Cellular Immunity and Inflammation in Atherosclerosis

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Dedicated to my parents

Summary

Cellular Immunity and Inflammation in Atherosclerosis

Atherosclerosis, the major cause of death and disability in western countries, is nowadays recognized as an inflammatory disease. Low density lipoprotein (LDL), the major carrier of cholesterol in plasma, is trapped in the subendothelial space, where compounds inside of LDL are chemically modified through lipid peroxidation. The oxidized LDL (oxLDL) is believed to initiate the inflammatory reaction in atherosclerosis. By activation of the endothelial cell layer, cells of the immune system (monocytes and T lymphocytes, T cells) are recruited to developing atherosclerotic lesions. Monocytes differentiated into macrophages, which engulf oxLDL through specialized scavenger receptors leading to accumulation of cholesterol in the vessel wall, so-called foam cell formation. Macrophages may also activate T cells, which in turn secrete cytokines. Previous data have shown that oxLDL can activate T cells, but the molecular mechanisms have not been identified.

Oxidation of fatty acids in LDL generates reactive aldehydes that modify proteins. Two important aldehydes are malondialdehyde (MDA) and 4-hydroxynonenal, which can form covalent adducts on proteins. To analyze the mechanisms for the activation of T cells by aldehyde-modified proteins, T cells that were activated by MDA- or HNE-modified self-proteins were established in a mouse model. MDA modified self-proteins triggered T cells that directly recognized the MDA adduct with their T cell receptors. This finding demonstrates that lipid peroxidation derived aldehydes may turn self-proteins into antigens that can initiate a T cell mediated inflammatory reaction. These T cells may also activate B cells to secrete antibodies that are directed either towards the modifications or towards unmodified sequences of altered proteins.

To study the inflammatory response during atherosclerotic plaque development gene expression array technology was applied in a mouse model of atherosclerosis. The expression of previously known atherosclerosis related genes, e.g. the expression of adhesion molecules and markers of blood derived inflammatory cells, were used to validate the experimental procedure. The gene array analysis revealed that the cytokine interleukin-15 (IL-15) was expressed in the vessel wall and upregulated in atherosclerotic plaques. IL-15 expression could be identified in macrophage rich areas of the lesions supporting the hypothesis that IL-15 may be involved in the recruitment of T cells to the atherosclerotic lesion.

The gene array analysis identified the expression of cellular retinoic acid binging protein-II, a gene that is regulated by vitamin A signaling pathways. This finding led us to study effects of vitamin A on foam cell formation. The vitamin A derivative all-trans retinoic acid was shown to upregulate the expression scavenger receptor CD36 through retinoic acid receptor (RAR) signaling path in the human monocytic cell line THP-1. The RAR signaling path was identified in human atherosclerotic lesion associated with infiltrating leukocytes. This suggests that RAR-signaling may contribute to the inflammation in the vessel wall and promote atheroslerosis.

Table of Contents

1 LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Wuttge DM, Bruzelius M and Stemme S. T-cell recognition of lipid peroxidation products breaks tolerance to self-proteins. *Immunology* 1999, 98:273-279.

II. Wuttge DM, Bruzelius M, Lundahl A, Paulsson G, Bergman T and Stemme S. Malondialdehyde epitopes are recognized as haptens by T Cells. *Manuscript*

III. Wuttge DM, Sirsjö A, Eriksson P and Stemme S. Gene expression in atherosclerotic lesion of ApoE deficient mice. *Molecular Medicine* 2001, 7(6): 383-392.

IV. Wuttge DM, Eriksson P, Sirsjö A, Hansson GK and Stemme S. Expression of IL-15 in mouse and human atherosclerotic lesions. *The American Journal of Pathology* 2001, 159(2): 417-423.

V. Wuttge DM, Rommert A, Eriksson U, Törmä H, Hansson GK and Sirsjö A. Induction of CD36 by all-trans retinoic acid: retinoic acid receptor signaling in the pathogenesis of atherosclerosis. *FASEB J.* (March 20, 2001) 10.1096/fj.00-0488fje

2 ABBREVIATIONS

APC	antigen presenting cell
Apo B-100	apolipoprotein B-100
Apo E-/-	apolipoprotein E deficient mice
BcR	B cell receptor
CRABP-II	cellular retinoic acid binding protein-II
CD	cluster of differentiation
HNE	4-hydroxynonenal
IL	interleukin
iNOS	inducible nitric oxide synthetase
ICAM-1	intercellular adhesion molecule-1
LXR	liver X receptor
MCP	monocyte chemotactic protein
M-CSF	macrophage-colony stimulating factor
MDA	malondialdehyde
MHC	major histocompatibility complex
MSA	mouse serum albumin
NF-κB	nuclear factor-κB
PPAR	peroxisome proliferator activated receptor
oxLDL	oxidatively modified low density lipoprotein
PCR	polymerase chain reaction
Sr-A	scavenger receptor-A I and II
RAR	retinoic acid receptor
RXR	retinoic X receptor
TcR	T cell receptor
VCAM-1	vascular cell adhesion molecule-1

3 INTRODUCTION

Atherosclerosis, the most important cause of premature death in western countries, is a complex and multifactorial disease [1]. First hallmarks of atherosclerosis, so-called fatty streaks, can be identified already in early childhood [2]. The histopathological demonstration of important cellular components of the immune system in early lesions has led to the recognition of atherosclerosis as an inflammatory disease [3].

