Atherosclerosis

Y Sherer, Y Shoenfeld

Is atherosclerosis a cellular or humoral mediated autoimmune disease?

Atherosclerosis is a complex and indolent histopathological process, which is considered to be the most common underlying process in cardiovascular morbidity and mortality. In recent years it has become apparent that in addition to the traditional risk factors for atherosclerosis, this condition is associated with infectious, inflammatory, and autoimmune factors.¹ Atherosclerosis fulfils all the four criteria delineated by Witebsky and Rose to define a condition as autoimmune in nature, and all arms of the immune system (including cellular components, autoantigens, and autoantibodies) play a part in atherosclerosis.² Thus one of the questions raised is whether atherosclerosis, as other autoimmune conditions, is mainly a humoral or cellular mediated autoimmune disease.

INVOLVEMENT OF CELLULAR COMPONENTS IN ATHEROSCLEROSIS

There is good evidence to suggest that cellular components of the immune system are involved in atherosclerosis. Studies using transgenic murine models show recruitment of mononuclear leucocytes through vascular leucocyte adhesion molecules and chemokines, differentiation of monocytes to macrophages, and endocytosis through scavenger receptors of oxidised LDL (oxLDL) in atherogenesis. The importance of T cells in atherosclerosis is emphasised in a study in which CD4+ and CD8+ T cell depletion reduced fatty streak formation in C57BL/6 mice, indicating that T cells aggravate fatty streak formation.³ A recent study emphasises also the importance of specific lymphocytes. Lymphocytes obtained from LDL receptor deficient mice immunised with β_2 glycoprotein I (β_2 GPI) were transferred intraperitoneally into syngeneic mice, producing larger fatty streaks in the recipients than in mice receiving lymphocytes from control mice.⁴ T cell depletion of lymphocytes failed to induce this effect. Hence, β_2 GPI reactive T cells could promote atherogenesis.

As for other autoimmune diseases, it is also of interest to determine whether atherosclerosis is mainly a Th1 or Th2 mediated condition. Although it is not

yet sufficiently clear, a recent study favours the former. In that study apo-E deficient mice were treated with an inhibitor of the Th1 differentiation pathway (PTX) or control for 12 weeks.⁵ The former mice reduced the size of atherosclerotic lesions by 60%, and developed lesions that were limited to the degree of fatty streaks. The control mice developed mature fibrofatty atherosclerotic lesions. Of note is that the lesion size correlated with the proportion of interferon γ (IFN γ) positive T cells. In vitro addition of PTX to cultured spleen cells did not modify the production of IFN γ , but increased the production of interleukin 10 (IL10) by T cells, indicating that PTX does not suppress IFN γ production but rather blocks Th1 polarisation while promoting Th2 polarisation.⁵ This study suggests that the Th1 immune response associated with atherosclerosis is deleterious. None the less, it seems that atherosclerosis, like other autoimmune conditions, involves both Th1 and Th2 related cytokines (an example with IL4 is provided below).

ASSOCIATION BETWEEN AUTOANTIBODIES AND ATHEROSCLEROSIS

Apart from the above data on the involvement of cellular components in atherosclerosis, the evidence also suggests an association between autoantibodies and atherosclerosis (reviewed by Shoenfeld *et al*⁶). When a pathological state is multifactorial, it is not surprising that many autoantibodies play a part in its pathogenesis. A brief summary of the most investigated autoantibodies follows.

Anti-oxLDL antibodies

Oxidation of LDL probably has an important role in the pathogenesis of atherosclerosis. It is not yet firmly established whether the immune response to oxLDL is pro-atherogenic or anti-atherogenic in vivo, or, alternatively, whether it is merely an epiphenomenon for the presence of oxLDL. Anti-oxLDL antibodies are raised in patients with early onset peripheral vascular disease,⁷ severe carotid atherosclerosis,⁸ and angiographically verified coronary artery disease.^{9,10} In addition, raised levels of oxLDL

antibodies were predictive of carotid atherosclerosis progression,¹¹ and myocardial infarction occurrence and mortality.¹² We have recently found raised levels of these antibodies in patients with coronary artery disease compared with healthy controls, regardless of the amount of coronary calcification.¹³ None the less, reduction of oxLDL antibody levels was reported in elderly patients with ischaemic stroke,¹⁴ and in animal models immunisation with oxLDL resulted in suppression rather than aggravation of early atherogenesis.^{15,16}

