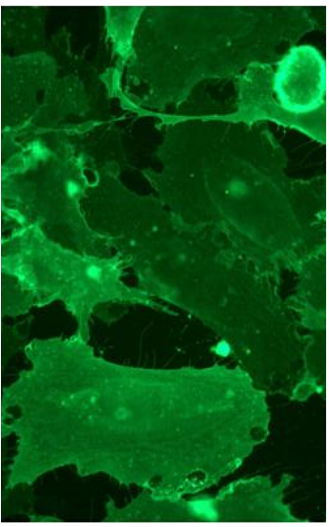

The role of endothelial progenitor cells in atherosclerosis

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Figure 1 (on front page). Mature endothelial cells stained with UEA (green) [18]

Abstract

Atherosclerosis is a progressive, inflammatory disease of the large and medium-sized arteries. Severe atherosclerotic lesions can result in myocardial infarction or stroke, which diseases contribute largely to the deaths worldwide. It is believed that endothelial dysfunction together with chronic inflammation initiates lesion formation. Endothelial progenitor cells (EPCs) are able to differentiate into endothelial cells and are believed to contribute to reendothelialization, neovascularization and neoangiogenesis. They are seen as critically for endothelial repair. Without sufficient levels of functioning EPCs, complete regeneration cannot take place. EPCs are currently seen as highly important factors in the pathogenesis of atherosclerosis. Whether EPCs perform positive or negative roles in atherosclerosis has not been clarified because of differences between studies. Also, mechanisms have not been unraveled yet. It is possible that EPCs help to repair endothelial injury and atherosclerotic lesions and that they may mediate interactions between cardiovascular risk factors in such a manner that these risk factors are ruled out. However, EPCs can also possibly lead to enhancement of atherogenesis. They might play a role in the progression or maintenance of lesions due to their pro-inflammatory and proangiogenic properties. A lot of progress has already been made, but much of this subject is not yet fully understood. At this point, one can only speculate about the role of EPCs in atherosclerosis. Negative effects could be the consequence of a single group of EPCs which exhibit strong pro-inflammatory and angiogenic properties, positive effects can then be subscribed to a group of EPCs which possess more differentiating ability. Nowadays, there are already clinical trials in which autologous bone marrow derived cells, EPCs or other cellular pools are transplanted in patients with ischemia. However, one should be very careful with these treatments, because precise mechanisms and consequences are yet unknown.

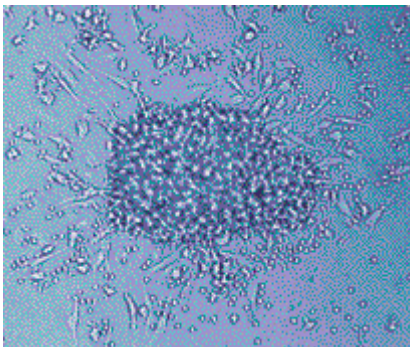

Figure 2. Phase-contrast micrograph of an endothelial progenitor cell colony characterized by a central cluster of rounded cells surrounded by radiating thin, flat cells. [8]

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1.0 Introduction

1.1 Cardiovascular disease

Cardiovascular diseases (CVD) are severe diseases which often cause morbidity and mortality in patients worldwide. Finding new treatments of CVD is important, because current treatments are often unsuccessful. According to the RIVM, CVD is still the number one cause of death in the Netherlands.
CVD are diseases associated with the cardiovascular system. These diseases are usually subdivided in coronary artery disease (CAD) and perivascular disease (PVD). A common CVD is atherosclerosis. Atherosclerosis is responsible for more than 50% of deaths in Western countries. [1] Atherosclerosis is an inflammatory disease that affects arteries in general, and thus is a CAD as well as a PVD. Severe atherosclerotic lesions can result in other CVD, such as myocardial infarction or stroke, which diseases also contribute enormously to the deaths caused by CVD.

Whether people are vulnerable for developing CVD is determined by the amount of cardiovascular risk factors they possess. These risk factors namely can induce damage to the endothelium, resulting in pro-inflammatory reactions, which can lead to the formation of atherosclerotic lesions. [2] Researchers have been investigating which risk factors determine the development of CVD in humans. For example, there has been the Framingham Heart Study, in which all residents of Framingham in America are involved. This study has shown that the occurrence of CAD in a patient in the future is dependent on the presence of the following risk factors: age, gender, total cholesterol level, high density lipoprotein cholesterol level, systolic blood pressure, cigarette smoking, glucose intolerance and cardiac enlargement. Using multivariate logistic functions, estimations can be made about the risk of CAD in the future. This is the Framingham risk score. [3]
A calculation of the risk for the development of atherosclerosis can also be made. One can do this by checking for the presence of metabolic syndrome. People with metabolic syndrome are at a high risk to develop atherosclerosis. Metabolic syndrome is the state of the body in which three out of the following components are present: visceral obesity (a waist circumference of 102 cm or higher in men and of 88 cm or higher in women), high triglycerides concentration (>150 mg/dL), low high-density lipoprotein cholesterol concentrations (below 50 mg/dL in women and 40 mg/dL in men), increased blood pressure (systolic blood pressure of 130 mmHg or higher, or diastolic blood pressure of 85 mmHg or higher), and hyperglycemia (>110 mg/dL, or >100 mg/dL according to the revised ATP III definition). [4]

Atherosclerosis and arteriosclerosis are often being confused. Arteriosclerosis is defined as any hardening of blood vessels and loss of elasticity, however, atherosclerosis is the hardening of blood vessels due to atherosclerotic plaque formation, and thus is a form of arteriosclerosis.

