Free radicals and endothelial dysfunction: Potential positive effects of TNF- α inhibitors

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Objectives: During the last decade many new biological immune modulators have entered the market as new therapeutic principles. Tumor necrosis factor (TNF)- α is a pro-inflammatory cytokine known to have a key role in the pathogenic mechanisms of various immune-mediated or inflammatory diseases. However, TNF- α also plays a key role in endothelial dysfunction and, thus, in the development and progression of atherosclerosis. What, then, is the potential therapeutic role of TNF- α inhibitors?

Methods: We analysed the current literature concerning the administration of TNF- α inhibitors and their potential benefits upon endothelial function.

Results: TNF- α inhibitors decrease the serum levels of inflammatory markers such as TNF- α itself, CRP, IL-6, and increased the tissue expression of endothelial NO synthase and the vasodilatory response to bradykinin.

Discussion: TNF- α inhibitors may change the progression of endothelial dysfunction and, thus, slow down the atherosclerotic process.

Keywords: Free radicals, Endothelial dysfunction, Atherosclerosis, TNF- α inhibitors, VEGF

Introduction

The endothelium represents the main regulator of vascular wall homeostasis and favors a relaxed vascular tone and low levels of oxidative stress by releasing mediators such as nitric oxide (NO), prostacyclin-2, and endothelin-1, as well as by controlling local angiotensin II activity.^{1,2} In particular, NO is continuously manufactured from healthy endothelial cells through the conversion of L-arginine by the endothelial NO synthase.^{1,3} However, NO can be also produced by macrophages in response to immunological stimuli via another, inducible, NO synthase.¹ In this review, we describe the role of free radicals in the development of endothelial dysfunction and discuss the potential positive effects of tumor necrosis factor (TNF)- α inhibitor treatment.

Endothelial dysfunction and atherosclerosis

Endothelium undergoes a phenotypic modulation from the normal state to a non-adaptive state known as endothelial dysfunction in response to different noxious stimuli.¹ Indeed, atherosclerosis is an inflammatory condition which starts as a 'response to injury' that adds to traditional cardiovascular and genetic risk factors and favors the endothelial dysfunction. Early changes in endothelial function include the

increase in permeability to lipoproteins and other plasma constituents, resulting in penetration of such lipids into the arterial wall and migration of monocytes and T-lymphocytes into the vessel intima.^{1,4} In particular, low density lipoproteins (LDLs) accumulate in the subendothelial space and modified or native LDL are uptaken by macrophages which become foam cells and play a key role in the development of fatty streaks.^{1,5} Pro-inflammatory cytokines such as TNF- α , interleukin (IL)-1 and IL-6, interferon- γ increase the expression of adhesion molecules including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin and, thus, favor the proliferation of the smooth muscle cells which migrate into the lesion and lead to the thickening of the vessel wall.¹ The accumulation of macrophages, T-lymphocytes, smooth muscle cells, and the formation of fibrous tissue induce the enlargement and remodeling of the lesion. A fibrous cap develops over the plaque and when it becomes unstable may favor plaque rupture and thrombosis.^{1,4} The defense of a normal artery depends on innate immune responses mounted by endothelial cells and, after an inflammatory stimulus, by macrophages and other cells of the immune response. Indeed, innate immunity plays a key role in the initiation of atherosclerosis.^{1,6,7} Lipid-loaded macrophages undergo apoptosis, become necrotic, and coalesce into the necrotic core of the vulnerable plaques.^{1,8} Recent evidence suggests