3.1 Pathogenesis of atherosclerosis

How it is started… LDL diffuses from plasma into the subendothelial space in a concentration dependent manner [4]. LDL there binds to extracellular matrix proteoglycans, which promote retention of LDL in the intima [5-7]. LDL is oxidatively modified through exposure to oxidative stress and obtains new properties that are believed to be decisive for the pathogenesis of atherosclerosis [8,9]. OxLDL activates the endothelium via activation of transcription factors; including the nuclear factor-κB (NF-κB) [10], which leads to upregulation of adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [11]. Also haemodynamic shear stress induces the expression of NF-κB signaling-dependent adhesion molecules in endothelial cells [12]. Monocytes and T lymphocytes (T cells), roll along the vessel wall and attach to the adhesion molecules. Following this firm adhesion, monocytes and T cells transmigrate through the endothelium and accumulate in the intima [13]. Extravasation is initiated and promoted by chemokines; e.g. MCP-1, which is a potent monocyte chemoattractant [14]. In the intima monocytes undergo M-CSF-mediated differentiation into macrophages [15]. Intimal oxLDL binds to macrophage scavenger receptors promoting endocytosis and transition of the macrophages into cholesterol loaded foam cells [16]. The importance of scavenger receptor-mediated uptake of oxLDL for the development of atherosclerosis has recently been investigated. Compound knockout mice, deficient in either scavenger receptor-A (Sr-A) or CD36 and apolipoprotein E (ApoE-/-) have reduced atherosclerotic lesions [17,18].

Fight of giants… In the lesion, macrophages may present antigens to T cells leading to T cell activation [19]. Both macrophages and activated T cells secrete potent cytokines leading to inflammation in the vessel wall [20-22]. Both oxLDL and cytokines promote the imigration of vascular smooth muscle cell from the media into the intima [23,24]. In the intima they differentiate, proliferate, produce extracellular matrix and form a fibrous cap in advanced atherosclerotic lesions [25]. They therefore play an important role for the stability of the atherosclerotic plaque [26].

Clinical complications… Plaque rupture in the shoulder regions of advanced plaques are believed to cause clinical complications, such as myocardial infarction and stroke [27]. The triggering cause for plaque rupture is unknown but accumulation of activated macrophages, T cells and mast cells are frequently seen at the sites of plaque ruptures [28]. Immunological events may lead to cell-mediated induction of programmed cell death in smooth muscle cells [29] and stimulate secretion of matrix metalloproteinases in smooth muscle cells, macrophages and endothelial cells [30-32]. These processes may contribute to the instability

of the plaque [33]. Both exposure of extracellular matrix to the blood, due to plaque rupture, and endothelial injury induced tissue factor activation trigger complement activation and lead to thrombus formation [34]. The thrombus may cause hypoxia of the tissue that is supplied by the affected vessel, which may result in tissue infarction [27].