1.3 Blood vessel anatomy

Before continuing, it is useful to know how the walls of arteries are constructed. The walls of blood vessels are composed of three layers: The tunica intima, which consist of a monolayer of endothelial cells which are aligned on a endothelial basement membrane, the tunica media, which consist of vascular smooth muscle cells and the tunica externa or tunica adventitia, which consist of fibroblasts and connective tissue, which is mainly collagen. Between the tunica intima and the tunica media lies the internal elastic lamina and between the tunica media and the tunica adventitia lies the external

elastic lamina. These three layers surround the lumen of the blood vessels and they all have their own specific function.[6]

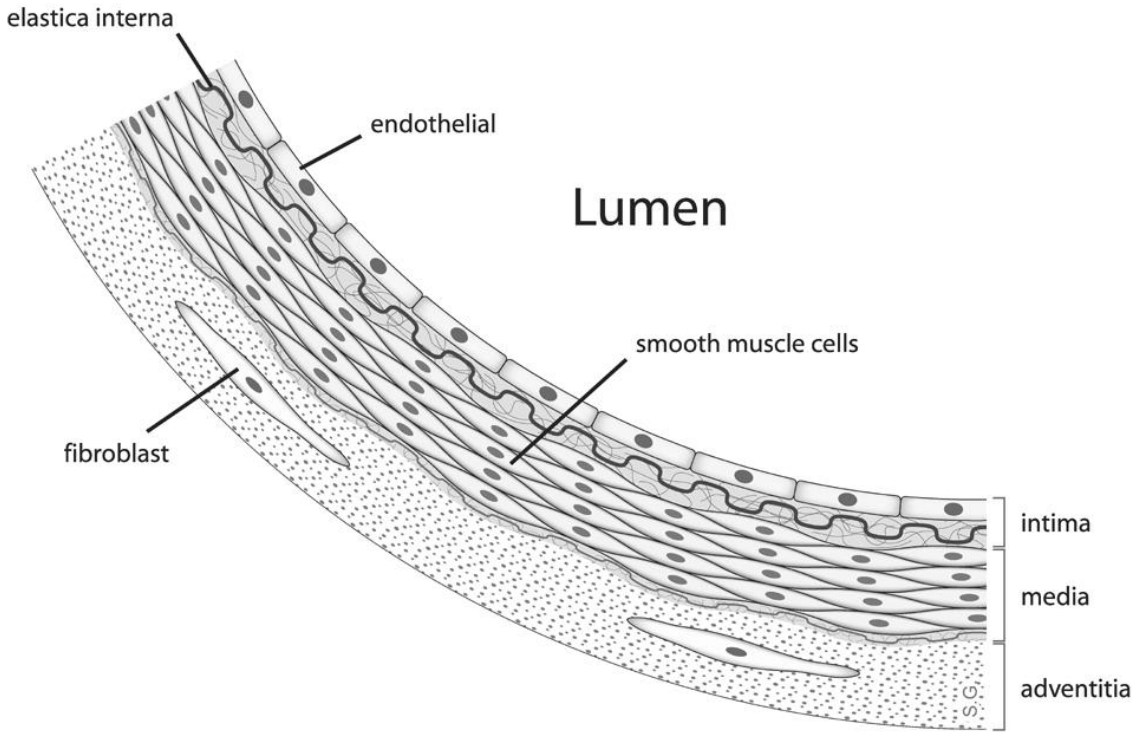

Figure 3. Blood vessel anatomy

1.3 Atherosclerosis and EPC

It is evident that endothelial progenitor cells (EPCs) play a major role in the pathogenesis of atherosclerosis and other cardiovascular diseases.[7] EPCs are thought to play a major role in neoangiogenesis, neovascularization and reendothelialization through paracrine signaling of EPCs and migration, differentiation and proliferation of EPCs in the vessel wall. These processes are essential for repair after injury. For example injury caused by atherosclerosis. EPCs are recruited from bone marrow upon stimulation through cytokines and chemokines released from endothelial cells in blood vessels at places of hypoxia. However, opinions differ regarding the positive and negative effects of EPCs.

1.4 Question

Recently, there has been a lot of research on this subject. EPCs seem to be a promising treatment of atherosclerosis and related diseases in the near future. To explain why one should be watchful when using these cells in treatments, in this paper I will answer the question: What is the role of endothelial progenitor cells in atherosclerosis? I will especially describe its benefits and its drawbacks. Before answering this question however, I will describe atherosclerosis and EPCs in more detail.

2.0 Atherosclerosis

2.1 Characteristics

Atherosclerosis is a progressive, inflammatory disease of the large and medium-sized arteries. It is probably caused by multiple factors which all contribute to atherogenesis. [9,10] Atherosclerosis is the formation of lesions in the arterial vessel wall due to the accumulation of inflammatory cells, lipids and ECM. At a late stage, atherosclerosis can result in ischemia of tissues (brain, heart or extremities) due to blockage of arteries. This causes infarction. [10]

What lesion formation initiates remains unclear. It has been thought that endothelial denudation is the main cause, however the most recent hypothesis is the response-to-injury hypothesis of atherosclerosis. In this theory one thinks that endothelial dysfunction together with chronic inflammation rather than endothelial denudation causes lesion formation. Endothelial dysfunction means increased adhesiveness to leukocytes and platelets, increased permeability, procoagulant instead of anticoagulant properties and forming of vasoactive molecules, cytokines and growth factors.[10] In young and healthy individuals local repair of the endothelium is complete and takes place rapidly, however, in elderly or in the presence of one or more cardiovascular risk factor, repair is ineffective and atherosclerotic lesions may develop due to smooth muscle proliferation and infiltration of mononuclear cells. [2,5] Cardiovascular risk factors induce endothelial dysfunction through chemical or mechanical injury. [11]

There can be different stages of the inflammatory process distinguished in atherosclerosis. Each stage in the process has its own characteristic lesion. [8]

2.2 Cardiovascular risk factors

Several factors have been investigated on their effect on CVD. The following factors are indicated as cardiovascular risk factors in many studies: Elevated LDL cholesterol levels and modified LDL cholesterol, cigarette smoking, hypertension, diabetes mellitus, genetic alterations (endothelial susceptibility, variations in cytokine responses, altered responses to blood-flow), elevated plasma homocysteine levels, presence of infectious micro-organisms (no direct evidence) and combinations of these factors. [9,10]

The mechanisms by which these factors cause endothelial dysfunction are different for each factor, however, each factor has something to do with modification of the endothelium.