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that neutrophils also play a fundamental role in the early phase of atherosclerosis and their transmigration and degranulation in the vessel wall is determined by modified or native LDL. Finally, neutrophil inflammatory signals trigger the intimal recruitment of monocytes.^{1,9} The analysis of human atherosclerotic plaques has proven the presence of activated-T-lymphocytes expressing major histocompatibility complex class II molecules with a pro-inflammatory T-helper (Th)-1 phenotype.^{1,10} The activation of this Th-1 response represents an autoimmune mechanism in which the adaptive immune system is targeted against self-antigens expressed by atherosclerotic plaques and modified by biochemical factors as oxidative stress and hypercholesterolemia and contributes to a more aggressive progression of the atherosclerosis.^{1,11} What mechanisms can induce the autoimmune process? The answer is neither easy nor unique. However, it has been reported that microorganisms sharing sequence homology with self-molecules (molecular mimicry theory) may favor autoimmune responses,^{1,12} impairments in apoptosis, and in clearance of apoptotic bodies can render apoptotic cells as a source of autoantigens,^{1,13} CD4⁺ T-lymphocyte responses against native apolipoprotein B-100 may help B-lymphocytes to produce antibodies against modified or native LDL^{1,14} and, finally, defects in central tolerance favor the persistence of LDL-specific effector memory lymphocytes. A very important role is played by dendritic cells (DCs) physiologically promoting tolerization to antigens by silencing T-lymphocytes. However, danger signals may activate DCs and lead to a switch from tolerance to activation of adaptive immunity.^{1,15} Moreover, the withdrawal of the suppressive effects of regulatory T-lymphocytes may sustain inflammation and exacerbate plaque growth.¹ Finally, the key role of the interaction between oxidative stress and inflammation in the pathogenesis of atherosclerosis is widely accepted. It also has been proven that autoimmune responses may be directed against self-molecules altered by high affinity ligand binding or by chemical damage such as oxidative stress.^{1,16}

Endothelial dysfunction, oxidative stress, and TNF- α

Arginase expressed in the endothelium serves as an endogenous competitor of NO synthase for L-arginine and, thus, plays a counteracting role in NO-mediated vasodilatory function.¹⁷ It is now widely recognized that the overproduction of reactive oxygen species (ROS) and/or a deficiency of antioxidant enzyme activity may contribute to the appearance of vascular lesions.^{1,18} Indeed, ROS may catalyze several modifications to nucleic acids, lipids, and proteins favoring the generation of neo-cryptic epitopes which may behave as autoantigens.^{1,19,20} Furthermore, the

oxidation process is also associated with major structural LDL modifications determining the formation of new antigenic epitopes that can be presented by DCs and give rise to clonal expansion of LDL-specific T-lymphocytes, which are detectable both in serum and in human atherosclerotic plaques.^{1,16,21,22} Notably, autoantibodies specific for modified or native LDL have been detected in humans and have been associated with cardiovascular diseases.^{1,23} Stress-induced heat shock proteins are also considered to be autoantigens that favor the progression of atherosclerosis.^{1,24} Indeed, under stress conditions, heat shock proteins are expressed both within the cells and on the cell surface and can be released in the intercellular space, stimulating an immune response.^{1,25} Oxidative stress changes the structure and function of the β 2-glycoprotein I which becomes a target antigen of a Th-1 lymphocyte response.^{1,19,26} It has been demonstrated that intraplaque hemorrhages release large amounts of hemoglobin (Hb), which is oxidized in the pro-inflammatory and pro-oxidant microenvironment. Both free Hb and oxidized Hb behave as autotigens.^{1,27} TNF- α is a pro-inflammatory cytokine known to have a central role in the initial host response to infection.^{28–35} However, TNF- α up-regulates the expression of arginase in endothelial cells during ischemia/reperfusion (I/R) injury, which causes a decrease in L-arginine availability to nitric oxidase synthase and consequently leads to O₂^{•-} production.¹⁷ The increased production of O₂^{•-} impairs the NO-mediated vasodilatation and favors endothelial dysfunction, which may play a key factor in the development and progression of I/R injury. These data confirm that the attenuation of NO synthesis in I/R injury may have devastating consequences.¹⁷ Furthermore, hypercholesterolemia impairs the L-arginine/NO pathway, inducing activation of the angiotensin II-type 1 receptor and, thus, vasoconstriction.¹ Viridis *et al.*³⁶ studied the small resistance arteries in the visceral fat of obese patients and demonstrated a marked up-regulation of TNF- α expression mainly in the media layer of these vessels. Overall, the vascular wall represents the source of TNF- α involved in endothelial dysfunction.³⁶ More recently, a potential specific role of inflammation from perivascular adipose tissue in the pathogenesis of endothelial dysfunction has been investigated. Greenstein *et al.*³⁷ confirmed the increased accumulation of TNF- α in the adipose tissue, which supported the theory that in obesity the adipose tissue may damage the surrounding vessels in a paracrine manner. Notably, the vessels are not only a target but also a source of inflammation, which contributes to the pathogenesis and progression of endothelial dysfunction by activating ROS production. Furthermore, TNF- α also reduces the density of insulin receptor content at the level of the endothelial cells, worsening

