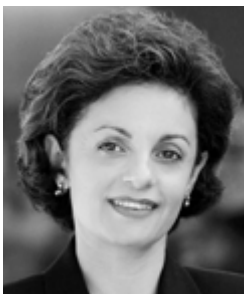
Editorial

Autoantibodies: Innocent Bystander or Key Player in Immunosenescence and Atherosclerosis?

Over the last couple of decades, the paradigm of premature immunosenescence in systemic autoimmune diseases such as rheumatoid arthritis (RA) has emerged[1,2]. The theory of immunosenescence suggests that immune dysfunction with aging is associated with increased autoantibody production. However, it is not entirely clear whether autoantibodies are innocent bystanders of the aging process or whether they play an important role in chronic diseases of aging such as atherosclerosis. There is evidence that certain autoantibodies may act directly in the pathogenesis of atherosclerosis. The role of autoantibodies in atherosclerosis and aging is particularly intriguing because there is an increased risk of cardiovascular (CV) disease and mortality in systemic autoimmune diseases, frequently associated with autoantibody production, that is not explained by traditional risk factors for atherosclerosis alone[3-10]. Further, recent evidence suggests that systemic autoimmune diseases may be associated with premature immunosenescence[1,2,11]. Could the autoantibodies produced through premature immunosenescence in systemic autoimmune diseases explain the increased atherosclerosis and CV mortality in these diseases? To examine this question, we review the current literature in the following areas: (1) the theory of immunosenescence and the role of autoantibodies in this process; (2) the role of autoantibodies in atherosclerosis and CV disease; (3) premature immunosenescence in systemic autoimmune diseases; and (4) prevalence studies of autoantibodies in the healthy elderly, which seem to support an "innocent bystander" effect of autoantibodies in select individuals.

IMMUNOSENESCENCE THEORY AND ROLE OF AUTOANTIBODIES IN IMMUNOSENESCENCE

The immunosenescence theory suggests that both cell- and antibody-mediated immune responses decline with age, with an increase in the numbers of senescent T cells with autoreactivity and proinflammatory capabilities. This immune dysfunction may cause dysregulation of cell homeostasis with a tendency to over-respond to endogenous antigens, and may be associated with increased neoplasia and susceptibility to infections in the general aging population[12]. Although B and T lymphocytes have a decline in response to antigenic stimulus with age, there is a paradoxical increase in autoantibodies. The naive T cell population decreases with aging, and there is a shift from Th1 to Th2 cytokine profiles with stimulation, which could augment B cell-mediated autoimmune disorders. In most cases, the numbers of autoreactive antibodies have no clinical significance and their numbers are kept low by tolerance mechanisms. As the immune system shifts to a Th2 response with aging, tolerance mechanisms fail and autoreactive antibodies increase[13,14].

In addition, there is evidence that supports the role of immunosenescence in diseases of aging. In a large Danish population-based cohort study, organ-specific autoantibodies were found to be more prevalent among centenarians with higher comorbidity than among less disabled subjects[15]. Further, low levels of autoantibodies against amyloid beta (Aß) peptide may lead to increased risk of development and/or progression of Alzheimer's disease[13,16]. These studies suggest that immune dysfunction through immunosenescence may lead to the presence of certain autoantibodies, which may be involved in development of chronic diseases of aging.

Alternatively, the autoantibodies that develop during immunologic aging may not necessarily mediate tissue damage. Rather, they may be markers for chronic diseases of aging, but not necessarily causal. Further scientific investigation of specific autoantibodies, including those that traditionally are used as markers of rheumatic diseases, is needed to better define whether they directly mediate tissue damage during the aging process.

AUTOANTIBODIES IN PATHOGENESIS OF ATHEROSCLEROSIS AND RISK OF CARDIOVASCULAR DISEASES

Before reviewing the literature on the potential atherogenic role of autoantibodies, we will first review the pathogenesis of atherosclerosis. Inflammation promotes endothelial cell (EC) activation, upregulation of adhesion molecules [such as P and E selectins, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1)], production of cytokines and upregulation of MHC class

II molecules on inflammatory cells. These events lead to the attachment and migration of leukocytes and monocytes into the vessel wall. The monocytes differentiate into macrophages in the intima, with uptake of lipids to form foam cells and the "fatty streak." Further inflammatory cell recruitment and proliferation of smooth muscle cells, through decreased availability of nitric oxide (NO) (a key feature of endothelial dysfunction), lead to the formation of plaque[4,17]. These known mechanisms of atherogenesis suggest that inflammation, which is one hallmark of systemic autoimmune diseases, mediates in part the premature atherosclerosis in these diseases.