Oxidative stress plays a central role in endothelial dysfunction. [12] It can reduce formation of NO, increase leukocyte adhesion and increase peripheral resistance in blood vessels. [10]
Oxidative stress can be induced by cigarette smoking through the formation of free radicals, by oxidized LDL cholesterol particles (oxLDL) and by hypertension. Once LDL cholesterol particles are trapped in an artery, they can turn into lipid radicals which subsequently turn into lipid peroxide radicals (oxLDL). [12] The lipid peroxide radicals are internalized by macrophages and facilitate cholesterol ester formation in these macrophages, which turns them into foam cells. [10] These

radicals also initiate oxidative stress in endothelial cells and smooth muscle cells. [12] LDL cholesterol has also other ways to induce damage. It is chemotactic for monocytes, can activate foam cells and can up-regulate gene expression of genes for macrophage colony-stimulating factor and monocyte chemotactic protein in endothelial cells. [10] Mediators of inflammation, such as tumor necrosis factor α, interleukin-1 and macrophage colony-stimulating factor make this process even worse. They increase binding of LDL cholesterol to endothelial and smooth muscle cells and up-regulate the transcription of the LDL-receptor gene. [10] Hypertension causes activation of the RAAS. Angiotensin II (Ang II) is up-regulated and vasoconstriction takes place, together with stimulation of growth of smooth muscle cells; Ang II binds on receptors, activates phospholipase C and increases intracellular calcium concentrations of smooth muscle cells. This causes smooth muscle contraction, increased protein synthesis, smooth muscle hypertrophy and increased lipoxygenase activity, which increases inflammation and LDL oxidation.

An elevated plasma homocysteine level is a cardiovascular risk factor because homocysteine is toxic for endothelium and prothrombotic. It increases the production of collagen and decreases the availability of nitric oxide (NO) in the artery wall. [10]

The factors that are summed above play a role in the initiation of atherosclerosis. Probably, these factors are not completely identical to the factors that play a role in lesion progression. [13]

2.3 Development of atherosclerosis without treatment

Atherogenesis starts with endothelial dysfunction based on the response-to-injury hypothesis mentioned above. The endothelium acquires increased adhesiveness to leukocytes and platelets, increased permeability, procoagulant instead of anticoagulant properties and formation of vasoactive molecules, cytokines and growth factors. [10] After these alterations, leukocytes can easily invade the vessel wall intima and smooth muscle cells are going to migrate and proliferate. The first stage in atherosclerosis, the fatty streak, is formed. [9] This first stage in atherosclerosis is a purely inflammatory lesion, with only monocyte-derived macrophages and T-lymphocytes present. [10]

The inflammatory response stimulates further proliferation and migration of smooth muscle cells. They can thicken the arterial wall, and dilation of the artery takes place to keep the lumen unaltered. This process is called remodeling. An intermediate lesion is formed. [10]

When the inflammation continues, increasing numbers of macrophages and T-lymphocytes migrate from the blood to the lesion and proliferate within the lesion. After activation, they release hydrolytic enzymes, cytokines, chemokines and growth factors, which can induce further damage and eventually lead to necrosis. A necrotic core develops. Also fibrous tissue is formed within the lesion. This process continues and the lesion becomes larger. Restructuring of the lesion takes place: the lesion gets a core of lipids and necrotic tissue and becomes covered with a fibrous cap. This is called the advanced lesion. [10]

At some time point, the thickening of the artery is so heavy that compensation by dilation is not sufficient anymore, the lesion may alter the blood flow in the artery. [10] In this way, an

atherosclerotic lesion can reduce blood supply to an organ, leading to symptoms such as angina pectoris, intermittent claudation, angina abdominis and renovascular hypertension. [5]

There are two types of advanced lesions: vulnerable advanced lesions and stable advanced lesions. Stable advanced lesions have firm, dense fibrous caps. Stable advanced lesions can become vulnerable advanced lesions due to erosion or uneven thinning of the fibrous cap. This may result from the influence of metalloproteinases. Metalloproteinases are, together with the tissue-factor procoagulant and other haemostatic factors, responsible for the development of plaque instability. Activated T-lymphocytes may stimulate metalloproteinase production by macrophages in the lesions.[10]

Plaque rupture and thrombosis easily can take place when eventually a vulnerable advanced lesion is formed. This will cause unstable coronary syndromes or arterial occlusion, which subsequently may lead to myocardial infarction or stroke. [5,10] Plaque rupture often occurs at the shoulders of the lesion, this is also the place where macrophages enter and accumulate. [10]

