dispensing tiny droplets of cell-alginate suspension into a solution of $CaCl_2$, which provides crosslinking ions that trap the cells inside tiny beads. The entire process takes about an hour, and the product can be stored for extensive periods of time (Microencapsulation Gene Therapy Group Homepage, 1997).

The process has a myriad of advantages. Since the microcapsules act as a barrier between the graft and the immune system, doctors can implant foreign cells into a patient without the use of immuno-suppressant drugs (Orive, 2003). The fact that the same cell-lines can be used for different recipients eliminates the need for customized genetic engineering of the patients' own cells using viruses. In addition, by changing the properties of the capsules, doctors can alter the rate at which the patient receives the protein products of the implanted cells (Dhoot, 2002).

That is not to say that microencapsulation techniques have been perfected. Much more research is needed to make the capsules biocompatible and stable *in vivo* (Orive, 2003). This necessitates collaboration with researchers from various disciplines to find novel substances from which to build the casing for the cells. Such substances may have new properties that give doctors more control over the way in which the protein products are released. In clinical trials involving diabetes patients, the capsules were found to be too large to use in the amount necessary to completely eliminate the need for insulin injections; therefore, there is an emphasis towards further miniaturization before microcapsules can be used to treat diseases.

Despite the many obstacles that researchers must still overcome, microencapsulation of non-autologous cells has the potential to become synonymous with hope for many patients afflicted with genetic disorder. M

LDL, HDL, and the Battle Against Heart Disease

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As North Americans progress into the 21st century, life has become increasingly chaotic and fast paced. The average North American lives in such a high-speed society that it is almost impossible to avoid fast food chains and express lines in the grocery markets. People now have no time to slow down their lives and adopt or maintain a healthy lifestyle. Consequently, heart disease, in particular atherosclerosis, has become the leading cause of death in North America (Ezekowitz et al., 2003). Atherosclerosis is a condition where deposits of fatty substances, cholesterol, cellular waste products, calcium, and other substances accumulate on the inner lining of an artery and form build-ups called plaque. If a clot forms and blocks a narrowed artery, it can result in a heart attack or stroke. Recently, there have been a number of breakthroughs in the fight against atherosclerosis, which have linked high-density lipoprotein (HDL) to antiatherogenic effects. The latest studies have linked HDL to the reversal of cholesterol transport, a process that removes excess cholesterol from the body.

Cholesterol is a normal constituent of most body tissues, especially vital in the brain, nervous system, liver, and blood system. Cholesterol is also needed to form the sex and adrenal hormones, bile in the liver, and vitamin D (Kumar et al., 2001). Unfortunately, high blood cholesterol levels increase the risk of developing health problems such as heart disease and atherosclerosis (Kumar, 2001).

The first major type of cholesterol is low-density lipoprotein (LDL) cholesterol; this form is often referred to as the "bad" cholesterol. LDL is a type of lipoprotein, which acts as a carrier for cholesterol and fats in the bloodstream. When too much LDL cholesterol circulates in the blood, it can slowly amass on the inner walls of arteries that supply the heart and brain. Together with other substances, plaque is formed. Evidence from observational studies suggests that higher total LDL cholesterol levels are associated with an increased risk of a variety of cardiovascular diseases, such as atherosclerosis (Anderson et al., 1994).

High-density lipoprotein (HDL) cholesterol is known as the "good" cholesterol. HDL is one of the most structurally complex and functionally versatile forms of lipoproteins in the human body. This type of lipoprotein has become a major focus of scientific and clinical investigation over the past decade. Scientific experimentation has found that HDL displays a variety of antiatherogenic effects – "HDL stimulates endothelial cell nitric oxide production, inhibits adhesion molecule expression, mediates antioxidant effects... and inhibits thrombosis and endothelial cell apoptosis among other processes" (Toth, 2003). These antiatherogenic properties act to prevent atherosclerosis.

Various scientific studies have compiled a large sum of research that indicates that HDL has the ability to clear hardened cholesterol from the arteries and aid in its removal from the body. Recent advances in atherosclerosis have led scientists to conclude that one of the most important antiatherogenic functions of HDL is its ability to drive reverse cholesterol transport, or RCT (Toth, 2003). RCT is the primary process through which excess cholesterol is extracted from peripheral tissues, delivered to the liver for conversion to bile salts, and removed from the body via secretion into bile. RCT is also critical for cholesterol delivery to other organs for conversion into steroid hormones (Toth, 2003).

Protein components of lipoproteins are called apolipoproteins (Zha, 2003). Apolipoprotein A1 (apoA1) is the main protein in HDL, and is involved in the RCT pathway. ApoA1 is produced by both the intestine and the liver, and once secreted, can interact with macrophages and fibroblasts to stimulate the transfer of phospholipids and cholesterol out of the cells. The action of apoA1 results in increased cholesterol efflux (Zha, 2003). Low levels of apoA1 are associated with an increased risk for atherosclerosis in humans. The over-expression of apoA1 in a number of animal species substantially reduces the risk of developing atherosclerosis in these organisms (Toth, 2003). Recent research also suggests that injection of recombinant apoA1 may be able to reduce and/or reverse atherosclerosis, and potentially, heart disease in humans (Nissen et. al., 2003).