However, besides inflammation, another hallmark of systemic autoimmune diseases is the presence of autoantibodies, which may also play an important role in endothelial dysfunction and hence atherogenesis. Certain autoantibodies, including antineutrophil cytoplasmic antibodies (ANCA) and antiphospholipid antibodies (aPL), have been shown to bind to and activate EC[18-20]. Specifically, in patients with Wegener's granulomatosis or microscopic polyangiitis, proteinase 3 (Pr3), myeloperoxidase (MPO), and anti-EC antibodies (AECA) were found *in vitro* to upregulate E-selectin and interleukin 6 (IL-6) production, which are early steps leading to vascular injury and atherosclerosis[18]. aPL have been shown to activate EC by increasing the expression of leukocyte adhesion molecules. EC activation is dependent upon the presence of $ß_2$-glycoprotein I[20]. Anti-$ß_2$-glycoprotein I antibodies upregulate mRNA expression of proinflammatory mediators through nuclear factor-κB translocation and the signaling cascade triggered by Toll-like receptors, quite comparable to that reported after EC incubation with standard proinflammatory agonists (IL-1, tumor necrosis factor-α, and lipopolysaccharide)[19]. The direct activation of EC through binding of such autoantibodies could directly explain accelerated atherosclerosis.

Further, Quadros, *et al* have shown that IgG AECA binding to human umbilical vein EC (HUVEC) *in vitro* is significantly higher in patients with RA, Felty's syndrome, systemic lupus erythematosus (SLE), and lupus anticoagulant compared to controls[21]. In RA and Felty's syndrome, rheumatoid factor (RF) was shown to augment immunoglobulin binding to EC. Treatment of RA sera to remove immune complexes and RF activity resulted in a decrease in IgG binding to HUVEC. Five of 9 RA sera contained cytotoxic IgG antibodies that fixed complement, and these patients also had vasculitis. These studies suggest that a group of autoantibodies in collagen vascular disorders may mediate endothelial dysfunction and may be important in vasculopathies associated with these disorders[21].

In addition, heat shock proteins (HSP) have been implicated in the pathogenesis of atherosclerosis, as EC express HSP upon activation[4,22,23]. Dying EC release soluble HSP, which are directly proatherogenic by stimulating macrophages to produce proinflammatory cytokines and upregulate expression of adhesion molecules on EC. Activated B cells produce autoantibodies to HSP, which can react with HSP expressed on activated EC[4]. Human serum anti-hsp65 antibodies were found *in vitro* to act as autoantibodies against hsp60 on stressed EC and were able to mediate endothelial cytotoxicity[24]. Thus, the humoral immune reaction to hsp60 may also play an important role in the pathogenesis of atherosclerosis.

A growing body of work has implicated the role of enhanced oxidation of lipids, namely lipid peroxidation of low density lipoprotein (LDL) into oxidized LDL (oxLDL), in atherogenesis, because it is chemotactic for macrophages and lymphocytes, activates T cells, and is taken up by macrophages in the atherosclerotic plaque[4,25-27]. Autoantibodies against oxLDL have been found to be related to atherosclerosis, with elevated anti-oxLDL antibodies found in atherosclerotic lesions, in patients with atherosclerosis, and in patients with SLE[4,23,27]. However, other studies have suggested that anti-oxLDL may actually prevent plaque formation by scavenger effect, clearing oxLDL from the circulation[28]. Whether the immune response to oxLDL is primarily proatherogenic or antiatherogenic is still controversial[4,23,27,29,30].