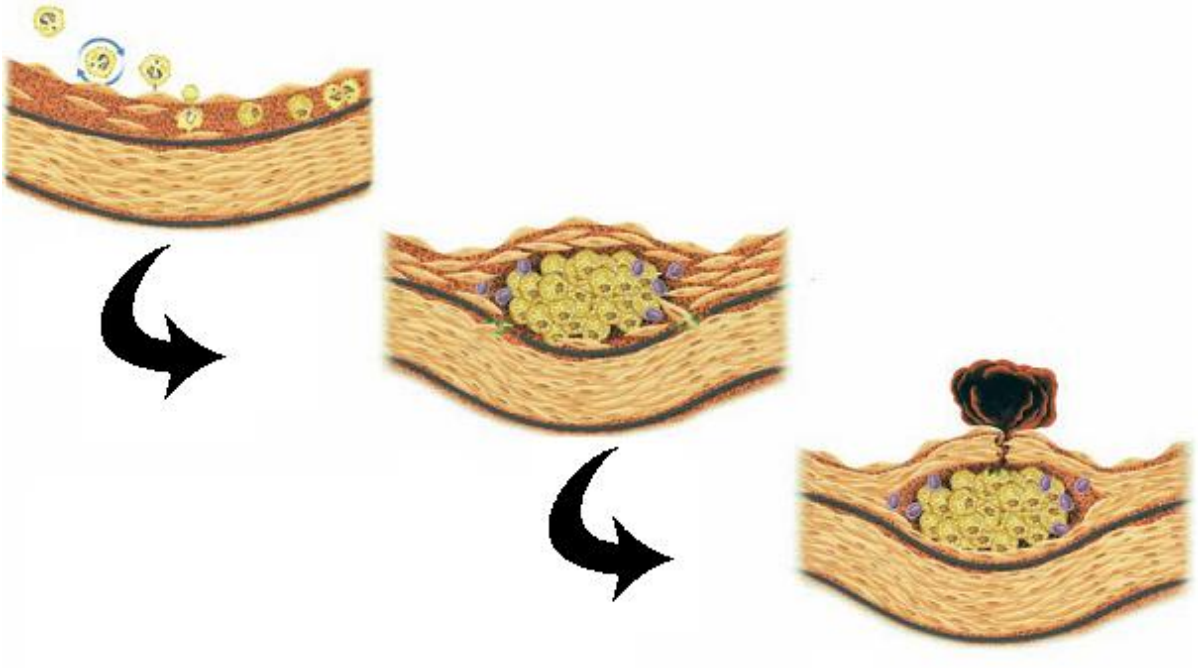

Figure 4. Atherosclerotic lesion formation [2]

2.4 Mediators of atherosclerosis

Endothelial cells, adhesion factors, chemokines, smooth muscle cells, platelets, myeloid leukocytes (monocyte subsets, polymorphonuclear leukocytes and mast cells), connective tissue and different subsets of T-lymphocytes (CD4 and CD8 positive T-lymphocytes) are important cells and components involved in atherogenesis. [9]

At places of endothelial dysfunction, adhesion factors appear on the endothelium. These adhesion molecules consist of selectines, vascular-cell adhesion molecules and intercellular adhesion molecules. These adhesion molecules act as receptors for ligands (glucoconjugates, integrines) on monocytes and T-lymphocytes and act together with chemokines from smooth muscle cells causing

monocyte and T-lymphocyte adherence and migration into the arterial wall. Smooth muscle cells also act as antigen presenting cells to T-lymphocytes, causing the activation of T-lymphocytes. However, because of their different embryonic origin, all smooth muscle cells have different characteristics and it depends on their origin how the cells respond to different stimuli. [10]

Platelets can adhere to dysfunctional endothelium, exposed collagen and macrophages. Platelets release granules with cytokines and growth factors upon activation. These factors cause migration and proliferation of smooth muscle cells and monocytes/macrophages. Activates platelets also form arachidonic acid, which is transformed into prostaglandins or leukotrienes. Vasoconstriction, platelet aggregation and immune response amplification take place. [10]

Myeloid leukocytes are together with T-lymphocytes the most important mediators of atherogenesis. Especially macrophages play a significant role. Macrophages are activated by interferon-γ. However, under certain circumstances, interferon-γ induces them to go into apoptosis. [10] Macrophages and foam cells are involved in all stages of atherogenesis, and are the most abundant myeloid cell types in the lesions. [9] They produce cytokines, proteolytic enzymes and growth factors, which cause damage and repair within the atherosclerotic lesion. They contribute for a great deal to the necrotic cores in advanced lesions. [10]

T-lymphocytes probably are activated upon recognition of oxidized LDL presented by macrophages and smooth muscle cells. After activation of T-lymphocytes, they secrete interferon-γ and tumor necrosis factor α and β. These cytokines amplify the immune response. [10]

All cells are surrounded by extracellular matrix. This matrix might also influences the process of atherogenesis. The exact effects of the extracellular matrix are not yet fully elucidated, but it is known that collagen, fibronectin and heparin sulfate inhibit the cell cycle and that cell-matrix interactions can lead to the production of chemokines by macrophages. [10]

2.5 Current treatments of atherosclerosis

Currently, atherosclerosis often cannot be fully cured. Treatments are based on removal of the symptoms or risk factors, deceleration of the disease progression and prevention of further complications. Sometimes the treatment is successful and the disease process can be reversed, but in most cases, full recovery is not achieved.

Lifestyle changes are prescribed first, which means a healthy diet and exercise. Drugs used for treatment are anti-platelet medications, cholesterol medications (For example statins, which cause lipid lowering, endothelial improvement and anti-inflammatory effects) [10], blood pressure medications (ACE inhibitors for example) or anticoagulants. These drugs help to slow the disease process, minimize risk of complications, and sometimes even reverse the disease process. However, in patients at high risk, or when medication is unsuccessful, surgical treatment is applied.

Surgical treatment consist of four different therapies. Angioplasty, in which narrowed vessels are opened with a balloon and often a stent is placed to keep the vessel open, bypass surgery, in which a

bypass is created using a vessel from some other part of the body or from synthetic origin, endarterectomy, in which lesions are surgically removed, or thrombolytic therapy, in which a clot dissolving drug is injected at the place of a blood clot. [14]