ATP-binding membrane cassette transport protein A1 (ABCA1) plays a major role in RCT, and its activity is found to be associated with "serum levels of HDL... and risk for atherosclerotic disease" (Toth, 2003). ABCA1 consumes energy to physically transport phospholipids from the cytosol into the extracellular space (Attie, 2001). Mutations in the gene for ABCA1 in humans lead to conditions known as Tangier's disease and familial HDL deficiency, which are characterized by severely decreased levels of HDL cholesterol in the blood and increased risk for atherosclerosis and heart disease (Hayden et. al., 2000). Similarly, studies with

HDL AND REVERSE CHOLESTEROL TRANSPORT

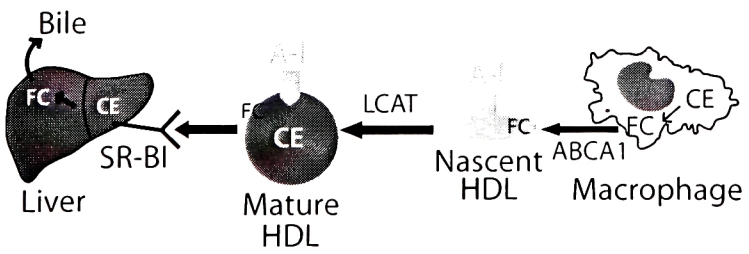

FIGURE 1
"HDL is believed to protect against atherosclerosis at least in part through the process of reverse cholesterol transport, whereby excess free cholesterol (FC) is removed from cells in peripheral tissues, such as macrophages within the arterial wall, and returned to the liver for excretion in the bile." (Ballantyne, 2000)

mice reveal that knocking out the gene for ABCA1 gives rise to similar losses of HDL and susceptibility as in atherosclerosis, whereas over-expressing human ABCA1 increases serum levels of HDL and protects against atherosclerosis by increasing RCT (Aiello et. al., 2003; Singaraja, 2002; Vaisman, 2001).

Another enzyme, lecithin-cholesterol acyltransferase (LCAT), catalyzes the esterification of cholesterol and the eventual formation of HDL molecules. In one rabbit model, LCAT over-expression was seen to decrease the risk for atherosclerosis (Hoeh, 1996). Cholesterol can also be transferred from HDL to other lipoproteins, including LDL, in the blood. This is the result of a protein called cholesteryl ester transfer protein (CETP). Since this shifts cholesterol from the "good" to the "bad" lipoprotein, researchers are testing whether blocking CETP may suppress atherosclerosis by helping to divert cholesterol away from LDL and towards pathways leading to its elimination. The delivery of HDL cholesterol to the liver for elimination in bile or conversion into bile salts is the final step in RCT, and appears to be mediated by an HDL receptor called SR-BI (Trigatti, 2000). Studies in mice revealed that knocking out SR-BI blocks delivery of HDL cholesterol to liver and increases atherosclerosis, whereas the transfer of SR-BI gene to increase its expression in the liver increases RCT and reduces atherosclerosis (Trigatti, 2000).

Various experiments have shown that it is not the absolute level of HDL that determines the risk for atherosclerotic disease, but rather the overall capacity of the RCT pathway to deliver excess cholesterol from the various parts of the body to the liver for disposal. Therefore, the ability of HDL to promote RCT prevents the accumulation of excess cholesterol in the circulatory system, which in turn prevents cholesterol from building up on the inner lining of arteries and blood vessels. Thus, the RCT pathway continues to receive a great deal of attention from researchers trying to develop strategies to prevent atherosclerosis.

As scientists are still looking for a treatment for atherosclerosis, prevention remains the best way to combat the disease. One of the most effective ways to prevent atherosclerosis is to combat the high levels of cholesterol within the population. Unhealthy diets, inactive lifestyles, and smoking also increase the risk of acquiring the condition. Subsequently, healthy lifestyle choices play a major role in protection against atherosclerosis and heart disease. Nevertheless, pharmaceutical intervention may be required for individuals whose condition is very serious. Statins are a class of drugs that are typically prescribed to treat high levels of cholesterol. These drugs work by partially blocking the synthesis of cholesterol in the liver, which leads to increased removal of cholesterol from the blood. These drugs are very effective in the treatment of hypercholesterolemia and prevention of atherosclerosis. It should be noted, however, that statins are usually used in conjunction with healthy lifestyle choices.

The latest statin to have received approval for treatment in Canada is Crestor® (Rosuvastatin Calcium). In several clinical trials, Rosuvastatin has been effective in significantly lowering blood cholesterol levels. In one particular trial, the new drug was more effective in lowering LDL cholesterol when compared to 3 other commonly used statins (Atorvastatin, Simvastatin, and Pravastatin). The most adverse side effects for statins that have been reported are pharyngitis, headaches, and diarrhea, but data regarding mortality and morbidity have not yet been made available (Rosuvastain/Crestor®, 2003).

A recent addition to the myriad of drugs used to combat high serum cholesterol is a drug called Ezetimibe. This drug blocks the absorption of cholesterol in the intestine, and has been shown in animal studies and human clinical trials to result in substantial reductions in LDL cholesterol and increased HDL cholesterol. More importantly, because Ezetimibe and statins act on different pathways, they can be used in combination to provide a more profound reduction in LDL cholesterol.

Owing to many years of research, the scientific community continually makes rapid progress in unravelling the many biological pathways that are involved in the development of atherosclerosis. As new pathways are uncovered and described, new targets for drug development emerge. This leads to an ever-increasing arsenal of drugs and treatments to control cholesterol levels and combat atherosclerosis. The next several years will be a crucial period, where many potential therapies undergo clinical trials and new therapies will be developed. M

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