Figure 1 Cells and molecules involved in endothelial dysfunction.

the insulin resistance that characterizes obese individuals and, thus, impairing the NO synthesis.³⁸ A few studies have demonstrated that women present higher serum TNF- α levels in postmenopausal period. The high levels of TNF- α associated with the low estrogen levels favor the progression of endothelial damage.^{39,40}

Cells and molecules involved in endothelial dysfunction are depicted in Fig. 1.

TNF- α inhibitors: emerging role in the prevention of endothelial dysfunction

TNF- α blockers have demonstrated efficacy in large, randomized controlled clinical trials either as monotherapy or in combination with other anti-

inflammatory or disease-modifying anti-rheumatic drugs in the treatment of chronic immune-mediated or inflammatory diseases.^{41–43} There are several TNF- α inhibitors available for clinical use including anti-TNF- α monoclonal antibodies (infliximab, adalimumab, golimumab, and certolizumab pegol) and a fusion protein that acts as a ‘decoy receptor’ for TNF- α (etanercept).^{28–32,44} Booth *et al.*⁴⁵ confirmed that the serum levels of TNF- α may promote the progression of the endothelial dysfunction. However, C-reactive protein, TNF- α , and IL-6 increase the cardiovascular risk.^{46–49} Furthermore, TNF- α seems to favor the expression of IL-6. Therefore, on the basis of this hypothesis, they decided to treat with

Figure 2 Potential beneficial effects of TNF- α inhibitors.

infliximab alone or in addition with conventional treatment for patients with anti-neutrophil cytoplasmic antibody-associated systemic vasculitis in order to address the effects of anti-inflammatory therapy upon both the systemic inflammation and endothelial dysfunction. The treatment decreased the serum levels of C-reactive protein and IL-6, but did not reduce the serum TNF- α level. Nevertheless, the current available ELISA assay measures the total TNF- α level but not those of free TNF- α which is virtually related to the disease activity. However, infliximab therapy improved both the disease activity and the endothelial function.⁴⁵ The forearm blood flow response to acetylcholine which resulted reduced in the patients also improved after the administration of infliximab. This finding confirms that the chronic inflammatory autoimmune diseases as systemic lupus erythematosus appear as an independent risk factor for the development of atherosclerosis and cardiovascular diseases.^{50,51} Therefore, the anti-inflammatory therapy may change both the clinical course of the autoimmune disease and the progression of the endothelial dysfunction and, thus, slow down the atherosclerotic process. Arenas *et al.*⁵² evaluated the effects of etanercept administration in ovariectomized rats which presented high levels of TNF- α . Etanercept decreased the serum TNF- α level and increased the tissue expression of endothelial NO synthase and vasodilatory response to bradykinin. Dulai *et al.*⁵³ confirmed that TNF- α inhibitors (etanercept, infliximab, adalimumab) may have a beneficial effect on arterial stiffness in patients with rheumatoid arthritis improving, thus, accelerated atherosclerosis and consequently reducing the cardiovascular risk. Szekanecz *et al.*⁵⁴ suggested that etanercept and adalimumab may exert beneficial effects on the lipid profile improving the endothelial dysfunction. Furthermore, TNF- α inhibitors are able to reduce the expression and production of vascular endothelial growth factor (VEGF), NO, and inducible NO synthase.³⁵ VEGF is a critical mediator of inflammation both in chronic immune-mediated and allergic diseases.^{1,55,56} It is known that VEGF is a pro-angiogenic factor which alters the microvascular network and, thus, correlates and may contribute to the development and progression of atherosclerosis. In summary, the administration of TNF- α inhibitors reduces the systemic inflammation in patients with chronic immune-mediated diseases, improves both the clinical course of the disease itself and the endothelial function and, thus, may decrease the risk of acute cardiovascular and/or cerebrovascular events.

