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Vaccination against atherosclerosis

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Chapter 1

**General introduction and
outline of the thesis**

Thomas van Es

1 ATHEROSCLEROSIS

1.1 GENERAL

Cardiovascular disease (CVD) is still the number one cause of death in the Western world. There are many different clinical manifestations of cardiovascular disease such as angina pectoris, cardiomyopathy, endo-myocarditis and aneurysm.

Atherosclerosis affects the medium and large sized arteries of the heart and blood vessels and is the underlying cause of many clinical symptoms of CVD. Major risk factors for CVD, such as smoking, obesity and a high fat diet are well recognized. In 2004, 45445 people died of the consequences of CVD, accounting for 33%¹ of all reported deaths in the Netherlands, whereas 28% (38824 people) of all reported deaths were related to cancer.² This states the importance of cardiovascular disease in the Dutch society.

The current treatment of atherosclerosis is mostly aimed at the reduction of risk factors by life style advice (stop smoking, more exercise, and lower cholesterol intake) and by subscribing drugs, such as statins, that lower plasma cholesterol levels. Additionally drugs that lower blood coagulation and blood pressure are prescribed. However, these interventions cannot prevent that CVD is still the leading cause of death in the Western world. Therefore, there is an urge to develop new therapies targeting the different molecular pathways and stages of atherosclerosis.

1.2 PATHOGENESIS OF ATHEROSCLEROSIS

1.2.1 Initiation of the lesion

The first stage of atherosclerosis is fatty-streak formation. Fatty streak formation is asymptomatic and is already found during the first decades of life in medium and large sized arteries, at predisposed sites. The typical atherosclerotic prone sites are characterized by low shear stress and high oscillatory shear stress, which increase adhesion of leukocytes and the expression of inflammatory genes.³

Under normal conditions, healthy endothelium is able to respond to physical and chemical signals by the production of a wide range of factors that regulate vascular tone, cellular adhesion, thrombus resistance, smooth muscle cell proliferation, and vessel wall inflammation.⁴ It is generally accepted that the activation of the endothelial cell layer, as a response to modulated gene expression, caused by hyperlipidemia, hypertension, diabetes mellitus and smoking, forms the first step in atherosclerosis, the fatty-streak formation.^{5,6}

Circulating lipoproteins, in particularly very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) infiltrate into the arterial intima and become modified through processes, such as oxidation, glycation, aggregation, association with proteoglycans or incorporation into immune complexes.⁷⁻¹⁰ The modified LDL particles are highly immunogenic and activate the endothelial cells.

Stimulated endothelial cells undergo a switch from a quiescent phenotype towards a phenotype that initiates a defense response. Most cardiovascular risk factors, such as smoking and high blood pressure, activate the molecular machinery in the endothelium, resulting in the expression of chemokines, cytokines and adhesion molecules designed to interact with leukocytes and platelets and designed to target inflammatory cells to specific tissues to clear invading microorganisms or to respond to vascular injury.¹¹ Activated endothelial cells express adhesion molecules, like vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin and E-selectin.^{3, 12} Leukocytes (i.e. monocytes and lymphocytes) express counter receptors for these adhesion molecules and decelerate via interaction with P- and E-selectin. Once slowed down, a more firm adhesion is facilitated via interaction with VCAM-1 and ICAM-1 with very late antigen-4 (VLA-4) and lymphocyte function associated antigen-1 (LFA-1), respectively, which are expressed on leukocytes.¹³⁻¹⁵

Once a firm adhesion is established, leukocytes migrate through the interendothelial junction into the subendothelial space (diapedesis) (Figure 1.2A). This process is facilitated by additional adhesion molecules, such as platelet/endothelial cell adhesion molecule-1 (PECAM-1) and junctional adhesion molecule-1 (JAM-1).^{16, 17} Furthermore, activated endothelial cells produce several chemokines and interleukins (IL), which enhance diapedesis¹⁸ and the recruitment of leukocytes into the lesion. CCL2 (MCP-1), produced by vascular endothelial cells, is an important chemoattractant for monocytes and T cells. These cells play an important role in lesion development.¹⁹⁻²² Activated vascular cells but also macrophages within the lesion continue to produce chemokines such as, CCL5 (RANTES), CXCL10 (IP-10) and CCL11 (eotaxin) to further enhance the immune response (Figure 1.2B).²³⁻²⁵ Within the lesion, monocytes differentiate into macrophages by stimulation of macrophage-colony-stimulating factor (M-CSF), which is produced by endothelial and smooth muscle cells.²⁶ The macrophages become activated by the uptake of modified LDL, thereby transforming into lipid loaded “foam cells”. This process will be discussed in more detail later on.

The above-described process will lead to focal fatty streak formation and remain asymptomatic for a long time and may even be reversible at this stage. However, during the progression of life these fatty streaks may develop into more advanced lesions, depending on the exposure to several risk factors. A schematic overview of the above-described processes are depicted in figure 1.1A.

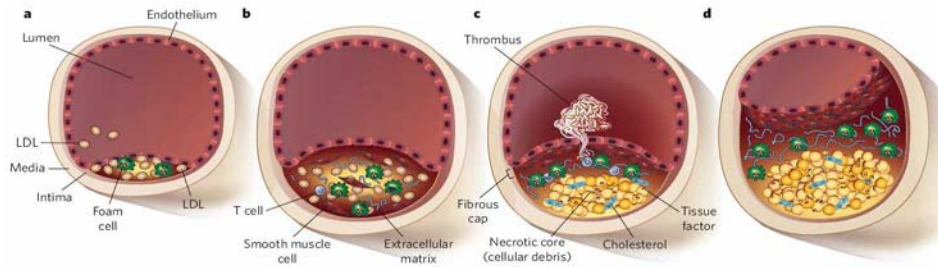


FIGURE 1.1: DEVELOPMENT OF ATHEROSCLEROSIS.

A, fatty streak formation: Endothelial cells in lesion prone areas become activated leading to permeability for lipoproteins, such as LDL. Within the intima, LDL is modified by processes, like oxidation and becomes immunogenic. Monocyte derived macrophages migrate into the intima and start to phagocytose the modified LDL particles and become activated. Macrophages, trapped in the intima and loaded with cholesterol are now called “foam cells”. B, progression of the plaque: vascular smooth muscle cells become activated. They migrate and produce extracellular matrix proteins, in particular collagen, to form a cap structure to protect the lesion from the blood flow. More leukocytes are recruited to the intima, such as T cells and monocytes and these cells enhance the inflammation. To compensate for the narrowed lumen, outward remodeling takes place. C, necrotic core and thrombus formation: “foam cells” become apoptotic and eventually form a necrotic core, consisting of cellular debris and free cholesterol. The necrotic core is highly immunogenic, which results in the recruitment of more inflammatory cells to the intima. The fibrous cap formed by the smooth muscle cells protects the lesion from the bloodflow, however this may not prevent rupture. When the plaque ruptures, a thrombus will form and can cause clinical symptoms, such as acute coronary syndrome. D, obstructive lesion: when the plaque does not rupture, the lesion can grow by the ongoing inflammation. When outward remodeling is not sufficient, the lesion becomes obstructive and causes clinical symptoms, such as angina pectoris. More details are described in the text. (Adapted from Rader and Daugherty).²³⁶

1.2.2 Progression of the lesion

Atherosclerotic lesion progression is associated with the continuous influx of inflammatory cells due to the local production of chemokines in the plaque. T cells interact with activated macrophages (“foam cells”), which express class II and class I histocompatibility complexes (MHC II and MHC I) and present antigens to T cells. CD4⁺ and CD8⁺ T cells are associated with all stages of atherosclerotic lesion development and activation of T cells results in a broad range of immune responses and the acquisition of many features of a chronic inflammatory state.²⁷ Activated T cells produce several cytokines such as IFN- γ and TNF- α .²⁸ These cytokines activate other cells within the lesion, such as endothelial cells, macrophages and smooth muscle cells.