Anti-heat shock protein (HSP) 60/65 antibodies

HSPs are a family of proteins that show a highly homologous sequence between different species, from bacteria to man. Sonographic assessment of carotid atherosclerotic lesions showed that subjects with such lesions had significantly raised levels of anti-HSP65 antibodies compared with controls.¹⁷ The possible pathogenicity of anti-HSP antibodies could be learnt from animal models. For example, rabbits that were immunised with material containing HSP65, either in the form of mycobacteria or recombinant HSP65 alone, developed atherosclerotic lesions.¹⁸ In another study C57BL/6 mice were injected with either HSP65, HSP65-rich *Mycobacterium tuberculosis*, or phosphate buffered saline (PBS). Early atherosclerosis was significantly enhanced in mice fed a high cholesterol diet that were immunised with HSP65 or *Mycobacterium tuberculosis*, compared with the mice injected with PBS.¹⁹ Recently, it has been suggested that IL4 has a crucial role in the progression of early atherosclerosis mediated by inflammation, as IL4 knockout mice immunised with HSP65 had significantly less fatty streak formation than lesions in C57BL/6 mice.²⁰

Anticardiolipin antibodies

The pathogenic potential of anticardiolipin in atherosclerosis has been demonstrated in a mouse model in which immunisation with this antibody resulted in the development of high titres of mouse anticardiolipin and increased atherosclerosis compared with controls.²¹ The presence of high levels of anticardiolipin was found to be an independent risk factor for myocardial infarction or cardiac death in middle aged men.²² We recently found raised levels of anticardiolipin antibodies in patients with coronary artery disease compared with healthy controls, regardless of the amount of coronary calcification,¹³ and these antibodies were raised in patients with typical chest pain compared with patients with atypical or no chest pain.²³

Anti- β_2 GPI antibodies

β_2 GPI is currently considered by most researchers as the autoantigen in antiphospholipid syndrome rather than a cofactor. Its association with atherosclerosis has been reviewed by Shoenfeld *et al.*²⁴ Probably the most important finding comes from animal models, in which immunisation of two different strains of mice with β_2 GPI led to the production of anti- β_2 GPI antibodies and acceleration of atherosclerosis.^{25–26}

INCLUSION OF BOTH CELLULAR AND HUMORAL COMPONENTS IN ATHEROSCLEROSIS

Atherosclerosis may also include both cellular and humoral components in its underlying pathophysiology, as occurs in many autoimmune diseases. A recent study determined the role of cellular and humoral immune responses to HSP65 in murine atherosclerosis. Lymph node cells, splenocytes, and IgG were obtained from the LDL receptor deficient mice immunised with HSP65.²⁷ Adoptive transfer of HSP65 reactive lymph node cells increased fatty streak formation in comparison with mice treated with bovine serum albumin primed cells. Similarly, repeated intraperitoneal administration of IgG from the serum of HSP65 immunised mice enhanced fatty streak formation in mice in comparison with controls.²⁷ This study provides direct evidence for the pro-atherogenic properties of cellular and humoral immunity to HSP65, and raises the possibility that both arms of the immune system have a synergistic pro-atherogenic effect.

ANTINUCLEAR ANTIBODIES IN ATHEROSCLEROSIS

In this issue of the *Annals* Grainger and Bethell provide evidence for the presence of raised levels of antinuclear antibodies (ANA) in patients with radiological evidence of advanced atherosclerosis.²⁸ Their discussion includes the possibility that these antibodies are merely an epiphenomenon or, alternatively, that they have a pathogenic role in atherosclerosis. Even though this study naturally does not provide answers to that crucial question, their finding itself is important and raises several thoughts and assumptions.

"Are ANA markers of advanced atherosclerosis or do they participate in its acceleration?"

