of autoantibodies in the development of ATS.

In fact, there are clear evidences that in SLE traditional risk factors including hypercholesterolemia, hypertension, age and cigarette smoking, play a major role in the progression of the atherogenic process, but the data on the role of autoantibodies are conflicting probably due to the influence of other factors including renal involvement and corticosteroid terapy.

It has been demonstrated that serum levels of anti-oxLDL, anti-β2GPI, anti-HSP60/65 and anti-oxLDL/β2GPI complexes are higher in SLE patients compared to healthy controls; very interestingly these autoantibodies were observed within atherosclerotic plaques. However, no relationship was found between the titers of these autoantibodies and the IMT in SLE patients.

In our cohort of patients, we detected circulating oxLDL/β_2GPI complexes in association with renal involvement and thrombotic events. Supporting these data, it has been demonstrated both in humans and in animal models of SLE, that the oxidative stress is enhanced, especially in patients with lupus glomerulonephritis.

Interestingly, we also found an inverse relationship between oxLDL/β_2GPI complexes and neurological involvement, whereas it has been shown that aPL are involved in the pathogenesis of thromboembolisms and neuropsychiatric manifestations of SLE.

No relationship between oxLDL/β_2GPI complexes and IMT or atherosclerotic plaque was found, but we observed that the titer of the complexes was associated to the number of risk factors for ATS. The accumulation of oxLDL/β_2GPI in the arterial wall seems to play a role in the formation and rupture of plaques and aPL may be related to atherothrombosis in SLE patients.

We did not observed any relationship between oxLDL/β_2GPI complexes and IgG or IgM anti-complexes. Although in our study we observed a positive correlation between IgG or IgM

anti-complexes antibodies and IgG or IgM anti-aCL antibodies, no relationship between these autoantibodies and IMT, plaque or number of risk factors for ATS was found.

In RA, not only a relationship between some traditional risk factors, such as hypertension and hypercholesterolemia, and titers of aCL antibodies, but also a correlation between titers of aCL and IMT was observed.

The highly conserved family of pentraxins are considered protective molecules in autoimmune diseases, but it has been shown that they actively participate in the progression of both SLE and atherogenesis throughout various mechanisms including the interaction with modified lipoproteins, such as oxLDL, leading to foam cell formation within the plaque, and the activation of the classical complement cascade. The levels of circulating pentraxins were found to be higher in patients with cardiovascular diseases (CVD) and are considered predictors of CVD in general population. Immunohistochemical studies conducted on human aortas showed that CRP, SAP and PTX3 are expressed in atherosclerotic plaques.

It has been shown that pentraxins can become autoantigens; however, the process leading to the transformation of pentraxins in autoantigens is still unknown. Anti-CRP and anti-SAP antibodies were observed in patients with autoimmune diseases, especially in SLE, where they seem to be related to disease activity and renal involvement.

To date, autoantibodies against PTX3 have never been reported. We demonstrated that anti-PTX3 antibodies and, interestingly, antibodies towards two PTX3-related peptides PTX3_1 and PTX3_2, having similar antigenic properties to PTX3_whole molecule, were significantly prevalent in SLE patients and their levels were higher in SLE than in healthy subjects.

Our results also demonstrated that anti-PTX3 or anti-PTX3 derived peptides play a different role from the anti-CRP and anti-SAP. In fact, they were found to be associated neither with disease activity nor with glomerulonephritis, which is considered the immune-complex prototypic feature in SLE.

The *in situ* release of PTX3 at the inflammation site, for example glomerula, could play a protective role promoting the clearance of apoptotic cells and immune-complexes by macrophages resident cells, thus preventing SLE onset. Anti-PTX antibodies can enhance this mechanism through the involvement of FcγR in macrophages.

In our patients, an association between anti-PTX3 and anti-PTX3_2 and aPL or aPL syndrome was found, but not between antibodies levels and thrombotic events, suggesting a protective effect from atherosclerotic plaque rupture.

For some of these autoantibodies it has been demonstrated an association with some clinical manifestations of SLE, but not with the disease activity. Moreover, their potential role in the development of ATS in SLE is difficult to demonstrate due to the influence of other immunoinflammatory mechanisms characteristic of the disease.

It has been shown that autoantibodies of IgM isotype can have a protective role for both SLE and atherogenesis, since they can reduce foam cells formation and plaque blocking the interaction between autoreactive clones and autoantigens.

Some investigators demonstrated the efficacy of induced oral tolerance or hyperimmunization with autoantigens to reduce plaque formation, causing an anergy/depletion of reactive clones. his has been shown in LDL-receptor deficient mice, in which oral tolerance to β2GPI suppresses early atherosclerosis as well as in apo-E-deficient mice hyperimmunized with oxLDL. Moreover, it has been demonstrated that immunization of LDL−/− mice with oxLDL, is protective from inflammation and plaque formation, inhibiting the uptake of oxLDL by macrophages..

Many other studies are required to explain the role of autoantibodies in the pathogenesis of ATS, in particular to demonstrate their role in accelerated ATS of SLE patients. Moreover, it is important to clarify if IgM autoantibodies can exert a protective role in ATS and if oral tolerance could be effective in reducing the foam cells formation.

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