The next step in atherosclerotic lesion development is the formation of a necrotic core. This necrotic core consists of extracellular lipids and cellular debris derived from apoptotic cells. This process involves pro- and anti-apoptotic proteins, including death receptors, proto-oncogenes and tumor suppressor genes. Oxidized sterols, present in oxLDL, promote apoptosis and necrosis of foam cells in the plaque, thereby releasing oxidized and insoluble lipids within the lesion.²⁹

The necrotic core becomes covered by a fibrous cap, which consists of smooth muscle cells and extracellular matrix proteins like collagen. The formation of the cap structure is facilitated by cytokines and growth factors, which are produced by activated macrophages and T cells. The cytokines and growth factors stimulate smooth muscle cells to proliferate and migrate to the cap.^{5, 6} Activated smooth muscle cells can migrate from the media to the intima, where they are able to internalize lipids and transform into smooth muscle cell derived foam cells and can produce matrix proteins.³⁰

In this stage of lesion development, the outward remodeling of the vessel takes place to compensate for the increase in lesion size. The process of outward remodeling is necessary to prevent severe narrowing of the vessel and to preserve blood flow.³¹ The above-described process is schematically depicted in figure 1.1B and C.

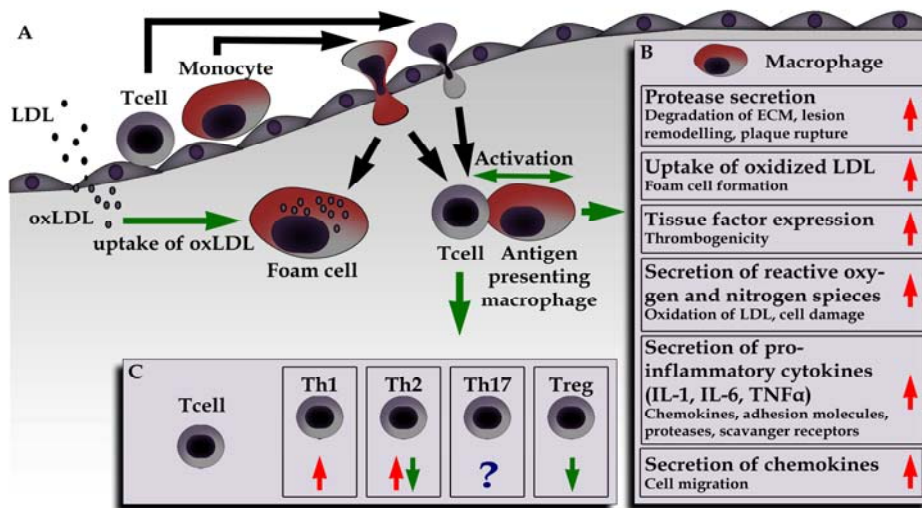


FIGURE 1.2: THE ROLE OF MACROPHAGES AND T CELLS IN ATHEROSCLEROSIS.

A, Monocyte and T cells are recruited to the intima of the vessel wall where they become activated via different stimuli. Depending on the microenvironment within the intima, the inflammation can be enhanced or dampened. B, Within the intima, macrophages take up modified LDL and become activated and exert several pro-atherogenic properties. C, Macrophages are able to take up modified LDL and present it to T cells, which recognize the antigen and become activated. Depending on the microenvironment, T cells can develop into different subtypes of T cells population. B and C, During atherosclerosis, T cells and macrophages tightly regulate each others activation and function, which make them important immune cells in bridging the innate (macrophages) and adaptive (T cells) immune system.

1.2.3 Lesion stability and rupture

When outward remodeling can not compensate for the reduced volume, the advanced atherosclerotic lesion leads to insufficient blood flow to distal tissues, causing clinical symptoms, such as angina pectoris (Figure 1.1D). However, acute cardiovascular events are associated with myocardial infarction and stroke as a result of a ruptured plaque and a subsequent thrombotic event (Figure 1.1C).^{32, 33}

Plaque rupture is the final outcome of plaque destabilization. During lesion development activated macrophages, T cells and other immune cells accumulate around the necrotic core and in the shoulder regions of the plaque.^{6, 34} Furthermore, activated macrophages, T cells, and mast cells have been observed at sites of plaque rupture, indicating their potential relation to the rupture process.³⁵⁻³⁷ T cells, predominantly of the T helper 1 phenotype, produce high amounts of IFN- γ , which inhibit the production of collagen by vascular smooth muscle cells and their cell proliferation, thereby negatively influencing plaque stability.^{28, 38}

Activated macrophages produce several proteases, which destabilize the plaque, such as matrix metalloproteinases (MMPs), cysteine proteases, and chymases.³⁹⁻⁴¹ Members of these families of enzymes are found in the atherosclerotic plaque and can degrade the matrix. Especially MMP-1, MMP-8, MMP-9 and MMP-13 may be important.⁴² In addition, macrophages induce apoptosis of vascular smooth muscle cells, thereby negatively influencing the collagen production and subsequent plaque stability.^{43, 44}

Since the weakened cap structure cannot withstand the hemodynamic forces, the unstable atherosclerotic lesion may rupture and consequently expose thrombogenic plaque material (lipids/necrotic core) to the blood. The subsequent aggregation of platelets and coagulation forms a thrombus, which obstructs blood flow and results in clinical symptoms such as myocardial infarction and stroke.³⁴

Plaques that are prone to rupture contain high numbers of activated immune cells. Furthermore, patients with acute coronary syndromes (ACS) demonstrate signs of inflammation, with elevated levels of circulating cytokines, acute phase reactants, and not only activated T cells.^{45, 46} Therefore the immune system and inflammation play an important role throughout atherosclerotic plaque development, but are also crucial in the final stage of atherosclerosis.

2 ATHEROSCLEROSIS: AN INFLAMMATORY DISEASE

During the last years, it has become more clear that atherosclerosis resembles a chronic autoimmune-like disease. Inflammation plays a pivotal role in the process of atherosclerotic lesion development. As atherosclerosis already starts in early life and gradually develops during life, it can be considered a chronic disease. Inflammation is tightly regulated by cells involved in the innate and adaptive

immune response, both with their specific role in host defense.^{47,48} This is illustrated in different mouse models for atherosclerosis with a specific depletion of components of the innate as well as the adaptive immune system.^{49, 50} For example, when LDL receptor deficient mice were cross-bred with lymphocyte-deficient (RAG1 deficient) mice, a reduction in lesion size was observed.⁵¹

Further evidence supporting the relation between atherosclerosis and inflammation is found in gene polymorphisms involved in inflammation, such as TLR4 polymorphisms.⁵²⁻⁵⁴ There are associations found between an increased risk of cardiovascular events and autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus.⁵⁵ However, besides inflammation, atherosclerosis is also associated with metabolic and hemodynamic factors, thereby placing atherosclerosis as an unique disease.

Metabolic and hemodynamic factors are likely to play a role in the initiation of autoimmunity by activation of endothelial cells and subsequent recruitment of immune cells. This process leads to initial atherosclerosis via the innate immune system and gradually evolves into a chronic, autoimmune like, inflammatory disease via the adaptive immune system. Therefore, the regulation and crosstalk between innate and adaptive immune cells is very important in the initiation and development of atherosclerosis.