The presence of ANA is the hallmark of systemic lupus erythematosus, and they are found in various frequencies in other autoimmune diseases as well. The accelerated atherosclerotic state found in

patients with systemic lupus erythematosus and antiphospholipid syndrome might result from the higher frequency of traditional risk factors in these patients as well as from the presence of anticardiolipin and anti- β_2 GPI antibodies.²⁹ However, ANA might also contribute to the accelerated atherosclerosis found in these patients. Further, the association of ANA with atherosclerosis raises the possibility that these antibodies play a part in atherogenesis or arteriosclerosis in other autoimmune and inflammatory states, such as vasculitides. As for other autoantibodies, the frequency of ANA is significantly higher in elderly people (10–37%) than in the young (0–6%).³⁰ As coronary artery disease resulting from atherosclerosis is also found mostly in the elderly, ANA may be markers of advanced atherosclerosis or, alternatively, participate in its acceleration.

It is obvious now that many autoantibodies, autoantigens, and cellular components of the immune system are involved in atherogenesis and progression of atherosclerosis. The association of ANA with atherosclerosis raises again the "cause and effect" question that is so common in autoimmunity and in the immune aspects of atherosclerosis in particular. The role of ANA in atherosclerosis should be further studied, but the association of ANA with atherosclerosis provides additional evidence for the autoimmune nature of this condition.

Ann Rheum Dis 2002;**61**:97–99

Authors' affiliations

Y Shoenfeld, Department of Medicine "B" and Centre of Autoimmune Diseases, Sheba Medical Centre, Tel-Hashomer, 52621 Israel
Y Sherer, Sackler Faculty of Medicine, Tel-Aviv University, Israel

Correspondence to: Professor Shoenfeld; Shoenfel@post.tau.ac.il

REFERENCES

- Shoenfeld Y, Sherer Y, Harats D. Atherosclerosis as an infectious, inflammatory and autoimmune disease. *Trends Immunol* 2001;**22**:293–295.
- Shoenfeld Y, Harats D, Wick G, eds. *Atherosclerosis and autoimmunity*. Amsterdam: Elsevier, 2001:1–370.
- Emeson EE, Shen ML, Bell CG, Qureshi A. Inhibition of atherosclerosis in CD4 T-cell-depleted and nude (nu/nu) C57BL/6 hyperlipidemic mice. *Am J Pathol* 1996;**149**:675–85.
- George J, Harats D, Gilburd B, Afek A, Shaish A, Kopolovic J, *et al.* Adoptive transfer of beta-2-glycoprotein I-reactive lymphocytes enhances early atherosclerosis in LDL receptor-deficient mice. *Circulation* 2000;**102**:1822–7.
- Laurat E, Poirier B, Tupin E, Caligiuri G, Hansson GK, Bariety J, *et al.* In vivo downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation* 2001;**104**:197–202.
- Shoenfeld Y, Sherer Y, George J, Harats D. Autoantibodies associated with atherosclerosis. *Ann Med* 2000;**32**(suppl 1):37–40.