In addition, AECA in patients with SLE has been shown *in vitro* to induce a dose-dependent EC activation by increasing functional adhesion of monocytes, upregulating adhesion molecules (E-selectin, ICAM-1, VCAM-1), and secretion of IL-6[31]. This EC activation was not seen at all in healthy controls or AECA-negative SLE patients' sera. Further, the AECA IgG effects were not related to aPL or anti-DNA activities, suggesting a specific pathogenic role of AECA in vessel damage in SLE[31].

Another potential mechanism of atherothrombosis mediated by aPL antibodies is through decreased binding of annexin V to EC[32]. Annexin V can form 2-dimensional crystals on lipid bilayers, creating a shield with antithrombic properties[33]. Cederholm, *et al*[32] showed that binding of annexin V to EC is significantly decreased when EC are cultured with plasma from SLE patients with CV disease, as compared with SLE patients without CV disease and with controls. It is hypothesized that an important early step in atherogenesis may be inhibition of annexin V binding, leading to increased phospholipase A_2 (PLA_2) levels, which then cause much of the proinflammatory effects of oxLDL[32].

In addition to the *in vitro* models of specific pathogenic autoantibodies discussed above, population-based studies also support the association between autoantibodies and increased CV disease and overall mortality. Gabriel, *et al*[34] found that RF positivity is a significant predictor of CV disease and mortality in the general population, even after adjusting for the presence of rheumatic diseases. Heliovaara, *et al*[35] found in a longitudinal population-based Finnish study that patients without arthritis with "false-positive RF" titers ≥ 128 were found to have an increased rel-

ative risk (RR) of CV deaths (RR 1.74, 95% confidence interval 1.06–2.86). Interestingly, for patients with arthritis, when adjusted for age, sex, and smoking, the RR of death from any cause in RF-positive subjects was 1.61 (95% CI 1.03–2.51), whereas RF-negative non-erosive arthritis was not associated with mortality (RR 1.03, 95% CI 0.72–1.49). Aho, *et al*[36] showed in a Finnish case-control study nested within a large population cohort that individuals with positivity for the autoantibodies RF or ANA had a relative risk of death due to CV causes of 3.3 ($p < 0.01$) compared to individuals without positive RF or ANA.

These population-based studies generate the hypothesis that autoantibodies like RF and ANA, even in the absence of inflammation from rheumatic diseases, may mediate increased atherosclerosis. Indeed, Aho, *et al*[36] raised the intriguing question whether these autoantibodies were only indirect indicators of some underlying process, or whether they have a direct pathogenetic role. Aho, *et al*[36] suggested that immunosenescence could contribute to shorter lifespans due to depressed T cell function, with a concomitant increase in autoantibodies with aging. This hypothesis would seem to be supported by the aforementioned pathogenetic and population-based studies implicating a significant role of autoantibodies in atherogenesis, CV disease, and mortality.

PREMATURE IMMUNOSENESCENCE IN SYSTEMIC AUTOIMMUNE DISEASES

There is already evidence that premature immunosenescence, or accelerated aging of the immune system, exists in systemic autoimmune diseases, particularly RA. Crowson, *et al*[11] recently performed a population-based study to examine whether the mortality pattern in seropositive patients with RA was consistent with the concept of accelerated aging by comparing the observed mortality rates in RA to age-accelerated mortality rates from the general population. The authors assembled a population-based inception cohort of 393 seropositive subjects with RA and followed them for vital status until January 1, 2006. Observed mortality was estimated using Kaplan-Meier methods, and acceleration factors were estimated for expected mortality using accelerated failure-time models. Mean followup was 15.9 years, and the optimal acceleration factor was estimated as 1.25. That is, after incidence, RA subjects' mortality was equivalent to aging 12.5 "effective" years for each 10 years of actual time. Similar results were noted for cardiovascular and respiratory mortality.

In addition, Weyand and Goronzy[1] have measured the lengths of telomeric sequences in lymphocytes of patients with RA. In individuals with RA, telomeres were already severely shortened at age 20, lending evidence for excessive proliferative turnover of cells and consequent prematurely senescent CD4 T cells. These senescent T cells have increased autoreactivity and other functional deviations[1]. In systemic autoimmune diseases, premature immunosenescence could lead to clinically significant autoantibodies at a much earlier age, resulting in both the disease phenotypes as well as premature atherosclerosis and mortality.