Although several exogenous stimuli, such as cytomegalovirus (CMV) and *Chlamydia pneumoniae* have been identified in atherosclerosis,⁵⁶ there is also evidence that endogenous stimuli are involved in the process of atherosclerosis.^{57, 58}

2.1 INNATE IMMUNE SYSTEM

The innate immune response is the first line of defense against pathogenic stimuli and is characterized by a natural selection of germline-encoded receptors, which focuses on highly conserved motifs in pathogens. It provides the first line of defense for the host and is characterized by fast (minutes to hours) and blunt (lacking exquisite structural specificity) responses. It is a very conserved system, which is already present in many lower organisms.

Important cells involved in the innate immune response are macrophages, neutrophils, mast cells and natural killer (NK) cells. The exact role of neutrophils in atherosclerotic lesion initiation is not known yet, but neutrophils are found in ruptured or eroded plaques, indicating that they are recruited in a later phase in response to injury.⁵⁹ However, upon endothelial activation, P-selectin and E-selectin are upregulated, which both bind neutrophils suggesting a non-confirmed role in the initial stages of atherosclerosis.⁶⁰⁻⁶³

Another kind of innate immune cells are mast cells. These cells are present in atherosclerotic lesions and are activated at sites of plaque rupture, indicating that mast cells are involved in the rupture process of advanced lesions.⁶⁴ Mast cells store granules, which contain growth factors, chymases and pro-inflammatory cytokines.^{65,66} Once stimulated, the mast cells degranulate and exocytose the granule-associated effector substances into their microenvironment, thereby negatively influencing plaque stability.^{67,68}

NK cells are found to play a role in early atherosclerosis.^{69,70} Their role however, is not yet completely understood. Reduced atherosclerosis was observed in LDLr^{-/-} mice after bone marrow transplantation from transgenic mice overexpressing the Ly49A receptor, which results in dysfunctional NK cells.⁷⁰ Noteworthy, in these transgenic mice, not only NK cells are affected, but also NK T cells, CD8⁺ cytotoxic cells and other lymphocytes expressing granzyme A. Therefore, it is difficult to determine the exact role of NK cells in the initiation of atherosclerosis.

The key inflammatory cell during atherosclerotic plaque formation is the macrophage. Macrophages are part of the innate immune system and have an important "bridge" function between the innate and adaptive immune system by presenting innate immune signals to the adaptive immune system. In atherosclerosis, macrophages play an important role in the various phases of lesion formation and progression.¹² Infiltrated monocytes differentiate into macrophages and start to express cytokines and receptors⁷¹ by stimulation via M-CSF, which is produced by endothelial and smooth muscle cells.²⁶ Monocyte-derived macrophages express pattern recognition receptors (PRRs), which are involved in the innate immune response. PRRs recognize a restricted pattern of ligands called pathogen-associated molecular patterns (PAMPs). PAMPs consist of many different ligands such as lipopolysaccharides, aldehyde-derivatized proteins, bacterial DNAs and denatured DNAs, resulting in endocytoses and lysosomal degradation of the ligand^{72,73} and activation of nuclear factor- κ B, resulting in an inflammatory response.⁷⁴

Two important groups of PRRs are the scavenger receptors (ScRs) and the toll-like receptors (TLRs), which are both expressed by macrophages.^{47,75} The ScR family, which includes CD36, CD68, SR-A and SR-B, mediates the internalization of modified lipoproteins (e.g. oxLDL) via endocytosis and contributes to foam cell formation, a hallmark of the atherosclerotic lesion.^{9,76,77} The uptake of modified lipoproteins and their constituents via ScRs is important in triggering the production of the mediators of innate immunity such as, IL-1 β and TNF- α .⁷⁸ Additionally, they are important in the activation of the adaptive immune system via presentation of internalized material on MHC class II molecules.⁷⁹

The other important pattern recognition receptor family, the TLRs, are involved in the activation of macrophages.⁸⁰ Studies with TLR4 and ApoE double knock out mice, demonstrate a reduction in atherosclerotic plaque development, thereby further illustrating the importance of the innate immune system in atherogenesis.⁸¹ Activation of ScRs and TLRs on macrophages within the plaque results in a proinflammatory environment capable of activation of other vascular and immune cells, thereby enhancing lesion progression.

Within the lesion, foam cells contribute to the production of reactive oxygen species, cytokines and other molecules, thereby amplifying and sustaining the inflammatory response in the plaque.⁸⁰ Additional cells in the early plaque, such as smooth muscle cells and endothelial cells become activated by these molecules and start producing interleukins and chemokines, i.e. IL-1 β , IL-6, IL-18, TNF- α and CCL2.⁸²⁻⁸⁴ Besides production of these cytokines, macrophages and other vascular cells start to produce T cells attractants, like CCL5, CXCR3, CXCL10 and CXCL11.²⁴ The pro-atherosclerotic effect of T cells in atherosclerosis is demonstrated by blocking for example CCL5 or CXCR3 and thereby attenuating atherosclerosis.^{85,25}

Besides attracting T cells, macrophages also express MHC class II in association with a specific epitope. This is a pivotal step in bridging the innate immune response to the adaptive immune response. Within the lesion MHC class II expressing macrophages (and also dendritic cells, discussed in more detail later on) can be detected close to T cells, which suggests that there is an ongoing immune activation of the adaptive immune response in the plaque.⁸⁶⁻⁸⁸

2.2 ADAPTIVE IMMUNE SYSTEM

The adaptive immune system is characterized by a slower response than the innate immune system and exerts a high specificity for its target. The high variation of antigen specificity of, for example T cell receptors (TCRs) and immunoglobulins, is a result of somatic rearrangements in blast cells. When T cells recognize a specific antigen, which is presented by an antigen-presenting cell (APC), an adaptive immune response against that specific antigen is initiated. Many cells are involved in the adaptive immune response, in particular DCs, T cells, B cells and macrophages. Upon stimulation, these cells demonstrate versatile effects, for instance a helper T cell response, regulatory T cell response, cytotoxic T cell response, stimulation of B cells and subsequent antibody production, production of chemokines and cytokines, which enhance and regulate the innate and adaptive immune cells.

The role of the adaptive immune system, especially the T and B cells, is nicely demonstrated in studies in which these cells were not functional by for example using severe combined immunodeficient (SCID) mice, resulting in decreased lesion formation.⁸⁹

2.3 AUTOANTIGENES

Many exogenous and endogenous antigens have been suggested to be presented in atherosclerosis. As mentioned before, T cells recognize specific antigens presented by APCs with their TCR. Upon activation, T cells that recognize a specific antigen will start proliferating, resulting in clonal T cell expansion. Clonal T cell expansion has been detected in atherosclerotic lesions of mice and humans, suggesting specific TCR activation by an (auto) antigen.⁹⁰⁻⁹²

Since inflammation has a prominent role in atherosclerosis, it has been suggested that exogenous stimuli may initiate atherosclerotic lesion development. Many virus and bacteria related antigens have been identified in atherosclerosis, but most extensively studied are the Cytomegalovirus (CMV)⁹³ and *Chlamydia pneumoniae*.^{94, 95} Both pathogens are associated with aggravating atherosclerosis in mice and humans, since antibodies against these pathogens have been correlated with the severity of cardiovascular disease in patients.^{93, 96-98} Furthermore, experimental data have shown that infection with *Chlamydia pneumoniae* enhances atherosclerosis.⁹⁷ However, large clinical trials on the treatment of cardiovascular patients with antibiotics directed against *Chlamydia pneumoniae* did not result in a reduction of cardiovascular events in antibiotic treated patients.^{99, 100}