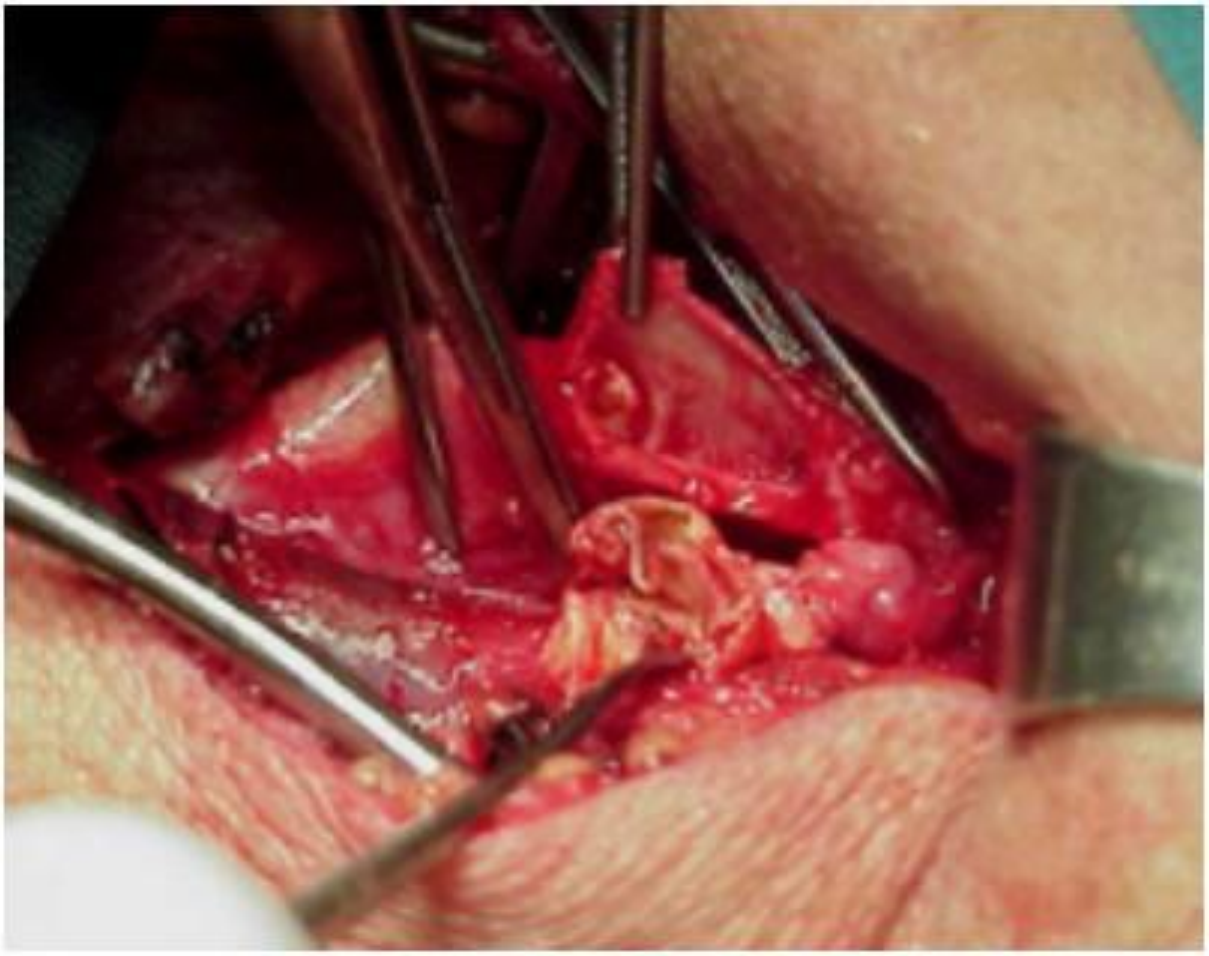

Figure 5. Carotid endarterectomy [26]

3.0 Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are currently seen as highly important factors in the pathogenesis of CVD. They are therefore the subject of many studies. EPCs are able to differentiate in endothelial cells and are believed to contribute to reendothelialization, neovascularization (de novo formation of blood vessels) and neoangiogenesis (sprouting from existing blood vessels). They are seen as critically for endothelial repair. Without sufficient levels of functioning EPCs, complete regeneration cannot take place. [5] EPCs are recruited from the bone marrow to the peripheral circulation in response to many stimuli derived from sites of hypoxia. Asahara et al. reported for the first time the existence of EPCs in the peripheral circulation. [15]

3.1 Characteristics

There are two types of EPCs: the early outgrowth EPCs and the late outgrowth EPCs. The early outgrowth EPCs are derived from the monocytic (CD14+) progenitor cell lineage. These cells co-express endothelial cell markers and markers from the myeloid lineage. The early outgrowth EPCs do not have fully proliferative potential and possess functions of both endothelial cells and monocytes/ macrophages. [6] The late outgrowth EPCs are derived from hematopoietic stem cells (CD34+). These cells are the real endothelial progenitor cells. They only express endothelial cell markers after cell culture, possess a self-renewal capacity and exhibit a wider proliferative potential. [6] In most studies researchers discuss only the late outgrowth EPCs when they discuss EPCs.