PREVALENCE STUDIES OF AUTOANTIBODIES IN THE HEALTHY ELDERLY

However, a competing hypothesis is that autoantibodies may be a benign bystander in the immunosenescence theory, supported by many prevalence studies of autoantibodies in the elderly[12,15,37-40]. Prevalence studies suggest that a large proportion of elderly people in the general population may have autoantibodies, which may be related to a higher exposure to infections or different medications over time[12]. RF is one of the most frequent autoantibodies in the elderly, with a frequency of 9%–48%, and ANA was found in 14% of 3462 patients studied, in a recent comprehensive review[12].

In a prevalence study, Potocka-Plazak, *et al* concluded that although the prevalence of autoantibodies including ANA, anti-smooth muscle, antimitochondrial, thyroid antimicrosomal, and antibodies to gastric parietal cells was more common in patients with ischemic heart disease (IHD) than in control healthy, very elderly subjects, there were no statistically significant differences[37]. However, it is notable that in elderly subjects with IHD ($n = 19$), ANA was found in 42.1%, versus only 10% of healthy control elderly subjects ($n = 20$), with $0.05 < p < 0.06$. The small sample size may have contributed to difficulty in detecting a statistically significant difference between the 2 groups, and there was a substantially higher prevalence of ANA in elderly subjects with IHD compared to controls without IHD.

Ioannidis, *et al*[41] addressed the question of whether the presence of autoantibodies confers an excess risk of death independent of other risk factors in a prevalence study of 156 very elderly nursing home subjects in Greece. All were 67 years of age or older and in good functional condition with no major medical problem suggestive of imminent risk of death at the time of autoantibody evaluation. The autoantibodies measured included RF, extracted nuclear antibodies to Ro/SSA, La/SSB, Sm, and U1RNP, IgG and IgM antibodies to single-stranded DNA (anti-ssDNA), double-stranded DNA, and anticardiolipin antibodies (aCL). Total followup time was 14.6 years after autoantibody measurements. There were no significant differences in mortality according to presence or absence of aCL, RF, anti-ssDNA, and any autoantibody among those assayed, even after adjusting for age and other comorbidities. The authors concluded that the results excluded the possibility that the autoantibodies evaluated substantially increase the risk of death in very elderly subjects in good functional condition.

However, it is important to note that the median age of subjects in Ioannidis's study[41] was 84 years at the time of autoantibody measurements, and the study population

specifically excluded subjects with major medical problems suggestive of imminent risk of death. As discussed by the authors, it seems likely that the study's results, especially for aCL, were influenced by a large "survivor" effect, where subjects with the presence of the autoantibodies assayed are at high risk of dying in late middle age and early old age and therefore may not survive to the late 80s and beyond.

CONCLUSION

Although prevalence studies seem to suggest that the high prevalence of autoantibodies in the elderly may be an "innocent bystander" effect of immunosenescence and cumulative exposure to infections with aging, it is important to note that most of the subjects in those studies are healthy elderly. Perhaps in the select subgroup of healthy very elderly individuals, the presence of autoantibodies may indeed be clinically insignificant and an epiphenomenon of immunosenescence. Further research is needed to determine whether the presence of specific autoantibodies, traditionally used as markers of systemic autoimmune diseases, may be markers for chronic diseases of aging such as atherosclerosis. However, the preponderance of current evidence on autoantibodies in the pathogenesis and epidemiology of atherosclerotic disease suggests that premature immunosenescence may be an attractive biologically plausible theory for the increased CV disease and mortality seen in systemic autoimmune diseases. Premature immunosenescence could lead to premature production of autoantibodies that may directly or indirectly lead to endothelial dysfunction, premature atherosclerosis, and increased mortality in these diseases.

KIMBERLY P. LIANG, MD,
Department of Medicine and Division of Rheumatology;
SHERINE E. GABRIEL, MD, MSc,
Department of Health Sciences Research,
Mayo Clinic and Mayo Graduate School of Medicine,
Rochester, Minnesota, USA

Address reprint requests to Dr. K.P. Liang, Department of Health Sciences Research, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.
E-mail: liang.kimberly@mayo.edu

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