Another group of antigens is of endogenous origin, but is related to exogenous antigens via a process called molecular mimicry.¹⁰¹ An example are the heat shock proteins (HSPs), a group of highly conserved proteins. These proteins are highly conserved and immune responses induced against bacterial HSP60 may cross-react with responses against endogenous hsp60. Endogenous HSP60 is induced when cells are exposed to different stress stimuli.¹⁰² HSP60 is expressed on endothelial cells, vascular smooth muscle cells and mononuclear cells in human atherosclerotic plaques.¹⁰³ Furthermore, circulating antibodies against HSP60 were detected in patients with atherosclerosis and HSP60 specific T cells were detected within the atherosclerotic plaque.^{104, 105} Therefore an immune response against HSP60 may contribute to endothelial damage and subsequent enhancement of atherosclerosis.^{106, 107} Interestingly, antibodies against HSP60 and its prokaryote homologue HSP65 are also detected in other autoimmune diseases such as rheumatoid arthritis.¹⁰⁸ In relation to this, patients with rheumatoid arthritis have a 2- to 5- fold higher risk of cardiovascular morbidity and mortality.¹⁰⁹

A third group of antigens is derived from endogenous sources. This group mainly contains altered self-proteins and the autoimmune response against these proteins is directed against the neo-epitopes of the altered proteins. T cells do not react with native LDL, as there is immunological tolerance against self-antigens. However, modifications of LDL lead to non-self epitopes (neo-epitopes) and increased autoreactivity of T cells in mice and human.^{110, 111} LDL in the atherosclerotic lesion can be modified by various processes as described before, and are accountable for the development of neo-epitopes.¹¹² An example of a neo-epitope related to LDL is oxLDL. OxLDL specific T cells are identified in human plaques and circulating antibodies against epitopes of oxLDL are detected in serum samples of patients with cardiovascular disease.^{110, 113} Furthermore, lymph nodes and spleens of ApoE-deficient mice gave rise to an oxLDL specific T and B cell line displaying a strong humoral and cellular immune response against these modified lipoproteins, indicating the role of oxLDL in immune activation.^{114, 115}

2.4 DENDRITIC CELLS

Although activated macrophages effectively present antigens to T cells, dendritic cells (DCs) are the most potent APCs of the immune system and are the key players in the regulation of the adaptive immune response.¹¹⁶

Immature DCs efficiently take “samples” of their antigenic microenvironment through macropinocytoses and receptor mediated endocytosis. Depending on the triggered PRRs, DCs present the antigen in context of either MHC class I or MHC class II and produce cytokines to evoke an appropriate immune response.¹¹⁷ Therefore, DCs are crucial for an adequate clearance of the infection, but DCs are also responsible for pathogenic immunological responses.

Dendritic cells are a component of the vasculature associated lymphoid tissue and low numbers are found in the intima of healthy, but susceptible arteries before atherosclerotic lesion development is initiated.¹¹⁸ Furthermore, DCs increase in number in the intima during the progression of atherosclerosis.¹¹⁹ This indicates a role for DCs in the initiation and regulation of arterial inflammation. Immature DCs capture antigens at the site of inflammation and migrate to secondary lymphoid organs, such as the spleen and lymph nodes. The migration is orchestrated by various chemokines, such as CCR-2, -5, -6, -7 and CXCR1 and CXCR2.^{120, 121} Within the secondary organs the matured DCs are able to stimulate antigen specific T cells, which is further enhanced by the fact that mature DCs starts to express co-stimulatory molecules, such as CD40, thereby enabling them to interact with CD40L expressing T cells.

Some macrophages take up antigens in tissues and differentiate into migratory cells resembling dendritic cells that emigrate to lymph nodes.¹²²

However, in hypercholesterolemic conditions these monocyte derived DCs are possibly not able to migrate out of the atherosclerotic lesion to the secondary lymph organs, thereby directly activating residential cells in the lesion such as T cells, which leads to aggravated atherosclerosis.¹²²⁻¹²⁴

2.5 T CELLS

T cells are activated in the lymphoid organs by APCs by recognizing a specific antigen and by costimulatory signals such as CD40L-CD40 and CD80/CD86-CD28 interactions.¹²⁵ The microenvironment determines the type of T cell response. For example IL-12 production by APCs lead to the development of T helper 1 (Th1) cells, which have been shown to aggravate atherosclerosis.¹²⁶ Activated T cells migrate from the lymphoid organs to the site of inflammation e.g. the atherosclerotic lesion via chemokine signaling and are reactivated upon recognition of the antigen presented by APCs. Interference in T cell migration via the inhibition of the CXCR3 and CCL5 pathway, results in reduced Th1 cell influx into the lesion and subsequently in reduced atherosclerosis.^{85, 127}

Most T cells within the atherosclerotic lesions are CD3⁺ CD4⁺ TCR αβ⁺ cells.^{128, 129} Although CD8⁺ T cells are also found in atherosclerotic plaques, their role in atherosclerosis is not yet clear. Furthermore, CD4⁻ CD8⁻ TCR γδ T cells are found in plaques and may play a role in relation to IL-17 production.¹²⁹ IL-17 will be discussed later in more detail.

In relation to the topic of this thesis, the discussed T cell populations will be limited to Th1, T helper 2 (Th2), T helper 17 (Th17) and regulatory T (Treg) cells (Figure 1.3).

2.5.1 T helper 1 cells

Data obtained from patients with CVD illustrated a predominant Th1 pattern within atherosclerotic plaques. This is also observed in mouse models for atherosclerosis. T cells differentiate into Th1 cells through stimulation of naive T cells with Th1 polarizing cytokines, such as IL-12 and IL-18.^{130, 131} The production of interferon (IFN)-γ hallmarks Th1 cells, which has pleiotropic pro-atherosclerotic effects. IFN-γ promotes activation of macrophages and endothelial cells to produce more adhesion molecules, proinflammatory cytokines and chemokines, which results in more T cell recruitment. Furthermore, IFN-γ promotes the production of proteases and inhibits collagen production, thereby interfering in plaque stability.¹³² Another Th1 associated cytokine is TNF-α, which also exerts pleiotropic pro-atherogenic effects.¹³³

The role of Th1 cells in the aggravation of atherosclerosis has already been demonstrated. For example, vaccination against IL-12 in LDLr^{-/-} mice results in a reduction in IFN- γ expression within the lesion and reduced atherosclerotic lesion development.¹³¹ Buono *et al.* demonstrated in LDLr and IFN- γ deficient mice a 75% reduction in lesion size, indicating the pro-atherosclerotic nature of Th1 cells.¹³⁴ Furthermore, IL-18 deficient ApoE^{-/-} mice also showed reduced atherosclerosis.¹³⁰