Potential beneficial effects of TNF- α inhibitors are depicted in Fig. 2.

Conclusion

Chronic systemic inflammation that characterizes the immune-mediated or inflammatory diseases plays a key role in the development and progression of endothelial dysfunction. Free radicals such as O₂^{-•}, cytokines such as TNF- α , adhesion molecules, dyslipidemia, and innate and adaptive immune responses may favor the vascular damage. Indeed, activated Th-1 lymphocytes contribute to a more aggressive progression of atherosclerosis, which can be viewed as an autoimmune disease in which the adaptive immune system is targeted against self-antigens modified by biochemical factors such as oxidative stress. Furthermore, TNF- α up-regulates the expression of arginase in endothelial cells and leads to O₂^{-•} production that impairs the NO-mediated vasodilatation and favors endothelial dysfunction. Notably, the vascular wall represents the source of TNF- α involved in endothelial dysfunction as demonstrated in obese individuals. TNF- α inhibitors by decreasing systemic inflammation and production of VEGF seem to have a beneficial effect upon the progression of atherosclerosis, reducing the risk of acute cardiovascular and/or cerebrovascular events.

References

- Murdaca G, Colombo BM, Cagnati P, Gulli R, Spanò F, Puppo F. Endothelial dysfunction in rheumatic autoimmune diseases. *Atherosclerosis* 2012;224(2):309–17.
- Gonzalez MA, Selwyn AP. Endothelial function, inflammation, and prognosis in cardiovascular disease. *Am J Med* 2003; 115(Suppl 8A):99S–106.
- Napoli C, Ignarro LJ. Nitric oxide and atherosclerosis. *Nitric Oxide* 2001;5(2):88–97.
- Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011; 473(7347):317–25.
- Tabas I, Williams KJ, Borén J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation* 2007;116(16):1832–44.
- Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol* 2011;12(3):204–12.
- Lundberg AM, Hansson GK. Innate immune signals in atherosclerosis. *Clin Immunol* 2010;134:5–24.
- Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat Rev Immunol* 2010;10:36–46.
- Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med* 2011;17(11):1410–22.
- Jonasson L, Holm J, Skalli O. Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis. *J Clin Invest* 1985;76(1):125–31.
- Nilsson J, Wigren M, Shah PK. Regulatory T cells and the control of modified lipoprotein autoimmunity-driven atherosclerosis. *Trends Cardiovasc Med* 2009;19(8):272–6.
- Blank M, Shoenfeld Y. Beta-2-glycoprotein-I, infections, anti-phospholipid syndrome and therapeutic considerations. *Clin Immunol* 2004;112(2):190–9.
- Hall JC, Casciola-Rosen L, Rosen A. Altered structure of auto-antigens during apoptosis. *Rheum Dis Clin North Am* 2004; 30(3):455–71.
- Hermansson A, Ketelhuth DF, Strodthoff D, Wurm M, Hansson EM, Nicoletti A, *et al.* Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. *J Exp Med* 2010;207(5):1081–93.
- Niessner A, Weyand CM. Dendritic cells in atherosclerotic disease. *Clin Immunol* 2010;134:25–32.
- Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques

- recognize oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 1995;92(9):3893–7.
- 17 Gao X, Xu X, Belmadani S, Park Y, Tang Z, Feldman AM, *et al*. TNF- α contributes to endothelial dysfunction by upregulating arginase in ischemia/reperfusion injury. *Arterioscler Thromb Vasc Biol* 2007;27(6):1269–75.
 - 18 Puddu P, Puddu GM, Cravero E, De Pascalis S, Muscari A. The emerging role of cardiovascular risk factor-induced mitochondrial dysfunction in atherogenesis. *J Biomed Sci* 2009;16:112.
 - 19 Buttari B, Profumo E, Mattei V, Siracusano A, Ortona E, Margutti P, *et al*. Oxidized b-glycoprotein I induces human dendritic cell maturation and promotes a T helper type 1 response. *Blood* 2005;106(12):3880–7.
 - 20 Arvieux J, Regnault V, Hachulla E, Darnige L, Berthou F, Youinou P. Oxidation of b2-glycoprotein I (b2-GPI) by the hydroxyl radical alters phospholipid binding and modulates recognition by b2-GPI autoantibodies. *Thromb Haemost* 2001;86(4):1070–6.
 - 21 Nilsson J, Hansson GK. Autoimmunity in atherosclerosis: a protective response losing control? *J Intern Med* 2008;263(5):464–78.
 - 22 Frostegard J, Wu R, Giscombe R, Holm G, Lefvert AK, Nilsson J. Induction of T-cell activation by oxidized low density lipoprotein. *Arterioscler Thromb* 1992;12(4):461–7.
 - 23 Nilsson J, Kovanen PT. Will autoantibodies help to determine severity and progression of atherosclerosis? *Curr Opin Lipidol* 2004;15(5):499–503.
 - 24 Wick G, Knoflach M, Xu Q. Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu Rev Immunol* 2004;22:361–403.
 - 25 Park HK, Park EC, Bae SW, Park MY, Kim SW, Yoo HS, *et al*. Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. *Circulation* 2006;114(9):886–93.
 - 26 Profumo E, Buttari B, Alessandri C, Conti F, Capoano R, Valesini G, *et al*. Beta2-glycoprotein I is a target of t cell reactivity in patients with advanced carotid atherosclerotic plaques. *Int J Immunopathol Pharmacol* 2010;23(1):73–80.
 - 27 Buttari B, Profumo E, Petrone L, Pietraforte D, Siracusano A, Margutti P, *et al*. Free hemoglobin: a dangerous signal for the immune system in patients with carotid atherosclerosis? *Ann N Y Acad Sci* 2007;1107:42–50.
 - 28 Murdaca G, Colombo BM, Cagnati P, Gulli R, Spanò F, Puppo F. Update upon efficacy and safety of TNF- α inhibitors. *Expert Opin Drug Saf* 2012;11(1):1–5.
 - 29 Murdaca G, Colombo BM, Puppo F. Emerging biological drugs: a new therapeutic approach for systemic lupus erythematosus. An update upon efficacy and adverse events. *Autoimmun Rev* 2011;11(1):56–60.
 - 30 Murdaca G, Colombo BM, Puppo F. Adalimumab for the treatment of immune-mediated diseases: an update on old and recent indications. *Drugs Today (Barc)* 2011;47(4):277–88.
 - 31 Murdaca G, Colombo BM, Puppo F. Anti-TNF- α inhibitors: a new therapeutic approach for inflammatory immune-mediated diseases: an update upon efficacy and adverse events. *Int J Immunopathol Pharmacol* 2009;22(3):557–65.
 - 32 Murdaca G, Colombo BM, Barabino G, Caiti M, Cagnati P, Puppo F. Anti-tumor necrosis factor- α treatment with infliximab for disseminated granuloma annulare. *Am J Clin Dermatol* 2010;11(6):437–9.
 - 33 Murdaca G, Colombo BM, Contini P, Puppo F. Determination of lymphotoxin- α levels in patients with psoriatic arthritis undergoing etanercept treatment. *J Interferon Cytokine Res* 2012;32(6):277–9.
 - 34 Murdaca G, Spanò F, Puppo F. Selective TNF- α inhibitor-induced injection site reactions. *Expert Opin Drug Saf* 2013;12(2):187–93.
 - 35 Murdaca G, Spanò F, Miglino M, Puppo F. Effects of TNF- α inhibitors upon the mechanisms of action of VEGF. *Immunotherapy* 2013;5(2):113–5.
 - 36 Viridis A, Santini F, Colucci R, Duranti E, Salvetti G, Rugani I, *et al*. Vascular generation of tumor necrosis factor- α reduces nitric oxide availability in small arteries from visceral fat of obese patients. *J Am Coll Cardiol* 2011;58(3):238–47.
