A Vaccine for Atherosclerosis
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Atherosclerotic vascular disease is the anatomic basis for angina, unstable angina, myocardial infarction, most cases of sudden death, ischemic stroke, claudication and gangrene with loss of limb. It is the leading cause of death among men and women and costs billions of dollars to the healthcare system and society on an annual basis. Over the last three decades, a considerable amount of progress against this disease has been made with improved diagnostic and preventive/therapeutic interventions, but a considerable residual burden of this disease remains. If current trends continue with respect to the increasing prevalence of obesity, due largely to unhealthy lifestyles, and its associated increased risk for type 2 diabetes, it is likely that all gains against atherosclerosis will be erased in the next 10 to 20 years. Developing and poor nations are experiencing a dramatic increase in cardiovascular disease, which was once thought to be a disease of the affluent West, and the next epidemic is likely to come from these countries.

Adoption of a therapeutic lifestyle (consumption of a heart-healthy diet, regular physical activity) coupled with risk factor modification, including use of cholesterol lowering and antithrombotic drugs, have clearly reduced the risk of atherothrombotic cardiovascular events. Despite these interventions, a significant number of atherothrombotic cardiovascular events continue to occur, and are particularly prevalent among those with metabolic syndrome/diabetes, chronic renal failure and advanced congestive heart failure. These observations highlight the need for additional new interventions geared to preventing, stabilizing or regressing atherosclerosis.

**Brief pathophysiology of atherosclerosis: role of the immune system**

Atherosclerosis results from vascular entry followed by subendothelial retention and oxidation of circulating atherogenic lipoproteins (apoB-100-containing lipoproteins) in the arterial wall. Genetic and hemodynamic factors, such as shear stress, modulate regional susceptibility for development of atherosclerosis. Lipoprotein retention triggers an activation of innate and adaptive immune responses against atherogenic autoantigens, resulting in recruitment of immune cells with subsequent activation of inflammatory cascade, and development of an atherosclerotic plaque consisting of lipids, matrix proteins, inflammatory and immune cells, neovascular channels and smooth muscle cells. The innate and adaptive immune responses appear to play a prominent role in atherogenesis, with evidence implicating the immune system in both promotion as well as amelioration of atherosclerosis.

Oxidized LDL is one of the best-studied autoantigens in which both a humoral as well as cellular immune response is detectable in humans and animal models of atherosclerosis. During subendothelial retention, apoB-containing lipoproteins, especially LDL, undergo oxidative modification with alteration of both apoB-100 as well as the phospholipids associated with LDL. These alterations expose putative autoantigens or neoantigens, which are then presented by antigen-presenting cells to the naïve T-cells. The naïve T-cells can then undergo polarization towards a more pro-inflammatory phenotype (Th1 type) or a less inflammatory and perhaps an anti-inflammatory phenotype (Th2 type and Treg type). Th1 polarization is athero-promoting, whereas Treg and possibly Th2 polarization is anti-inflammatory and athero-protective (Figure 1).

Although the role of the immune system has been recognized for many years, the concept of exploiting the immune response to reduce atherosclerosis is relatively recent. In 1996, our laboratory at the Cedars-Sinai Heart Institute reported that immunizing hyperlipidemic rabbits with a vaccine made from homologous oxidized LDL coupled to an adjuvant substantially reduced aortic atherosclerosis without a significant change.