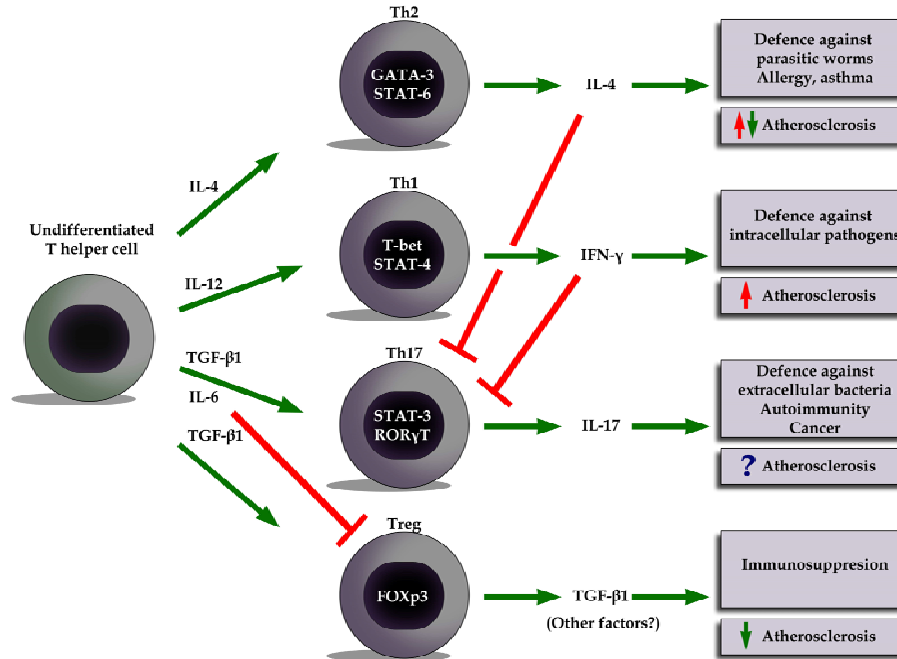


FIGURE 1.3: DIFFERENTIATION OF T HELPER CELLS.

Naive T cells differentiate into either Th1, Th2, Th17 or Treg cells upon stimulation with specific interleukins. Every Th cell subset has its own specific function in the immune response. However, a disproportional proliferation of a Th cell subset may lead to autoimmune diseases or chronic inflammation. The role of T helper cells in atherosclerosis is also depicted in this figure. See text for a more detailed description of the different Th subsets. (Adapted and modified from Tato and O'Shea)²³⁷

2.5.2 T helper 2 cells

Traditionally, Th2 cells are considered anti-atherosclerotic due to the production of IL-4, IL-5, IL-9, IL-13, IL-10 and IL-3.¹³⁵ An athero-protective role for these cells is suggested since the Th2 interleukins inhibit Th1 cells. Overexpressing IL-10 in LDLr^{-/-} mice for example, exerts anti-atherosclerotic effects.^{136, 137} Furthermore, IL-5 exerts an indirect anti-atherosclerotic effect by stimulation of B-1 cells. These B-1 cells produce natural occurring T15/EO6 IgM antibodies directed against oxLDL. These natural antibodies can block the uptake of oxLDL by macrophages and

thereby attenuate atherosclerosis.¹³⁸ This effect is nicely illustrated by Binder *et al.*, where IL-5 deficient bone marrow is transplanted into a LDLr^{-/-} recipient mice leading to aggravated atherosclerosis and a decrease in natural autoantibodies.¹³⁹

Conflicting data exist on the role of IL-4 in atherosclerotic plaque development. An athero-protective effect on initial plaque development was found after injection of IL-4 into mild hypercholesterolemic mice.¹⁴⁰ On the other hand IL-4 deficient ApoE^{-/-} mice demonstrate reduced atherosclerosis, illustrating a pro-atherosclerotic effect of IL-4.¹⁴¹ Moreover, IL-4 is associated with increasing MMP-1 production by macrophages and may therefore have negative effects on plaque stability.¹⁴²

For long, it was thought that a disturbed balance between Th1 and Th2 cells was causative for many autoimmune diseases, including atherosclerosis. However, the aforementioned findings on Th2 cells show that the classical Th1/Th2 balance cannot adequately explain the inflammatory process in atherosclerosis.

Recently, two new T cell subsets were identified: the Th17 cell and the Treg cell. These cells provide more complexity and may provide an opportunity to explain the observed conflicting experimental effects.

2.5.3 Regulatory T cells

The regulatory T cells (Tregs) are important in maintaining immune homeostasis and preventing autoimmunity.¹⁴³ Tregs develop in the thymus and display a diverse TCR repertoire specific for autoantigens. Tregs migrate from the thymus into the peripheral tissues and exert their anti-inflammatory response by recognition of specific autoantigens.¹⁴⁴

There are various mechanisms of immune suppression by Tregs.¹⁸² Firstly, the suppression of immune cells by cytolysis, via granzymes and perforins. Secondly, suppression by metabolic disruption, via “consuming” IL-2. Thirdly, suppression by inhibitory cytokines, such as TGF- β and IL-10. Finally, suppression by targeting DCs, via the interference in maturation and function of DCs, for example by cell-cell inhibition via CTLA4 (on Tregs) and CD80/CD86 (on APCs).¹⁴⁶

Tregs can generally be divided into two groups, the natural occurring Treg (nTreg) cells and the inducible T regulatory (iTreg) cells.^{147, 148} nTreg cells express CD4, CD25 (IL-2R α), cytotoxic T lymphocyte antigen (CTLA)-4 and forkhead box P3 (Foxp3).¹⁴⁵ nTreg cells exert their immunosuppressive action predominantly by expressing membrane bound TGF- β , which suppress cells via cell-cell contact in paracrine fashion.^{146, 149} Furthermore, nTreg cells are able to bind to CD80 and/or CD86 via CTLA-4, thereby suppressing the immune system.¹⁵⁰

There are also Foxp3 negative Treg cells leaving the thymus, which can be induced in the periphery to become immunosuppressive Treg cells, called inducible Tregs (iTregs). Depending on the suppressive action, these cells can be divided in Tr1 cells, which predominantly produce IL-10¹⁵¹ and Th3 cells, which exert their immunosuppressive function predominantly via TGF- β . Interestingly, Th3 cells do transiently express FoxP3.^{152, 153}

Mallat *et al.* hypothesized that adaptive or natural regulatory T cells may play an important role in the regulation of pathogenic T cells in atherosclerosis. There are several studies underlining this hypothesis. Depletion of Treg cells by treatment with anti-CD25 antibodies results in aggravated atherosclerosis in ApoE^{-/-} mice.¹²⁵ Furthermore, van Puijvelde *et al.* demonstrated that the induction of Treg cells via tolerance induction against oxLDL or HSP60 leads to attenuated atherosclerosis.¹⁵⁴ Moreover, Foxp3 expression has been detected in human atherosclerotic plaques, indicating that Treg cells are present.¹⁵⁵

2.5.4 T helper 17 cells

Th17 cells are a novel T cell subset with a separate lineage. Langrish *et al.* showed that IL-23 selectively induces the proliferation of *in vivo*-primed IL-17-expressing Th cells and that these cells do not produce IL-4 or IFN- γ , indicating a separate Th subset.¹⁵⁶ Harrington *et al.* and Park *et al.* further established the idea of a separated lineage of Th cells distinct from the T helper type 1 and 2 lineages. A naive precursor T cell is potently inhibited by IFN- γ and IL-4 in differentiation towards Th17 cells, whereas committed Th17 cells are resistant to suppression by Th1 or Th2 cytokines.¹⁵⁷ Together these data provide evidence for a new Th subset, which is regulated by cytokines of Th1/Th2 cells and is involved in autoimmunity.

Hence the name, IL-17 is the most prominent cytokine produced by Th17 cells. The IL-17 family consists of six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. IL-17A and F are most related to each other and share a 50% homology in protein sequence.¹⁵⁸ As IL-17A was the first member of the IL-17 family which was identified, it is mostly designated as IL-17.