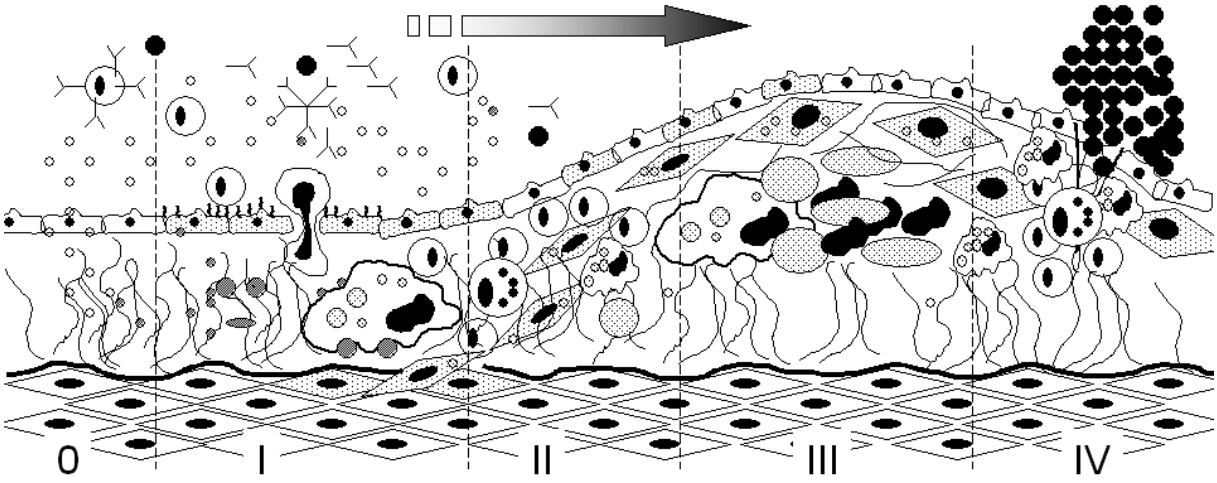

Figure 1 Pathogenesis of atherosclerosis

0) *Normal artery*. LDL diffuse from the vessel lumen to the intima where it binds to matrix proteoglycans. Some particels get modified.
I) *Fatty streak*. Modified LDL activates EC to express adhesion molecules and chemokines. Leukocytes adhere to the EC's and migrate in to the intima. Differentiated MØ and SMCs phagocytoses modified/oxidizied LDL, accumulate intracellular cholesterol ester and activate T cells to secrete cytokines and chemokines.
II) *Intermediate atherosclerotic lesion*. Cytokine/chemokine-actictivated smooth muscle cells migrate in to the intima. Increased accumulation of blood derived cells and inflammation. Extracellular lipid deposits appears.
III) *Advanced atherosclerotic plaque*. Under a fibrous cap mainly consisting of intimal SMCs, a necrotic calcified core with lipid deposits and cell debris has formed.
IV) *Complicated lesion*. Due to the secretion of matrix proteases by MØ, mast cells and SMCs the extracellular matrix becomes unstabil leading to injury of the endothlial cells. Extracellular matrix becomes exposed to the vessel lumen that in turn leads to the activation of the clotting system an thrombus formation.

3.2 Lipid peroxidation and oxLDL

3.2.1 LDL

LDL is a large particle and the major carrier of cholesterol in the blood [35]. LDL contains a lipid core of cholesteryl ester and triglycerides that is surrounded by phospholipids, free cholesterol and a protein, the apolipoprotein B-100 (Apo B-100) [36]. The main components are cholesteryl esters (40-44%), phospholipids (20-24%) (mainly phosphatidylcholine and sphingomyelin), protein (21-26%), free cholesterol (10%) and triglycerides (3-5%). The fatty acid fraction contains both saturated (predominately palmic acid (16:0) and stearic acid (18.0)), monounsaturated (mainly oleic acid (18:1) and palmitoleic acid (16:1)), and polyunsaturated fatty acids [37]. Both linoleic acid (18:2), that is bound to cholesteryl esters, and arachidonic acid (20:4) bound to phospholipids are the dominating polyunsaturated fatty acids in LDL. The protein moiety Apo B-100 contains 4536 amino acids, is glycosylated with galactose, mannose, N-acetylglucosamine and contains sialic acid residues [38].

3.2.2 Prerequisites for lipid peroxidation in the intima

The demonstration of oxidatively modified LDL in the intima suggests that this process takes place locally [8,39,40]. After the extravasation by transcytosis, LDL binds to extracellular matrix proteoglycans [5,41]. Clusters of positively charged lysine and arginine groups mediate the binding of the ApoB-100 protein chain to chondroitin sulfate and heparin containing proteoglycans [42,43]. Binding of LDL to extracellular matrix proteoglycans promotes retention of LDL in the intima and facilitates enzymatic and oxidative modification of LDL [42]. Several oxidant-generating systems are associated with atherosclerosis, but appear to influence progression of the disease differently. ApoE compound knockout mice deficient in either 15-lipoxygenase or inducible nitric oxide synthetase (iNOS) showed decreased lipid peroxidation and decreased lesion sizes compard to control ApoE-/- mice [44-46]. Paradoxically, myeloperoxidase-deficient mice showed aggravated development of atherosclerotic lesions [47]. Importantly, all vascular cell types have been shown to mediate LDL oxidation in vitro. However, metal ions have frequently been used to study the oxidation of LDL in vitro and may also be of importance in vivo [48,49].

3.2.3 Lipid peroxidation

Lipid peroxidation is initiated by free radicals that remove hydrogen atoms from polyunsaturated fatty acids to form a carbon-centred lipid radical [50]. An increased amount of double bounds in the fatty acid chain facilitates the removal of hydrogen atoms and initiation of lipid peroxidation. Lipid radicals react with molecular oxygen resulting in lipid peroxyl radicals. Lipid peroxyl radicals, in turn, remove hydrogen atoms from adjacent lipids resulting in lipid hydroperoxides and new lipid radicals. The latter then propagate lipid peroxidation. Lipid peroxidation results in the decomposition of lipid hydroperoxides to lipid alkoxyl radicals, and β-cleavage reactions of the alkoxy radicals generate a number of different aldehydes [51]. The oxidation of fatty acids results in both saturated and unsaturated aldehydes. Hexanal is the most abundantly formed saturated aldehyde during LDL oxidation in vitro, while malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are the predominant unsaturated aldehydes. The quantities of the individual aldehydes formed during oxidation depend on the amount of different fatty acids present in the LDL. Hexanal and HNE are derived from oxidation of linoleic acid and arachidonic acid, whereas arachidonic acid is the major source of MDA. The generation of MDA requires fatty acids that contain more than 3 double bounds.