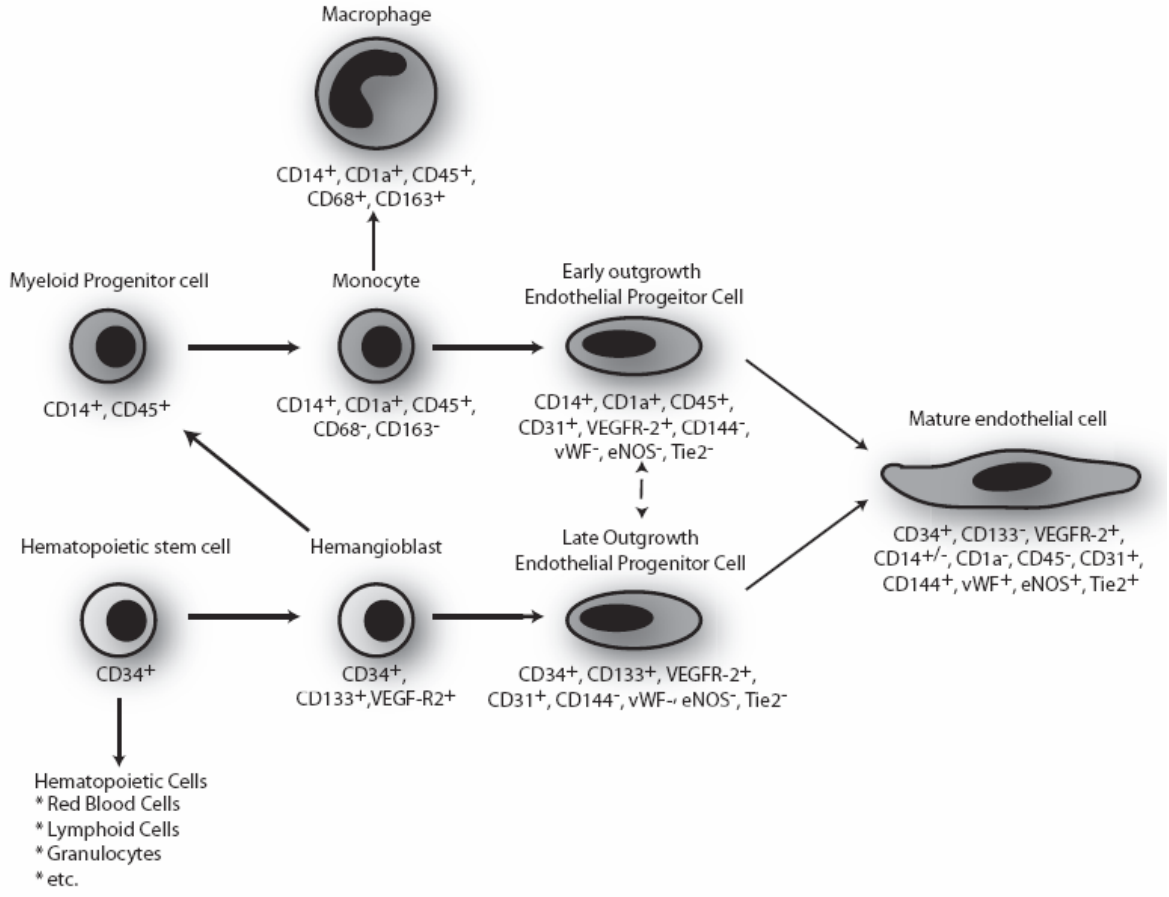

Figure 6. Origin of EPCs and characteristics [6]

Because EPCs share many surface markers with other cell types, no precise definition based on marker expression exists. EPCs are in fact a whole group of cells in different stages of differentiation ranging from the early progenitors to mature endothelial cells. [16] Due to this lack of unequivocality, different studies are poorly comparable. [5] Thus, one should be watchful when comparing the results of different studies concerning EPCs. The mostly used markers for identification of EPCs however, are the markers CD34, KDR/VEGFR2 and CD133/AC133 on mononuclear cell populations. [17]

EPCs have the ability to differentiate into a variety of cells, including hematopoietic cells, smooth muscle cells, pericytes and fibroblasts. It depends largely on culture or *in vivo* conditions which cell type the EPCs become. [1,6,7,18] Through this multipotency, EPCs can directly contribute to the formation of new blood vessels through maturing into the cell types that are needed. EPCs can also have supportive paracrine effects on endothelial cells by secreting angiogenic factors (for example VEGF, angiopoietins and fibroblast growth factor-2 (FGF-2)) However, EPCs are insufficient to completely regenerate all tissues which are lost after ischemia. [19] Contribution of EPCs to neovessel formation may range from 5% to 25% in response to granulation tissue formation or growth factor-induced neovascularization. [16]

There are different sources of progenitor cells, including: perivascular adventitia, heart, liver, spleen, gut, adipose tissue, muscle and bone marrow. [20] Most circulating EPCs however, are recruited from the bone marrow. EPCs are recruited from bone marrow in response to stimuli due to hypoxia which occurs after tissue ischemia or vascular damage. These stimuli are cytokines, growth factors and chemokines. The most important stimuli are: granulocyte macrophage colony stimulating factor (GMCSF), granulocyte colony stimulating factor (G-CSF), angiopoietin-1, monocyte chemo attractive protein-1 (MCP-1), vascular endothelial growth factor (VEGF) and stromal derived factor-1-α (SDF-1α). The last two factors are under transcription of HIF-1α (hypoxia inducible factor-1-α) which is specifically activated in hypoxic tissues. [4,19] These stimuli reach the bone marrow and stimulate the release of EPCs through eNOS and matrix metalloproteinase (MMP) dependant pathways. [5] NO activates metalloproteinase-9 in the bone marrow and modulates proliferation, differentiation and apoptosis in a variety of cell types. [21] NO bioavailability has thus revealed to be critical for the regulation of circulating EPC concentration. NO bioavailability is influenced by cardiovascular risk factors. [5,7]

Once EPCs are in circulation, they specifically home to sites of ischemia or vascular damage through specific receptors which recognize chemokines produced in injured tissues. [4] Enhancement of EPC mobilization limits the resulting damage, while inhibition of mobilization potentiates it. [5]

3.2 Factors that influence EPC numbers and function

EPC number and function is influenced by various factors, including cytokines, cardiovascular risk factors, pharmacological therapy and lifestyle modification. [7,11] These factors act synergistically on various mechanisms which culminate in reduced EPC number and function. [22] Lowering of peripheral EPC numbers seems to be caused by decreased mobilization from bone marrow or by reduced metabolism or reduced survival and/or differentiation rather than by depletion of stem

cells. [7,22] However, the inverse correlation between cardiovascular risk factors and EPC number and function has not been established. A lot of studies report the opposite according to Xiao et al. In their population based study, EPC numbers increased with increasing Framingham risk score.[17]

The factors that influence EPC numbers and function fulfill their effects through different mechanisms. However, many factors cause oxidative stress. Oxidative stress elicits multiple effects, including cell damage, genome instability, reduced telomerase activity, inactivation of the AKT/eNOS pathway, decreased NO bioavailability, decreased proliferation capacity and apoptosis of EPCs. Cell damage, genome instability and reduced telomerase activity cause cellular senescence of EPC which decreases EPC function. Inactivation of the Akt/eNOS pathway and a decrease of NO bioavailability cause impairment of adhesion, migration and tube forming capacities, more oxidative stress, release of pro-inflammatory mediators and apoptosis of EPCs, which affects EPC number and function. [4,5,7,19]

3.3 Treatment of low EPC numbers

Low EPC numbers or dysfunctional EPCs can be treated with cytokines (G-CSF, VEGF and SDF-1α), pharmacological therapy, lifestyle modification or transplantation of autologous EPCs. Most treatments are still subject of studies, but some of these treatments are already clinically applied for treatment of CVD without understanding the influences on EPCs.