*Continued on page 2 (see “Vaccine”)
Developing an atherosclerosis vaccine

Over the last 12 years, our laboratory and that of our collaborator, Jan Nilsson, MD, PhD, of Sweden, have been working on developing the concept of a vaccine for atherosclerosis using synthetic peptide fragments that contain amino acid sequences within the human apoB-100. Human apoB-100 contains 4,536 amino acids. We generated a library of 302 peptides (each 20 amino acids long with a 5 amino acid overlap), and identified nearly 100 of these peptide sequences to which humoral immune response could be detected in pooled human serum. Through further experiments, we have narrowed the list down to about a dozen sequences that have athero-protective effects. Immunization of mice using a vaccine formulation containing a single peptide, such as peptide 2 (Figures 3 and 4), peptide 45 or peptide 210, reduced atherosclerosis by 40 to 60 percent compared to nonimmunized mice. Similarly, vaccine containing multiple peptides (peptide 143 and peptide 210) also conferred a 60 percent reduction in atherosclerosis. In addition to reducing atherosclerosis, the vaccines also reduced plaque inflammation. We have shown that splenocytes are important in mediating the observed athero-protective effects, and further studies, published recently from our laboratory, have defined an important role for CD8 T-cells in mediating athero-protective effects of vaccination with the p210 peptide antigen. Current studies are focused on optimizing the formulation of the vaccine in anticipation of human trials, which are hoped to begin within about a year from now, pending FDA approval.
Vaccine for hypertension and aortic aneurysm

Given that hypertension or aortic aneurysm is an important risk factor (or closely associated with) atherosclerosis, we also expanded our vaccine research focus to hypertension and aortic aneurysm. Tomoyuki Honjo, PhD, a postdoctoral fellow in the Oppenheimer Atherosclerosis Research Center at the Cedars-Sinai Heart Institute, is currently working on an angiotensin II infusion model in mice to determine if apoB peptide vaccination could modulate hypertension or aneurysm and aneurysm rupture. Preliminary results are very encouraging, showing that the vaccine has blood pressure–lowering effects and protects animals from death due to aneurysm rupture. These results were presented for the first time at the AHA Scientific Sessions in November 2011.

Anti-oxidized LDL antibody for plaque regression

Working collaboratively with a Swedish biotech company, BioInvent, and Dr. Nilsson’s laboratory, we have also demonstrated that a high-affinity monoclonal antibody designed against oxidized p45 epitope, another apoB-100-related peptide antigen, shrank pre-existing plaque (regression) in experimental animals after three weekly injections (Figure 5). Based on this work, a phase 1 human trial was completed and a phase 2 proof-of-concept trial has finished patient enrollment and results are expected in the near future.

Conclusion and perspective

Clean water and vaccines have been the most important and effective public health measures in the last 150 years. Vaccines have proven safe and effective against a variety of infectious diseases, and more recently, the concept of vaccination is being extended to noninfectious immune-mediated disorders such as atherosclerosis. The possibility that someday our children could receive a vaccine that reduces their future risk of atherosclerotic cardiovascular disease is very appealing, and our work in this field has certainly provided for optimism that such an approach is possible. Similarly, our recent findings also raise the tantalizing possibility that a vaccine for high blood pressure and aortic aneurysm may also be feasible.

References

5. Ibid.

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Adult Cardiac Care for Patients with Duchenne and Becker Muscular Dystrophies

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Duchenne muscular dystrophy (DMD) is a devastating, X-linked muscle-wasting disease for which there is as yet no specific treatment. The disease gene encodes dystrophin, a large membrane-bound structural protein in skeletal muscle as well as respiratory muscle, cardiac muscle and vascular smooth muscle. Due to frameshift mutations in the dystrophin gene, patients with DMD make no dystrophin. Without dystrophin, muscular contraction causes tears in the muscle cell membrane, with excessive calcium influx and other defects in cell signaling. Affecting one in 3,500 male births, DMD accounts for 80 percent of all cases of muscular dystrophy. Boys are diagnosed as toddlers because of weak leg muscles. Most are wheelchair bound by age 15. Death often occurs by age 20 from respiratory muscle failure and dilated cardiomyopathy. More patients are surviving to age 30 thanks to noninvasive home ventilation and corticosteroids, which can prolong ambulation by two years, reduce the risk of scoliosis and temper pulmonary and cardiac decline in the second decade. Adult cardiologists need to be more involved in the care of post-pediatric patients with DMD and those with Becker muscular dystrophy (BMD), a milder form of the disease with later onset and median survival to age 50.