3.2.3.1 Aldehydes (figure 2)

MDA is the dominating hydrophilic aldehyde and can diffuse out of the LDL particle into the aqueous phase, whereas the other aldehydes are mainly lipophilic and therefore remain within LDL [52]. The formed aldehydes are strong electrophiles reacting with nucleophiles e.g. amino groups of proteins, increasing the negative surface charge of the protein. MDA mainly forms Michael adducts with lysine residues of proteins or with the amino-containing headgroups of phospholipids [53]. The dominant adduct is N^{ε}- (2-propenal) lysine, but also other products are formed including dihydropyridine-lysine, 1-amino-3-iminopropene and pyridium-dihydropyridine [53,54]. The latter two products lead to both intra- and intermolecular cross-linking of lysine groups. HNE also forms Michael adducts, mainly with cysteine, histidine and lysine residues; but reacts also with amino-containing headgroups of phospholipids. These reactions generate hemiacetal pyrrole adducts as well as fluorescent cross-links [55,56]. In this way, generation of covalent aldehydes-adducts during LDL oxidation leads to the posttranslational modification of Apo B-100 and phospholipids.

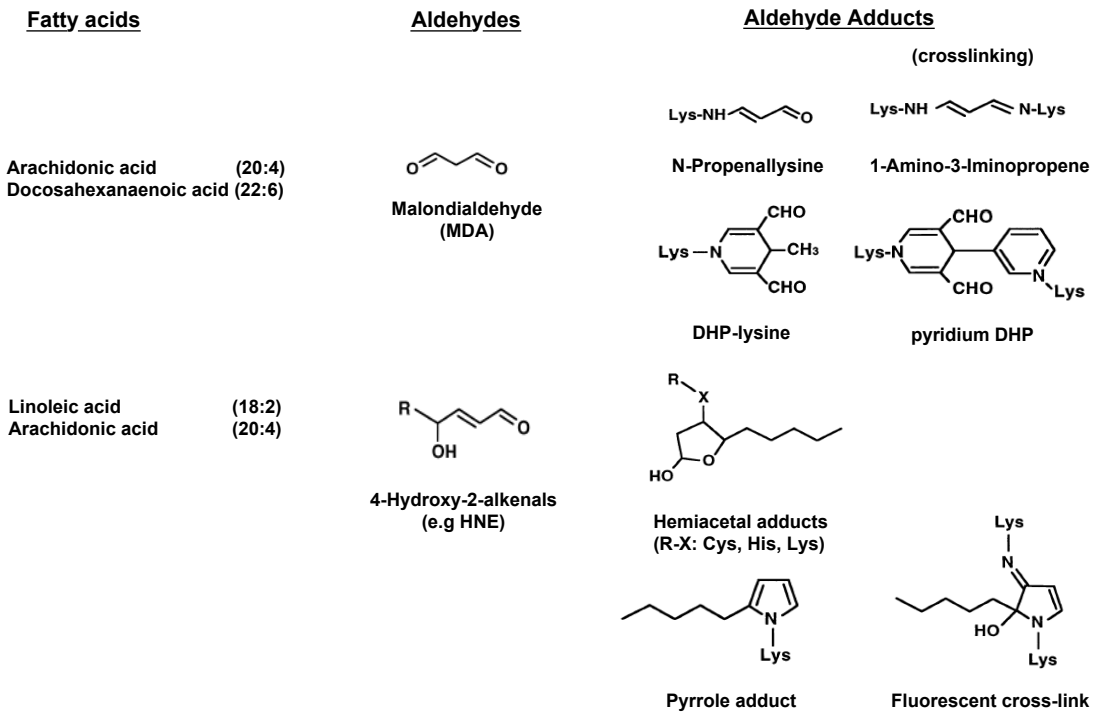

Figure 2 Formation of aldehyde adducts

The oxidation of several fatty acids in the LDL particle results in the formation of Malondialdehyde (MDA) and 4-hydroxynanenal (HNE) that reacts covalently with e.g. lysine groups of proteins (adopted from Uchida K. [54])

3.2.3.2 Other changes in oxidized LDL

In addition to aldehyde adduct formation, oxidation of LDL leads to fragmentation of Apo B-100 and promotes aggregation of LDL particles [57-59]. Lipid peroxidation of fatty acids results also in formation of fatty acid hydroperoxides, e.g. hydroxy linoleic acid (9-HODE, 13-HODE) and hydroxy arachidonic acid (11-HETE, 12-HETE, 15-HETE) [52]. Finally, oxidation of cholesteryl esters leads to formation of oxysterols e.g. 7- and 26-hydroxycholesterols [60].

3.2.4 Some mediators of oxLDL on inflammation

The biological effects of oxLDL depend on the degree of modification [9]. Minimally modified LDL induces the expression of MCP-1 and P-selectin [61,62], whereas extensively oxidized LDL is highly cytotoxic [63]. Both oxidized phospholipids and lysophosphatidylcholine have been shown to mediate several effects of oxLDL. Lysophosphatidylcholine is formed during the hydrolysis of oxidized phospholipids by LDL-bound phospholipase A2 and by the secretory phospholipase A2 bound to the extracellular matrix [64,65]. Lysophophatidylcholine activates the NF-κB signaling pathway resulting in the expression of adhesion molecules in endothelial cells [66-68]. Recently, 9-HODE and 13-HODE have been identified as ligands for the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) [69]. Through this signaling pathway oxLDL has been suggested to promote foam cells formation and macrophage differentiation [69,70].