Pharmacological therapy can be accomplished with a variety of drugs, including RAS inhibitors, AMD-3100, statins, EPO, estrogen replacement, agents able to decrease oxidative stress, ACE inhibitors, insulin and thiazolidinediones (class of PPAR-γ agonists). These drugs increase number and function of EPCs through enhanced mobilization and differentiation of EPCs, prevention of apoptosis, reduced senescence, improved migratory function, improved cardiomyogenic differentiation of EPCs and lowering blood pressure and blood glucose levels. [5,7,19]

Lifestyle modification includes physical exercise and smoking cessation. Physical exercise up-regulates the bioavailability of NO and VEGF. EPCs are actively mobilized from bone marrow, which restores EPC number. Smoking cessation reduces ROS formation and restores indeed partially EPC number and function. [5,7,19]

Transplantation of autologous EPCs or other cellular pools enriched with vascular progenitors can be applied to restore EPC level and function. These transplantations gave marginal and successful results in different studies that targeted treatment of atherosclerosis. [5]

3.4 Methods of quantification and functional assessment

At the moment, different methods of quantification and functional assessment of EPCs are available. Manual counting, flow cytometric analysis, incorporation into vascular networks on matrigel (matrigel assay), adhesion to mature endothelial cells or to matrix molecules, assay for migration in a Boyden-like chamber, staining for uptake of 1,1'-dioctadecyl-3,3',3'-tetramethylindo-carbocyanide-labeled acetyl low density lipoprotein (DiI-Ac-LDL) and binding of ulexlectin, standardized apoptosis-, senescence-, colony forming- and proliferative assays are all possibilities. [5, 19]

4.0 Role of EPCs in atherosclerosis

4.1 Difficulties around the role of EPCs in atherosclerosis

Whether EPCs perform positive or negative roles in atherosclerosis has not been clarified because of differences between studies. Also mechanisms have not been unraveled yet.
The behavior of EPCs in general is still the subject of many present studies. How EPCs are mobilized from bone marrow, how they home to specific sites in the body, what effects they have on blood vessels, what their functions are in different processes and whether some of their actions are pathogenic or not. Therefore, it is very difficult to find out their role in atherosclerosis when even their general role has not been elucidated yet. Studies which focus on the connection between EPCs and atherosclerosis are therefore hard and mostly without draught.

Also the results from equivalent studies differ. Many studies contradict each other. Such discrepancy is maybe due to differences in the mode of administration of EPCs, differences in nature of delivered cells (different subpopulations [17]), differences in stage of atherosclerosis when transplantation of EPCs is performed or differences in time point when results are obtained. (It is shown that EPC numbers exhibit a diurnal variation.[17]) An accurate comparison of these studies is therefore complex and might even be impossible. [13]

In addition, there have been many studies performed which concentrate on the combination of EPCs and different forms of CVD. However, less studies concentrate on the development of atherosclerosis and EPC. Most studies concentrate on the consequences of atherosclerosis, for example ischemia or myocard infarct. There are also many studies which concentrate on the connection between cardiovascular risk factors and EPCs and not on the diseases themselves.

Below, an overview of the various opinions and findings is given.

4.2 Positive roles of EPCs

EPCs play a major role in the pathogenesis of atherosclerosis. It is thought by many researchers that EPCs help to repair endothelial injury by replacing dysfunctional endothelial cells or incorporating at places of endothelial denudation and that due to depletion of EPCs in the presence of cardiovascular risk factors or absence of competent bone marrow, dysfunctional endothelium and atherosclerotic lesions cannot be repaired. [22] This lack of repair contributes to intensifying of the inflammation and atherosclerotic lesion size leading to progressive senescence and dysfunctional remodeling of the arterial wall according to Zhang M et al. [11] In late stages of atherosclerosis this may lead to poor collateralization. [5] There is a balance between the quantity of injury and the capacity for repair. When the injury overrules the capacity for repair, the endothelium is damaged and atherosclerotic lesions might develop. [22] Another mechanism by which EPCs can have a positive effect is that they may mediate interactions between cardiovascular risk factors in such a manner that these risk factors are ruled out. [4]

Regulation of the number and function of EPCs directly influences the development and maintenance of atherosclerotic lesions. Schmidt-Lucke C et al. documented from their study that reduced levels of circulating EPCs, partly due to cardiovascular risk factors, are associated with impaired vascular integrity and predict atherosclerotic disease progression. [22] This finding is confirmed by others. [4,19] Enhancement of EPC mobilization from bone marrow lowers the resulting damage, while inhibition of mobilization potentiates it. [5,7] The continuous repair of endothelial damage eventually will deplete EPCs in the circulation. This will trigger subsequent steps in the atherogenesis because of a reduced ability to repair the endothelium. [22]

Transplantation of autologous EPCs or other cellular pools enriched with vascular progenitors in atherosclerosis resulted in marginal and successful results in different studies. [5] Xiao Q et al. have performed a large population based study in which the data obtained indicate that EPCs might protect against atherogenesis, but that their relevance increases when lesions get more advanced and in symptomatic CVD. [17]