The IL-17R family consists of five members, designated as IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE.¹⁵⁸ The best-studied IL-17R is IL-17RA, also designated as IL-17R, and is expressed ubiquitously through the body, which explains the pleiotropic effects of IL-17. The primary source of IL-17A and F are Th17 cells.¹⁵⁹ However, there are other cells of the innate and adaptive immune system which produce IL-17A and F. It has been shown that CD8⁺ T cells produce IL-17A and F as well as the $\gamma\delta$ T cells.^{160, 161} In many different cell types, binding of IL-17A and/or F to its receptor results in the upregulation of a number of pro-inflammatory interleukins and chemokines.^{158, 162-165} IL-17A and F also exert a

chemotactic effect in recruiting and activating neutrophils, providing a mechanism by which Th17 cells can mediate the crosstalk between innate and adaptive immune responses.^{158,165,166} IL-17 exhibits pleiotropic biological effects on various atherosclerotic lesion-associated cell types, such as endothelial cells, vascular smooth muscle cells and macrophages.¹⁶⁷⁻¹⁶⁹ Upon activation by IL-17 these cells produce pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs), including IL-6, CXCL8, CCL2 and MMP-9.^{169, 170} However, the role of IL-17 in atherosclerosis is not investigated yet. In this thesis, the role of IL-17 and its receptor in atherosclerosis will be discussed in chapter 3 and 4.

Besides IL-17, Th17 cells also produce IL-21, IL-22 and CCL20. IL-21 is expressed by Th17 cells¹⁷¹, but also by other cell types, such as IL-6 stimulated T cells.^{172,173} The receptor for IL-21 is expressed only on lymphoid cells and predominantly on B cells.¹⁷⁴ IL-21 has pleiotropic effects, such as stimulating proliferation and differentiation of CD8⁺ T cells¹⁷⁵ and it promotes differentiation and isotype switching in B cells.¹⁷⁶ IL-21 induces CXCL8 expression in macrophages¹⁷⁷, which is involved in the recruitment of monocytes to the early lesion, thereby aggravating atherosclerosis.¹⁷⁸ Furthermore, IL-21 regulates the differentiation of CD4⁺ T cells to Th17 cells in an autocrine manner.^{171, 179}

IL-22 is a member of the IL-10 family¹⁸⁰ and the IL-22 receptor subunits are primarily expressed on epithelial and parenchymal tissues.¹⁸¹ IL-22 protects against liver damage in an acute inflammation model.¹⁸²

Finally, Th17 cells produce CCL20.^{183, 184} Interestingly, its receptor CCR6, is also expressed by Th17 cells.^{183, 185} This may imply an autocrine mechanism to regulate its own recruitment in inflamed tissues. The development pathway of the Th17 cell lineage is actively investigated. Initially it was thought that IL-23 was the driving force behind Th17 development.¹⁵⁶

IL-23 is a heterodimeric interleukin consisting of a p40 and a p19 subunit. IL-23 is closely related to IL-12 as they both share the p40 subunit.¹⁸⁶ The receptor of IL-23 consists of a heterodimeric complex consisting of IL-12R β 1 shared with the IL-12 receptor and a unique IL-23R. The IL-23R shares many features with IL-12R β 2, which is the other part of the IL-12 receptor. The IL-23R is mainly expressed on effector T cells and not on naive T cells, suggesting an important role for IL-23 in ongoing inflammation.^{186, 187}

IL-23 is mainly produced by macrophages and dendritic cells. Like the subunits of IL-27, EB13 and p28, p19 must be expressed together with p40 in the same cell in order to be functionally excreted as IL-23.¹⁸⁶ Recently, it is demonstrated that the development of gut inflammation in T-cell-deficient mice depends on IL-23, in that the loss of IL-23 but not IL-12 was associated with a decrease in gut inflammation. Most striking is the role of IL-23 in autoimmune diseases, such as EAE and RA. The role of IL-23 is identified by the observation

that IL-23p19-deficient animals do not develop EAE and do not develop IL-17-producing T cells.¹⁸⁸ In patients with Crohn's disease, a single nucleotide polymorphism (SNP) in the coding sequence of IL-23R results in a strong protection against this disease, indicating a pathogenic role of IL-23 in chronic inflammation. Since IL-23 can expand a population of IL-17-producing pathogenic cells, an important role of IL-23 in the development of autoimmune diseases was suggested.¹⁵⁶ These data imply that IL-23 may be responsible for the differentiation of Th17 cells. However, *in vivo* experiments demonstrated that IL-23 functioned more like a maintenance interleukin for the Th17 cell population.¹⁸⁹ Therefore, it has been proposed that IL-23 may play a role in maintaining or stabilizing the Th17 cell phenotype, or in the survival of Th17 cells (Figure 1.4).¹⁹⁰

Interestingly, as research continued, two "old" cytokines with opposing effects, IL-6 and TGF- β , were associated with Th17 cell differentiation.^{171, 191} IL-6 is a pro-inflammatory cytokine and was shown to aggravate atherosclerosis.¹⁹² On the other hand, TGF- β is an anti-inflammatory cytokine, which is associated with the differentiation of natural Treg cells and attenuation of atherosclerosis.^{193, 194}

Most research on Th17 cells is done in mice. However, there are differences observed in Th17 development between mice and humans. Recently, some studies showed that also TGF- β , in combination with IL-1 β , IL-6 or IL-21 is able to induce the differentiation of human Th17 cells,^{195, 196} indicating some overlap. More research has to be performed to further clarify the different aspects of Th17 cell development in different organisms.

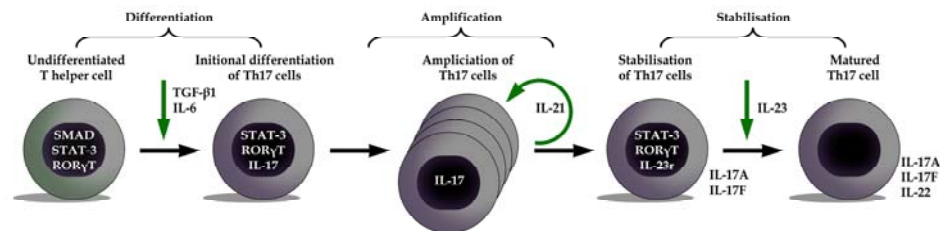


FIGURE 1.4: DETAILED OVERVIEW OF TH17 CELL DIFFERENTIATION.

Since the discovery of Th17 cells many research has been done to elaborate the developmental pathway of Th17 cells. The insight in the role of IL-23 in Th17 cell development changed over time and is now assigned as a stabilizing factor for Th17 cell differentiation. TGF- β and IL-6 are associated with the differentiation of naive T cells to Th17 cells. Furthermore, the recently identified IL-21 is assigned to amplify Th17 cell development via an autocrine positive feedback. (Adapted and modified from Bettelli and Kuchroo)¹⁹⁰

The discovery of Th17 cells has changed the view on initiation and development of autoimmune diseases. There is, however, almost no literature available which associates Th17 cells with cardiovascular disease, although Cheng

et al. recently identified increased Th17 cells in patients with acute coronary syndrome.¹⁹⁷

Nevertheless, there are indications that Th17 cells may play an important role in atherosclerosis. The prominent effector cytokine, IL-17 is discussed in chapter 3 and 4 of this thesis. Furthermore, the fact that IL-23 is involved in several autoimmune diseases, together with the finding that IL-23 is involved in the Th17 pathway, which is likely to be pro-atherosclerotic, indicates a role for IL-23 in atherosclerosis. Again, there are hardly any reports addressing IL-23 in atherosclerosis. In chapter 5 the role of the IL-23R in atherosclerosis will be discussed. Finally, we also studied the role of IL-27, which can suppress the development of Th17 cells,¹⁹⁸ as is described in chapter 6.