3.2.4.1 Nuclear receptors

Nuclear receptors are a family of transcription factors that mediate the regulation of important physiological processes [71]. These receptors have initially been identified to mediate ligand-dependent gene transcription of steroids, retinoids (via retinoic acid receptor (RAR) and retinoic X receptor (RXR)), and thyroid hormone and vitamin D. However, the number of nuclear receptors has rapidly increased [72]. Nuclear receptors bind to the DNA as monomers, homodimers or heterodimers [73]. PPAR-γ forms solely heterodimers with RXR, whereas RXR is a promiscuous nuclear receptor that forms heterodimers with different other nuclear receptors, such RAR or the liver X receptor (LXR). Binding of small lipophilic ligands to the so-called ligand-binding domain results in a conformational change of the nuclear receptor and its DNA-binding domain, which mediates DNA-binding. Both ligands to PPAR-γ and ligands to RXR, 9-cis retinoic acid [74], mediate the transcriptional activity of the PPAR-γ/RXR heterodimer [71].

3.3 Innate and adaptive immunity in atherosclerosis

3.3.1 Adhesion molecules

Leukocytes interact with endothelial adhesion molecules called selectins, which mediate leukocyte "rolling" along the endothelium [75]. When encountering VCAM-1 and ICAM-1 on activated endothelial cells at sites of inflammation or tissue damage, leukocytes stop rolling and attach firmly to the endothelium [76]. Several studies have demonstrated the importance of selectins and integrins for atherogenesis in experimental animal models. The lack of both P- and E-selectin in knockout mice reduces both early and advanced atherosclerosis in the LDL-/- mouse model, and similar results are seen in P-selectin and apoE compound knockout mice [77,78]. Blocking of the VCAM-1/VLA-4 integrin or the ICAM-1/LFA-1 interaction reduces fatty streak formation [79-81].

3.3.2 Chemokines

Hypercholesterolemia may activate the complement cascade and initiate expression of MCP-1 [82,83]. Interestingly, hypercholesterolemia also induces the MCP-1 receptor CCR-2 [84]. The recruitment of monocytes, guided by the interaction of MCP-1 with CCR2, is an important event in atherosclerosis [14,85]. However, other chemokines also appear to be involved in monocyte recruitment, since interruption of the CXCR2/IL-8 is associated with decreased atherosclerosis [86].

3.3.3 Macrophages

3.3.3.1 Role for macrophages in atherosclerosis

Macrophages accumulation is a dominant feature of early stages of human atherosclerosis [2]. Blood-derived monocytes differentiate into tissue macrophages, a process which is driven by macrophage-colony stimulating factor (M-CSF) [15,87]. The role of M-CSF for the development of atherosclerosis was tested in experiments with op mice that are deficient in M-CSF. These mice are deficient in macrophage function and develop very small lesions, even on high cholesterol diet [15].

3.3.3.2 Scavenger receptors

Macrophages are phagocytes and constitute a link between the innate and adaptive immune systems. They express evolutionarily conserved pattern recognition receptors that bind

bacterial membrane proteins and lipids [88]. Receptor ligation activates macrophages and induces cytokine secretion [89]. Scavenger receptors are also evolutionarily conserved membrane receptors with pattern recognition properties [90]. However, scavenger receptors mainly mediate uptake of their ligands. They were originally defined by their ability to mediate the binding and uptake of acetylated or oxidized LDL [91,92]. Increases in negative surface charge, e.g. due to modification of lysine-groups with covalent adducts, render a protein susceptible to binding by the classical scavenger receptor-A (Sr-A). Nevertheless, also lipid residues of membranes have been shown to mediate the binding of apoptotic cells, bacteria or oxLDL to several scavenger receptors [90]. Since the cloning of Sr-A, several other scavenger receptors have been evolved with either SrA-related or -unrelated structures [93,94]. The function of Sr-A both in the pathogenesis of atherosclerosis as well as in the immune defense has been investigated in the Sr-A and ApoE compound knockout mouse [17]. These mice show reduced lesion size but an increased susceptibility to infection. Interestingly, Sr-A has recently been shown to take part in antigen presentation of modified MSA and oxLDL to T cells, and may therefore play a role in initiating the adaptive immunity [95,96]. Another scavenger receptor, the thrombospondin receptor CD36, is currently investigated in the context of atherosclerosis. CD36 and ApoE compound knockout mice developed less lesions compared to ApoE-/- control mice, indicating an important role for this receptor in atherosclerosis [18]. Other scavenger receptors have emerged such as CD32, CD64, LOX and the recently cloned SR-PSOX [94,97].