4.3 Negative roles of EPCs

Recently, it is shown in some studies that EPCs not only fulfill positive roles in atherosclerosis because of their repair capacities, but that their actions can also lead to enhancement of atherogenesis. [19] EPCs might play a role in the progression or maintenance of pre-existing lesions. [18] Peinado VI et al. found that the number of progenitor cells attached to the endothelium in arteries correlated with the thickness of the arterial wall, suggesting an increase in lesion size. [23] This might be due to the pro-inflammatory and proangiogenic properties of EPCs. In several studies with bone marrow derived mononuclear cells, an increase in IL-6 and MCP-1 and a decrease in IL-10 is seen after administration of cells. This suggest a pro-inflammatory state and might implicate a partial skewing from Th-2 to Th-1 response. [13,24]

Several studies were done in a mouse model for atherosclerosis, the apolipoprotein (apoE)-knockout (KO) mice. These mice lack a functional *Apoe* gene and are therefore unable to produce apoE, which is essential for the transport and metabolism of lipids. The mice are healthy when born, but develop atherosclerotic lesions within 3 months. (Taconic Transgenic Models)
George J et al. found that apoE KO mice which received spleen cell-derived EPCs showed lesions with larger lipid cores, thinner fibrous caps and higher antibody levels against oxLDL cholesterol than controls. These data suggest that EPCs can cause plaque instability and acceleration of the atherosclerotic disease process. They also transferred bone marrow derived cells. Bone marrow derived cells may result in an increase in atherosclerotic lesion size, whereas EPC transfer could also potentiate lesion instability. [13] Silvestre J et al. studied transplantation of bone marrow derived mononuclear cells in apoE KO mice in a later stage of atherosclerosis, while ischemic injury had occurred. The cells stimulated angiogenesis at the site of ischemia, but as a side effect the cells also enhanced atherosclerotic plaque size. Plaque stability however, was not altered. [24] On the other hand, Tateishi-Yuyama et al. performed an equivalent study in patients with ischemic limbs, but they did not see any side effects and reported that transplantation was safe and effective for achievement of therapeutic angiogenesis. [25]

4.4 Levels of circulating EPCs can be used as a test for cardiovascular risk

There is a strong correlation between number of circulating EPCs and the Framingham risk factor score. [11] However, a lot of studies conclude that levels of circulating EPCs and function of these cells better predict the degree of endothelial turnover and damage and thus the associative cardiovascular events which can occur in the future than cardiovascular risk factors or the Framingham risk score and thus can be used as a novel surrogate prognostic biomarker. [4,8] A reproducible laboratory test measuring peripheral EPC number and function is needed for this purpose. [20] This prognostic value has been confirmed by two important studies according to Fadini GP et al. [5]

4.5 Possible treatments/therapies

Two possible new treatments of atherosclerosis using EPCs are therapeutic neovascularization and designer blood vessels. [6] Therapeutic neovascularization is the enhancement of circulating EPCs to increase vascular repair neovascularization and neoangiogenesis. Enhancement of circulating EPCs can be achieved by EPC transplantation or in vivo EPC induction. Designer blood vessels are vessels which are composed of in vitro grown EPCs with smooth muscle cells and degradable biomaterials. These designer vessels are then transplanted in damaged vessels where the biomaterial degrades and new extracellular matrix is formed. Hopefully this will lead to a healthy blood vessel.[6]

4.6 State of development

A lot of effort has been made, however precise action of EPCs in atherogenesis is still unknown. Unraveling functions and mechanisms of EPCs has just been started. A lot of progress has already been made, but much of this subject is not yet fully understood. There is still a long way to go before everything is elucidated.

4.7 Future Perspectives

At this point, it is unclear if new results can be obtained in the very near future. To get new results a lot of studies are needed, especially intelligent ones. Studies have to be more structured and new ways to study the subject are very welcome if we want to obtain new concepts, understandings and ideas. First, a general and straightforward definition of EPCs is crucial. Second, EPC isolation should be standardized. After that the following subjects should be studied in the near future: The molecules, cytokines and cofactors which are involved in the whole process and their role in altering EPC number and function, the actions and mechanisms of EPCs during early and late stages of atherogenesis, the mechanism through which development from stable to vulnerable plaque occurs, the cell types to which EPCs develop in atherosclerosis (do EPCs differentiate into SMCs in atherosclerotic plaques?) and the consequences of these cell types.

5.0 Discussion/Conclusions

At this point, one can only speculate about the role of EPCs in atherosclerosis. It seems that EPCs have positive and negative roles in the progress of the disease, however it is too early to make valid statements. In fact, a lot of researchers have been trying to do so; there are a lot of reviews on this subject but only a few relevant studies. In nearly all studies about this subject, circulating EPC levels are enhanced or transplanted and effects are observed. I think that there should also be other, more intelligent study designs which treat this subject in the future, otherwise mechanisms are never going to be unraveled. I also believe that the protocols of studies with EPCs must be standardized, differences between studies should be abolished (as described above) so that everyone performs their studies in the exact same way. This way, studies are going to be easily comparable and conclusions can be drawn safely. The subjects which I think that should be investigated are already summed up above.

The opinions on the actions of EPCs in atherosclerosis can be subdivided into two groups: negative effects are mediated through paracrine angiogenic and pro-inflammatory effects of the EPCs on endothelium and atherosclerotic lesions and positive effects are a consequence of the repair capacities of EPCs or through the deleterious effects on cardiovascular risk factors.

It is hard to draw a conclusion with this little information, but I think EPCs have both positive and negative roles in atherosclerosis in general. It depends highly on the subpopulation of EPCs which actions are performed. Negative effects could be the consequences of a single group of EPCs which exhibit strong pro-inflammatory and angiogenic properties, positive effects can then be subscribed to a group of EPCs which possess more differentiating ability. Every cell is specialized for its own task. To use EPCs in treatments it is useful to know if this hypothesis is true, because harmful subpopulations can be avoided.

Nowadays, there are already clinical trials in which autologous bone marrow derived cells, EPCs or other cellular pools are transplanted in patients with ischemia. I think one should be very careful with these treatments, because precise mechanisms and consequences are not yet known.

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