2.5.5 Th17 and Treg cells, two different subsets with a close relationship

Both, the Th17 lineage as well as the Treg lineage requires TGF- β for their development. Therefore, a reciprocal relationship between these two cell populations is likely.¹⁹¹ TGF- β is needed for both cell types and IL-6 has a pivotal role in shifting the balance toward Th17 cells.^{171, 191} Furthermore Laurence *et al.* demonstrated that IL-2, which is necessary for Treg cells, inhibits Th17 cells in a STAT5 dependent way.¹⁸⁹ Interestingly, IL-21 synergizes with TGF- β to promote the differentiation of Th17 cells in mice and, as mentioned before, IL-21 has an autocrine loop to enhance its own production by inducing proliferation and recruitment of Th17 cells.^{171, 179}

There is also an interesting relation between Treg cells and Th17 cells on the level of transcription factors (Figure 1.5). The Th17 cell specific transcription factors, ROR γ and ROR α , are able to bind and antagonize Foxp3, a Treg transcription factor, and vice versa.^{199, 200} To underline this fact, conditional deletion of Foxp3 protein in Treg cells *in vivo* results in an increase in ROR γ and subsequent upregulated IL-17 and IL-21 expression.^{201, 202} These data may be interesting for the treatment of chronic inflammation and autoimmunity.

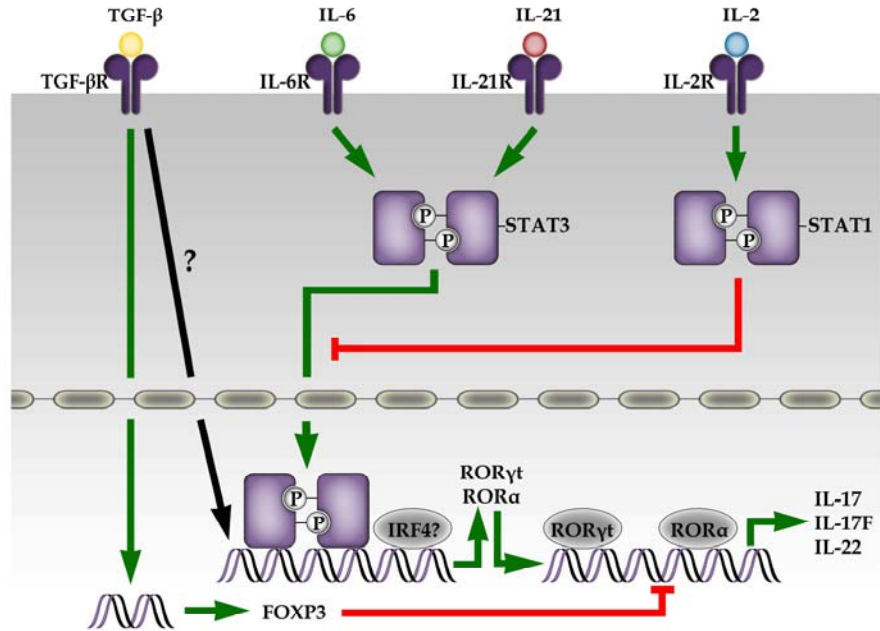


FIGURE 1.5: REGULATION OF Th17 AND TREG SPECIFIC TRANSCRIPTION FACTORS.

The transcription factor STAT3 is crucial for Th17 development. IL-6 and IL-21 activate this pathway, however the exact role for TGF- β on transcriptional level remains to be elucidated (black arrow). Recently, IL-27 has been shown to inhibit Th17 cells and this process is probably mediated by STAT1 dependent STAT3 inhibition. Furthermore, IL-2 is able to suppress Th17 development in a STAT1 dependent way. These data suggest a reciprocal relationship between Th17 and Treg cells. (Adapted and modified from Dong)²³⁸

2.6 B CELLS

B cells are also an important cell type of the adaptive immune system and are pivotal in the production of antibodies against specific antigens. Depletion of B cells results in aggravated atherosclerosis.^{203, 204} A specific subpopulation of B cells, B-1 cells, produce IgM class antibodies (T15/EO6) directed against antigens that exert an atheroprotective function.^{139, 205} Therefore, it is speculated that B cells are atheroprotective.²⁰⁶

However, B cells are also able to present antigens and produce cytokines. Positive correlations have been found between atherosclerosis burden in the carotid artery and activated B cells in the circulation.^{207, 208} Furthermore, these B cells produce IgG antibodies against autoantigens and are associated with aggravating atherosclerosis.²⁰⁹

3 COMMUNICATION BETWEEN IMMUNE CELLS, A CRUCIAL STEP

The immune system is a complex system capable of defending its host against many different pathogenic organisms. However, although tightly regulated, the immune system can become pathogenic when targeting autoantigens or by overexerting the immune response against a certain pathogenic antigen. The adaptive immune system depends on signals of the innate immune response to adjust and adapt the response against the antigen. However, when this line of communication is disturbed or deregulated, it may result in chronic or autoimmune diseases.

Within atherosclerosis, the adaptive and innate immune response have an important role in all stages of the disease. The innate immune system initiates atherosclerosis and the adaptive immune system is involved in development of the lesion. An important way of communication between immune cells and non-immune cells is done by interleukins. A number of interleukins of special interest for this thesis, as they are involved in autoimmune diseases or in the polarization of autoimmune associated immune cell population, are discussed below.

3.1 INTERLEUKIN 15

Interleukin 15 (IL-15) is a pro-inflammatory cytokine which is first described in 1994 by Grabstein *et al.* as a T cell activating factor with structural resemblance to IL-2.²¹⁰ Although, little primary homology on protein level is found, high homology on secondary level is observed between IL-15 and IL-2. In addition, IL-15 is designated as a member of the α -helix bundle cytokine family, which also includes IL-2.²¹⁰

The IL-15 receptor shares two subunits, the β and γ_c subunit, with the IL-2 receptor. The third subunit is formed by a unique α -chain, IL-15R α .²¹¹ The third subunit of the IL-2R is the IL-2R α (CD25).

IL-15 is expressed by several immune cells, in particular by monocytes and macrophages, but also by some non-lymphoid cells, such as fibroblasts.²¹² IL-15 is also involved in expansion and survival of Natural Killer T (NKT) cells, which form an important link between the innate and adaptive immune response and enhance atherosclerosis.²¹³ IL-15 is also expressed in a biologically active form in association with IL-15R α on the surface of monocytes and activated macrophages. This surface expressed IL-15 is approximately five times more effective than soluble IL-15 in the induction of T cell proliferation and is able to signal in a *cis* and *trans* fashion to neighboring cells.²¹⁴⁻²¹⁶ Since soluble IL-15 is difficult to detect in biological fluids, it is suggested that the membrane bound IL-15 exerts a more important function in inflammation.²¹⁷

IL-15 is expressed in human and murine atherosclerotic lesions^{218, 219} and may therefore affect T cells within the plaque. Besides activating T cells, IL-15 is a strong chemo-attractant for T cells and Natural killer (NK) cells^{220, 221} and it enhances CD44 mediated T cell adhesion to endothelial cells.^{220, 222} Sanchi *et al.* demonstrated that in the presence of IL-15, activated endothelium at sites of chronic inflammation is able to recruit and activate peripheral blood T cells to the site of inflammation.²²³ Furthermore, IL-15 can indirectly aggravate atherosclerosis by autocrine regulation of the production of pro-inflammatory cytokines by macrophages, such as TNF- α , IL-6 and IL-1 β ²²⁴ and fibroblasts produce matrix metalloproteinases upon stimulation by IL-15.²²⁵

These data suggest a direct and indirect role for IL-15 in atherosclerosis. The role of IL-15 in atherosclerosis will be addressed in chapter 2 of this thesis.