 - 37 Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, Jeziorska M, *et al*. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. *Circulation* 2009;119:1661–70.
 - 38 Aljada A, Ghanim H, Assian E, Dandona P. Tumor necrosis factor- α inhibits insulin-induced increase in endothelial nitric oxide synthase and reduces insulin receptor content and phosphorylation in human aortic endothelial cells. *Metabolism* 2002;51:487–91.
 - 39 Kamada M, Irahara M, Maegawa M, Ohmoto Y, Takeji T, Yasui T, *et al*. Postmenopausal changes in serum cytokine levels and hormone replacement therapy. *Am J Obstet Gynecol* 2001;184:309–14.
 - 40 Sites CK, Toth MJ, Cushman M, L'Honnemiedieu GD, Tchernof A, Tracy RP, *et al*. Menopause-related differences in inflammation markers and their relationship to body fat distribution and insulin-stimulated glucose disposal. *Fertil Steril* 2002;77(1):128–35.
 - 41 Taylor PC. Antibody therapy for rheumatoid arthritis. *Curr Opin Pharmacol* 2003;3:323–8.
 - 42 Chang J, Girgis L. Clinical use of anti-TNF- α biological agents – a guide for GPs. *Aust Fam Physician* 2007;36:1035–8.
 - 43 Puppo F, Murdaca G, Ghio M, Indiveri F. Emerging biologic drugs for the treatment of rheumatoid arthritis. *Autoimmun Rev* 2005;4(8):537–41.
 - 44 Hochberg MC, Lebowitz MG, Plevy SE, Hobbs KF, Yocum DE. The benefit/risk profile of TNF-blocking agents: findings of a consensus panel. *Semin Arthritis Rheum* 2005;34:819–36.
 - 45 Booth AD, Jayne DR, Kharbanda RK, McEniery CM, Mackenzie IS, Brown J, *et al*. Infliximab improves endothelial dysfunction in systemic vasculitis: a model of vascular inflammation. *Circulation* 2004;109(14):1718–23.
 - 46 Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, *et al*. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. *Thrombolysis in myocardial infarction. J Am Coll Cardiol* 1998;31(7):1460–5.
 - 47 Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973–9.
 - 48 Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor- α and increased risk of recurrent coronary events after myocardial infarction. *Circulation* 2000;101:2149–53.
 - 49 Biasucci LM, Vitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C, *et al*. Elevated levels of interleukin-6 in unstable angina. *Circulation* 1996;94:874–7.
 - 50 Colombo BM, Cacciapaglia F, Puntoni M, Murdaca G, Rossi E, Rodriguez G, *et al*. Traditional and non traditional risk factors in accelerated atherosclerosis in systemic lupus erythematosus: role of vascular endothelial growth factor (VEGATS Study). *Autoimmun Rev* 2009;8(4):309–15.
 - 51 Esdaile JM, Abrahamowicz M, Grodzicky T, Li Y, Panaritis C, du Berger R, *et al*. Traditional risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum* 2001;44:2331–7.
 - 52 Arenas IA, Armstrong SJ, Xu Y, Davidge ST. Chronic tumor necrosis factor- α inhibition enhances NO modulation of vascular function in estrogen-deficient rats. *Hypertension* 2005;46(1):76–81.
 - 53 Dulai R, Perry M, Twycross-Lewis R, Morrissey D, Atzeni F, Greenwald S. The effect of tumor necrosis factor- α antagonists on arterial stiffness in rheumatoid arthritis: a literature review. *Semin Arthritis Rheum* 2012;42(1):1–8.
 - 54 Szekanecz Z, Kerekes G, Soltész P. Vascular effects of biologic agents in RA and spondyloarthropathies. *Nat Rev Rheumatol* 2009;5(12):677–84.
 - 55 Ciprandi G, Murdaca G, Colombo BM, De Amici M, Marseglia GL. Serum vascular endothelial growth factor in allergic rhinitis and systemic lupus erythematosus. *Hum Immunol* 2008;69(8):510–2.
 - 56 Ciprandi G, Colombo BM, Murdaca G, De Amici M. Serum vascular endothelial growth factor and sublingual immunotherapy. *Allergy* 2008;63(7):945–6.