3.3.3.3 Antigen presenting cells and MHC molecules

Macrophages can trigger different T cells subsets through the presentation of protein derived peptide sequences, antigens, to the T cells [98]. They are therefore called professional antigen presenting cells (APCs). Extracellular proteins, that have been internalized either by pinocytosis or receptor mediated endocytosis, are subjected to proteolytic cleavage in the early and late endosomal compartment (figure 3) [99-101]. Several peptides are transferred from the endosomes into the so-called peptide-loading compartment where they are loaded into peptide-binding grooves of major histocompatibility complex (MHC) molecules [102,103]. MHC molecules are membrane molecules consisting of two chains and are mainly divided into MHC class I and class II molecules. MHC is the genomic region that encodes the proteins of MHC molecules [104,105]. MHC class I and class II genes are highly polymorphic and the individual variants of the genes are called alleles. It is believed that polymorphism in the MHC has evolved to ensure that sufficient sets of different peptide binding properties are available in the population. Each individual has therefore different MHC molecules with different binding affinities for peptides. In the mouse, the MHC class II genes are designated I-A, and individual alleles (haplotypes) are denoted eg I-A^b, I-A^e I-A^d, etc. MHC restriction means that T cells from an I-A^b strain mouse cannot respond to antigens presented by APC from an I-A^d mouse. During intracellular processing binding of peptides with particular amino acids' fitting into so-called anchor positions of the MHC peptide-binding grooves leads to stabilization of the molecular complex [106]. The MHC-peptide complex is then transported to the cell surface where the peptides are presented to T cells. Short intracellular derived peptides (8-10 amino acids) are loaded on the closed binding groove of MHC class I molecules and presented to CD8+ T cells. In contrast, peptides derived from extracellular proteins are longer (13-18 amino acids) and end-up on a binding groove of MHC class II molecules for presentation to CD4+ T cells. However, leakage between the two compartments may occur [107]. In addition, Macrophages express the costimulatory molecules B7 that are necessary for the activation and priming of T cell [108].

3.3.4 T cells

3.3.4.1 Evidence for T cell in atherosclerosis

T cells have been identified in human atherosclerotic lesion and in atherosclerotic lesions of ApoE-/- mice [13,109]. In humans, CD8+ T cells were predominantly present in early atherosclerotic lesions, whereas activated CD4+ memory cells were mainly detected in advanced lesions [19,110,111].

3.3.4.2 T cell receptors and antigen recognition

The specificity mediated by the T cell receptor (TcR) is a key feature of the adaptive immune system. The TcR consists of Vα- and Vβ-chain together forming an antigen binding region that is unique to each mature T cell and its progeny. In order to create a diverse repertoire of TcR, to fit all possible antigen challenges, the TcR gene is organized in a cluster of gene segments that randomly rearrange in each T cell during maturation in the thymus [112]. The resulting variable region of the TcR specifically recognizes amino acids of a peptide that is bound to an MHC molecule exposed on the surface of an antigen presenting cell, but also needs to recognize some areas of the MHC molecule itself. This need for presentation of antigen on the individuals own particular MHC molecules is termed MHC-restriction [113]. MHC class II restricted CD4+ T cells commonly recognize peptides containing 13-18 amino acids in an MHC binding groove which is open in both ends [114]. It is crucial that the potent T cell-mediated responses are turned against foreign antigens, but not against the individuals own tissue. Therefore T cells undergo a selection in the thymus, where T cells immunity is trimmed not to start immune reactions against tissue proteins of the individual [115]. Mature T cells that have not encountered their antigens yet are termed naive T cells [116]. These cells become activated when they receive a signal from the TcR together with signals from costimulatory molecules [108]. Upon activation these cells proliferate, produce cytokines and thereby initiate a primary immune response. It is important that the response is kept under control and T cell responses are therefore downregulated by the interaction of CTLA-4 on T cells with B7 on APC's. Many of the naive T cells are removed by Fas-Fas ligand-mediated programmed cell death [116]. After activation a few cells develop into so-called memory cells that keep the "memory" of the initial antigen. These memory T cells are characterized by the expression of certain membrane molecules, such as CD45RO and CD44 [117]. Memory cells can start a new potent immune response in case of re-encountering of the antigen, even without the need of costimulatory molecules. T cells are divided into CD4+ "helper" (Th) T cells and CD8+ "cytotoxic" (Tc) T cells according to their MHC restriction and function. The immune responses of CD4+ T cells are mainly mediated by the production of cytokines, but also directly through cell-cell contact such as CD40-CD40 ligand interaction [118,119]. While effector mechanisms of CD8+ T cells are mainly characterized by perforin- and Fas-mediated cytotoxicity, they also include the production of different cytokines [120].

3.3.4.2.1 Cytokines

Two opposing T cell categories are defined by the production of interferon-gamma (IFN-γ) and interleukin-4 (IL-4) [120]. IFN-γ is a potent proinflammatory cytokine that is secreted by TH1 CD4+ and Tc1 CD8+ T cells and exerts a large variety of actions on different cells of the cellular immune system. E.g. IFN-γ increases the expression of MHC molecules leading to enhanced antigen processing and presentation [121]. The finding of MHC class II expressing smooth muscle cells and macrophages in atherosclerotic lesions suggested the presence and activity of IFN-γ in atherosclerotic lesion [20]. mRNA analysis confirmed the expression of

this cytokine both in human and mouse atherosclerotic lesions [21,22]. In the context of atherosclerosis, IFN-γ inhibits smooth muscle proliferation, smooth muscle contractility and collagen synthesis but induces the secretory phospholipase A2 [122-125]. IFN-γ induces the expression of CD40 in smooth muscle cell, endothelial cells and macrophages, which may promote inflammatory cell-cell interactions [126]. Two studies have suggested a proatherogenic role of IFN-γ signaling in atherosclerosis. When IFN-γ signaling was abolished, a reduction of atherosclerosis was observed [127], whereas an increased lesion size resulted from exogenous administration of IFN-g [128].