Cardiac disease in DMD, BMD

Cardiac disease in DMD manifests most often as a cardiomyopathy and/or cardiac arrhythmia (with sinus tachycardia beginning in the first decade of life). The main cardiac pathology is patchy fibrosis that begins at birth in the posterior left ventricular wall, progresses to the lateral and anterior walls and eventually involves the entire myocardium. With improved pulmonary care, progressive cardiomyopathy has become the major cause of death in DMD. Heart failure can be the initial presentation of BMD if mild leg muscle weakness escapes diagnosis; a positive family history and calf pseudohyper trophy (due to muscle fibrosis) suggest the correct diagnosis.

The screening test for BMD (or DMD) is a dramatically elevated creatine kinase (CK) level. Index cases should be referred to a chemiluminescence immunoassay (CLIA)-certified laboratory for genetic testing to confirm the diagnosis, as well as all family members for testing and genetic counseling. A carrier mother has a 50 percent risk of having a son with DMD/BMD and a 50 percent risk of daughters being carriers. Most carriers are asymptomatic, but one in five develops some degree of muscle weakness or cardiomyopathy.

Cardiac evaluation

Cardiac evaluation should be part of comprehensive multispecialty care involving referral to a Muscular Dystrophy Association-sponsored neuromuscular disease clinic. Current guidelines recommend baseline cardiac evaluation for DMD at age six, biannual evaluation until age 10 and at least annual evaluation thereafter. Patients with BMD should have cardiac evaluation at the time of diagnosis and at least biannually thereafter. All female carriers should undergo cardiac evaluation. Minimal cardiac evaluation includes an electrocardiogram and a transthoracic echocardiogram.

Left ventricular ejection fraction (LVEF) is an insensitive measure, however. Advanced imaging techniques show that patients with DMD and BMD clearly have disease in their myocardium long before they develop global systolic dysfunction as indicated by decreased LVEF. Diastolic dysfunction with preserved LVEF precedes dilated cardiomyopathy. Tissue strain measurements by echocardiography or by cardiac magnetic resonance imaging (MRI) detect areas of abnormal myocardial contractile activity in younger patients with preserved LVEF. Contrast-enhanced cardiac MRI indicates areas of myocardial fibrosis, shown by late gadolinium enhancement. All these subclinical abnormalities worsen over time and predict faster onset of heart failure. How the newer imaging modalities will impact treatment needs more research.

Cardiac treatment

First-line treatment of heart failure in DMD and BMD is with angiotensin-converting-enzyme inhibitors (ACEI) followed by β-blockers and diuretics. When started before the onset of dilated cardiomyopathy, ACEI therapy may slow progression to heart failure; initial evidence supports this preemptive practice but awaits confirmation in larger clinical trials. Glucocorticoid-induced hypertension should be treated; the steroid dose may need to be reduced and prednisone may be replaced with deflazacort, which causes less hypertension. Prophylactic anticoagulation may be considered in advanced dilated cardiomyopathy. An implantable cardiac defibrillator should be considered for LVEF <35%, although more research is needed to establish the benefit in this population. The best exercise prescription for cardiac conditioning also needs more research; patients should avoid strenuous exercise and eccentric (e.g., downhill) exercise, which can damage vulnerable muscle. Cardiac transplantation is a viable option, particularly for patients with BMD.

Investigational treatment

Although basic science on DMD has flourished, clinical translation has not. An expanding clinical trial pipeline includes gene therapy, stem cell therapy, and, recently, drug therapy with sildenafil and tadalafil, inhibitors of the phosphodiesterase 5A (PDE5A) gene. In the mouse model of DMD, these PDE5A inhibitors—which boost nitric oxide/cGMP-mediated vasodilation—relieve spasm of skeletal muscle microvessels and allow the mice to perform more exercise with less skeletal muscle damage; cardiac function also is greatly improved by sildenafil. Dystrophin binds an isoform of nitric oxide synthase, which therefore does not function normally in dystrophin-deficient muscle.

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Dr. Victor is principal investigator on clinical trials sponsored by the Muscular Dystrophy Association and the Parent Project Muscular Dystrophy to determine if PDE5A inhibitors improve skeletal muscle function and cardiac function in patients with DMD and BMD and is the recipient of research grant U34AR062893 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases to plan for a multicenter clinical trial.