3.2 INTERLEUKIN 27

Recently, a new interleukin was identified with structural resemblance with IL-12 and IL-23, called IL-27 and is composed of Epstein-Barr virus induced gene 3 (EBI3) and p28.²²⁶ It is produced by activated antigen presenting cells and by resident macrophages.²²⁷ The IL-27 p28 is poorly secreted unless it is co-expressed with its partner EBI3 and thus creating a situation where expression of IL-27 can be tightly controlled during an immune response.¹⁸⁷ IL-27 is therefore an important regulator of the adaptive immune response by interpreting signals of the innate immune system.

The receptor for IL-27 is a heterodimeric complex of gp130 (part of the IL-6 receptor) and the novel IL-27R (also designated as WSX-1 or TCCR).²²⁸ IL-27 receptor is expressed on lymphocytes, such as B cells and Tregs, but also on natural killer (NK) cells, NK T cells, activated endothelial cells, activated epithelial cells, activated DCs, monocytes and mast cells.¹⁸⁷

Interestingly, IL-27 is also associated with several autoimmune diseases. Initially IL-27 was assigned to have proinflammatory properties, based on early reports of the group of Goldberg *et al.* They illustrated that vaccination against p28 resulted in the suppression of EAE and adjuvant induced arthritis.^{229, 230} Recent studies showed a more complex dualistic role for IL-27 with anti- and pro-inflammatory properties.

IL-27 is able to induce differentiation of naive CD4⁺ T cells to Th1 cells, but is also able to suppress the development of Th17 cells in EAE models thereby attenuating the disease.^{198, 227, 231} Furthermore, IL-27 inhibits the development of Tregs and Th2 cells.^{232, 233} IL-27 has an unexpected activity in the immune system, as in some events it has a proinflammatory activity and in other events it shows anti-inflammatory activities by suppression of immune hyperactivity. Yoshimura

et al. proposed a mechanism where IL-27 stimulates STAT1 and STAT3 in naive Th cells, whereas only STAT3 is activated in activated Th cells.²³⁴ Thus, IL-27 is capable to inhibit or stimulate T cells, depending on the IL-27R expression of the target cells and subsequent signal transduction. However, more research needs to be done to study the divergent effects of IL-27 on different cell types under different conditions.

The role of IL-27 in atherosclerosis is not yet investigated. The complex role of IL-27 in atherosclerosis is studied in this thesis and will be discussed in chapter 6.

4 VACCINATION AS RESEARCH AND THERAPEUTIC TOOL

The current treatment of atherosclerosis is focused on reducing risk factors, such as hypercholesterolemia by the administration of statins and a change in life style. Since the inflammatory aspects of atherosclerosis are getting more and more elucidated, novel strategies may arise as potential therapy, such as vaccination. Vaccination is an ideal tool to generate a desired immune response against an antigen. In atherosclerosis, vaccinations may be directed against one or several autoantigens involved in this disease. This approach is already successfully demonstrated by vaccinating mice against oxLDL¹⁵⁴ and HSP60²³⁵ (chapter 7), which resulted in attenuated atherosclerosis. Another possibility is the targeting of certain cell types to restore or shift a balance towards a favorable outcome. For example, vaccination against IL-12 resulted in a decrease of Th1 cells and subsequently in the reduction of atherosclerosis.¹³¹

A relative new development is DNA vaccination and this may prove to be a promising strategy in the future. DNA vaccination is based on, hence the name, DNA and can be produced at relatively low costs. Furthermore, the manufacturing and storage conditions are less stringent compared to protein-based vaccines and thereby facilitating a broader distribution and availability of anti-atherosclerotic medicine. As atherosclerosis and its related symptoms are getting pandemic proportions, these issues may be considered.

Furthermore, DNA vaccination may provide a good and again relatively cheap research tool to investigate the function of certain cell types, by inducing a cytotoxic response against these cells. Signaling molecules, such as interleukins, can be neutralized by raising a humoral response against them. Additionally, the effect of depletion or neutralization of the targets can be studied in different phases of the disease. This contributes to a better understanding of the disease and ultimately to its cure.

Detailed information and perspectives about vaccination against atherosclerosis is described in chapter 9 of this thesis as a perspective review.

5 OUTLINE OF THE THESIS

In this thesis, the role of several key interleukins and inflammatory cells is studied in relation to atherosclerosis.

In chapter 2 a DNA vaccination strategy is used, which makes use of a living carrier, the *Salmonella typhimurium* to induce a cytotoxic T cell response. In this study, we investigated the effect of IL-15 neutralization in atherosclerosis. We observed a strong reduction in atherosclerosis, which suggest an important role for this cytokine in the initiation of atherosclerosis.

Another proinflammatory interleukin, IL-17, is studied in chapter 3. In this study we made use of a novel vaccination strategy, where we inject a DNA vaccine in the muscle. By neutralizing IL-17 in LDLr^{-/-} mice with a HEL-IL-17 DNA vaccine, a dramatic decrease in atherosclerotic lesion development was observed.

In chapter 4 the role of IL-17 signaling is studied by performing a bone marrow transplantation of IL-17 receptor deficient bone marrow into LDLr^{-/-} recipient mice. This study illustrates an important role for IL-17 signaling in atherosclerosis.

In chapter 5, another bone marrow transplantation is described with p19 (a subunit of IL-23) deficient bone marrow to study the contribution of IL-23 signaling in atherosclerosis. However, there is no change in plaque size observed in this experiment.

In chapter 6, again a DNA vaccination strategy was used, although with another immunodominant T helper cell epitope, PADRE, to break the tolerance against the p28 subunit of IL-27. In this study, the effect of IL-27 depletion in atherosclerosis results in aggravated atherosclerosis, indicating an atherosclerotic protective role for IL-27.

To study whether regulatory T cells can be induced against an atherosclerotic related autoantigen, we induced tolerance against HSP60 as described in chapter 7. The tolerance induction results in an increased number of Treg cells and attenuated atherosclerosis. These results further establish the protective role of regulatory T cells in atherosclerosis.

Dendritic cell based vaccination is described in chapter 8. Here we used a novel mRNA based vaccine against the specific regulatory T cell transcription factor, Foxp3. Via electroporation, the dendritic cells are "loaded" with the mRNA and subsequently injected in LDLr^{-/-} mice. We demonstrate again that regulatory T cells have a protective role in atherosclerosis, as we observed aggravated atherosclerosis in Foxp3 vaccinated mice.

In chapter 9, a prospective review describes the possibilities of vaccination in atherosclerosis. Based on our work and the work of others, we think that immunomodulation can provide a very useful approach against atherosclerosis.

Furthermore, we discuss, to our opinion, the best approach to vaccinate against atherosclerosis.

Finally, in chapter 10, all results described in this thesis will be discussed in relation to the mechanism of atherosclerosis and possible future treatment of this disease in patients.

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