Th2 CD4+ and Tc2 CD8+ T cells produce IL-4. The expression of IL-4 is less abundant in atherosclerotic lesion than the expression of IFN-γ [22]. IL-4 expression could be detected in atherosclerotic lesions in ApoE-/- mice on high cholesterol diet [21]. IL-4 has been shown to induce 12/15-lipoxygenase to increase the production of PPAR-γ ligands. This resulted in a CD36-mediated induction of foam cell formation [129]. IL-4 inhibits inducible NO synthetase and cyclooxygenase 2 [130,131]. In vivo however, IL-4 did not influence the development of early atherosclerotic lesions in C57BL/6 mice on high cholesterol diet [132].

3.3.4.2.2 Cell/cell contact

In addition to cytokine secretion, T cells may exert activity by cell-cell contact during atherogenesis. CD40 ligand is expressed on the surface of macrophages, smooth muscle cells and endothelial cells in the vessel walls [126]. Interruption of CD40-CD40 ligand signaling by administration of anti-CD40 ligand antibodies reduced atherosclerosis in ApoE-/- mice [133]. Smooth muscle cells expressing Fas and T cells expressing Fas ligands have been demonstrated in advanced lesions [134,135].

3.3.5 B cells

3.3.5.1 Evidence for B cells in atherosclerosis

The involvement of B cells in atherosclerosis differs from that of macrophages and T cell. B cells have not been detected in human intimal lesions, but could be demonstrated in two animal models of atherosclerosis [110,136,137]. B cells have also been demonstrated in the adventitia of atherosclerotic vessels [138]. However, although B cells themselves are not present in human atherosclerotic plaques, high titres of antibodies towards atherosclerosis-related antigens are common in humans with advanced atherosclerotic lesions and animal models of atherosclerosis [39,40,139]. B cells may therefore have a “remote control” role in the atherogenesis.

3.3.5.2 B cell activation and antibody production

B cells may exert a role in atherogenesis by two mechanisms [140]. First of all the B cell gives rise to plasma cells producing antibodies, some of which are directed to atherosclerosis-related antigens. Another important, though less discussed, function is the role of B cells as potent antigen presenting cells. As for the TcR, B cell receptor (BcR) diversity is obtained through a recombination mechanism [112]. The BcR is a membrane-bound immunoglobulin consisting of two heavy and light chain pairs. The production of antibodies is triggered by the activation of the B cells [141]. Cross-linking of several BcRs by the antigen leads to the internalization of the antigen and a first activation signal. The antigen is processed and loaded onto MHC molecules for the presention to T cells [142]. If a T cell becomes activated, CD40-CD40 ligand interactions between T and B cell mediates a second activation signal to the B cell [143]. B cell activation leads to the production of soluble IgM antibodies. Depending on the cytokine environment, an isotype switch may take place resulting in the production of

other antibody subclasses, e.g. IgG [144,145]. An alternative form of B cell activation was recently demonstrated [141]. Antigens with repetitive motifs have been shown to induce antibody production independently of T cell help [146,147]. The nature of the second signals, which activates these B cells, is currently being investigated [146]. In addition, deletion of B cells with high affinity towards self-proteins in the bone marrow and periferal T cell dependent regulation provides the some mechanisms for tolerance towards body tissues [148].

3.3.6 OxLDL as antigen to the adaptive immunity

3.3.6.1 B cell responses

Since oxLDL is recognized as a key mediator in the pathogenesis of atherosclerosis [4], several studies have been performed to investigate immune responses towards oxLDL. Elevated titers of antibodies that recognize oxLDL are found in humans with extensive disease and in all animal models of atherosclerosis [39,40,139]. Both IgG and IgM antibodies have been demonstrated recognizing oxLDL, predominately MDA-adducts or oxidized phospholipids, e.g. POVPC (1-palmitoyl-2-5-oxvaloryl) [149]. Several of these antibodies recognize solely adducts, independently of the sequence of carrier-molecules [149]. By definition, haptens are small chemical adducts which by themselves do not trigger an immune response with antibody production, but may do so after coupling to a larger protein molecule [150]. Later research demonstrated that the effect of the protein was to provide T cell epitopes for triggering of the T cell help activity necessary for induction of antibody production [151,152].