- Bergmark C, Wu R, de Faire U, Lefvert AK, Swenberg J. Patients with early-onset peripheral vascular disease have increased levels of autoantibodies against oxidized LDL. *Arterioscler Thromb Vasc Biol* 1995;**15**:441–5.
- Maggi E, Chiesa R, Melissano G, Castellano R, Astore D, Grossi A, *et al.* LDL oxidation in patients with severe carotid atherosclerosis: a study in vitro and in vivo oxidation markers. *Arterioscler Thromb* 1994;**14**:1892–9.
- Lehtimäki T, Lehtinen S, Solakivi T, Nikkila M, Jaakkola O, Jokela H, *et al.* Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *Arterioscler Thromb Vasc Biol* 1999;**19**:23–7.
- Bui MN, Sack MN, Moutsatsos G, Lu DY, Katz P, McCown R, *et al.* Autoantibody titers to oxidized low-density lipoprotein in patients with coronary atherosclerosis. *Am Heart J* 1996;**131**:663–7.
- Salonen JT, Yla-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, *et al.* Autoantibodies against oxidized LDL and progression of carotid atherosclerosis. *Lancet* 1992;**339**:883–7.
- Wu R, Nityanand S, Berglund L, Lithell H, Holm G, Lefvert AK. Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction. *Arterioscler Thromb Vasc Biol* 1997;**17**:3159–63.
- Sherer Y, Tenenbaum A, Praprotnik S, Shemesh J, Blank M, Fisman EZ, *et al.* Coronary artery disease but not coronary calcification is associated with elevated levels of cardiolipin, beta-2-glycoprotein-I, and oxidized LDL antibodies. *Cardiology* 2001;**95**:20–4.
- Cherubini A, Mecocci P, Senin U. Autoantibodies against low-density lipoproteins in older stroke patients. *J Am Geriatr Soc* 1997;**45**:125.
- George J, Afek A, Gilburd B, Levkovitz H, Shaish A, Goldberg I, *et al.* Hyperimmunization of apo-E mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis* 1998;**138**:147–52.
- Ameli S, Hultgardh-Nilsson A, Regnstrom J, Calara F, Yano J, Cercek B, *et al.* Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 1996;**16**:1074–9.
- Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollenzer F, Kiechl S, *et al.* Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet* 1993;**341**:255–9.
- Xu Q, Dietrich H, Steiner HJ, Gown AM, Schoel B, Mikuz G, *et al.* Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. *Arterioscler Thromb* 1992;**12**:789–99.
- George J, Shoenfeld Y, Afek A, Gilburd B, Keren P, Shaish A, *et al.* Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein-65. *Arterioscler Thromb Vasc Biol* 1999;**19**:505–10.
- George J, Shoenfeld Y, Gilburd B, Afek A, Shaish A, Harats D. Requisite role for interleukin-4 in the acceleration of fatty streaks induced by heat shock protein 65 or *Mycobacterium tuberculosis*. *Circ Res* 2000;**86**:1203–10.
- George J, Afek A, Gilburd B, Levy Y, Blank M, Kopolovic J, *et al.* Atherosclerosis in LDL-receptor knockout mice is accelerated by immunization with anticardiolipin antibodies. *Lupus* 1997;**6**:723–9.
- Vaara O, Manttari M, Manninen V, Tenkanen L, Puurunen M, Aho K, *et al.* Anti-cardiolipin antibodies and risk of myocardial infarction in a prospective cohort of middle-aged men. *Circulation* 1995;**91**:23–7.
- Sherer Y, Shemesh J, Tenenbaum A, Blank M, Harats D, Fisman EZ, *et al.* Coronary calcium

- and anti-cardiolipin antibody are elevated in patients with typical chest pain. *Am J Cardiol* 2000;86:1306–11.
- 24 **Shoenfeld Y**, Sherer Y, George Y, Harats D. β 2-glycoprotein I in human and murine atherosclerosis. *Isr Med Assoc J* 2001;3:85–7.
 - 25 **George J**, Afek A, Gilburd B, Blank M, Levy Y, Aron-Maor A, *et al*. Induction of early atherosclerosis in LDL-receptor-deficient mice immunized with beta2-glycoprotein I. *Circulation* 1998;98:1108–15.
 - 26 **Afek A**, George J, Shoenfeld Y, Gilburd B, Levy Y, Shaish A, *et al*. Enhancement of atherosclerosis in beta-2-glycoprotein I-immunized apolipoprotein E-deficient mice. *Pathobiology* 1999;67:19–25.
 - 27 **George J**, Afek A, Gilburd B, Shoenfeld Y, Harats D. Cellular and humoral immune responses to heat shock protein 65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice. *J Am Coll Cardiol* 2001;38:900–5.
 - 28 **Grainger DJ**, Bethell HWL. High titres of serum antinuclear antibodies, mostly directed against nucleolar antigens, are associated with the presence of coronary atherosclerosis. *Ann Rheum Dis* 2002;61:110–14.
 - 29 **Sherer Y**, Shoenfeld Y. Antiphospholipid syndrome, antiphospholipid antibodies, and atherosclerosis. *Curr Atheroscler Rep* 2001;3:328–33.
 - 30 **Moulias R**, Proust J, Wang A, Congy F, Marescot MR, Deville Chabrolle A, *et al*. Age related increase in autoantibodies. *Lancet* 1984;ii:1128–9.

Annals of the Rheumatic Diseases through the ages

Browse the Archive

Annals of the Rheumatic Diseases online has an archive of content dating back to 1965.
Full text from 1997; abstracts from 1975; table of contents from 1965

www.annrheumdis.com