3.3.6.2 T cells responses

The demonstration of T cells in atherosclerotic plaques and evidence of Ig-class switch of naturally occurring anti-oxLDL antibodies suggested a role for antigen specific T cells in atherogenesis [149,153]. Therefore, oxLDL was tested as antigen for T cells. T cell clones that were isolated form human atherosclerotic lesion were activated by oxLDL, but not by native LDL [154]. Similar T cell responses towards oxLDL were demonstrated from blood derived cell culture from humans and spleen culture from ApoE-/- mice [155-158]. The exact epitope that is recognized oxLDL specific T cells is unknown. However, it is likely that T cells may recognize new epitopes, neoepitopes, that are generated by oxidation of LDL. However, no proof for this hypothesis has been provided yet.

3.3.6.2.1 Posttranslational modifications and antigen recognition (figure 3)

Posttranslational modification of proteins can interfere at several levels in order to create neoepitopes for T cells (figure 3). The modification can render the protein susceptible for either the binding to specific membrane receptors such as scavenger receptors or for Ig-mediated Fc receptor binding [159-162]. Receptor-mediated uptake by different APCs may in turn lead to differential processing of the proteins by several different mechanisms [99]. Modifications of the internalized protein may interfere with intracellular proteases, the activity of which may be increased, delayed or inhibited [163-165]. This may result in different proteolytic pattern and may result in the presentation of so-called cryptic (native) peptides [95]. Another possibility is that the binding affinity to the MHC molecule may be changed, depending on the modification [166,167]. The steric conformation of the MHC-peptide complex maybe changed, which may result in the affinity of a new TcR-subtype for the MHC-peptide complex. Finally, the adduct may in itself be recognized by the TcR either together with adjacent native amino acid residues or independently of the peptide backbone, similar to hapten recognition by immunoglobulins [168].

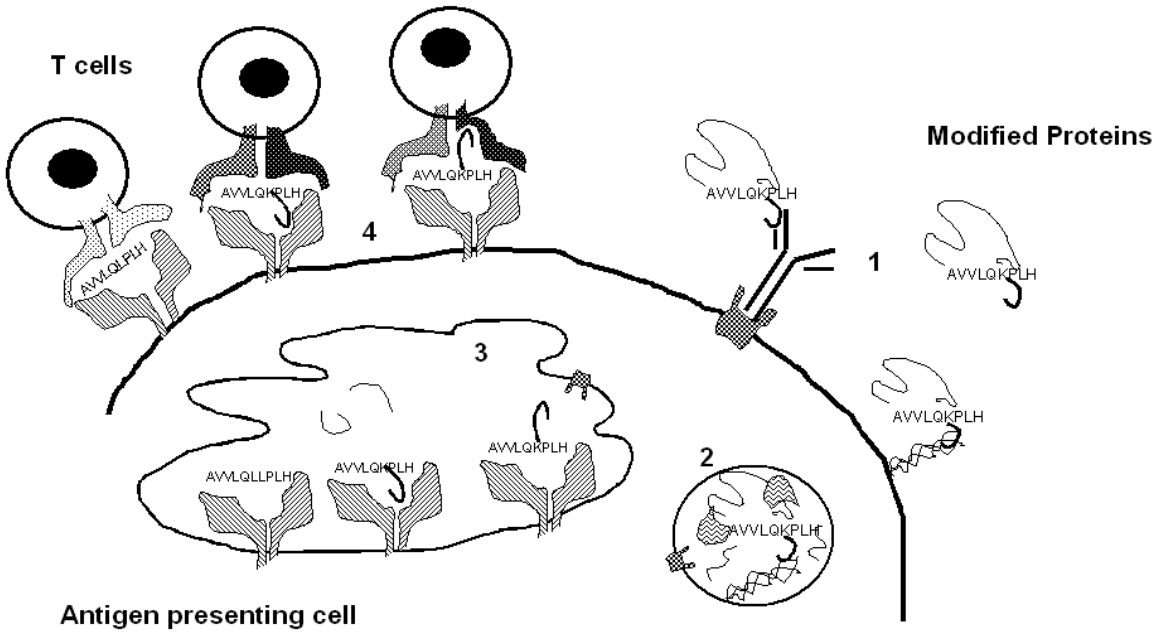

Figure 3 Processing and presentation of modified proteins

Receptor- or antibody-mediated uptake of modified proteins (**1**), digestion of proteins in the early and late endosomal compartments (**2**). Proteases can be inhibited by the modification and digestion patterns may be changed due to the binding to different receptors. Transfer and loading of peptides on to MHC class II molecules in the peptide loading compartment (**3**). Modifications can increase the binding of the peptides to the MHC molecules or native-cryptic peptides may be loaded. MHC class II Restricted antigen-presentation of the the modified and/or cryptic peptides to T cells with different specificities (**4**). The modification can directly interact with the T cell receptor.

3.3.6.2.2 MDA, a candidate aldehyde for the T cell recognition

From the discussion above, it is evident that oxidative modification of LDL may generate many possible neoepitopes that could be recognized by T cells. However, high titers of anti-MDA antibodies are present in humans and animal models of atherosclerosis [39,40,149]. Immunizations with MDA-LDL have been shown to reduce atherosclerosis in different mouse models [169-173]. This evidence suggests an MDA-dependent immune mechanism that involves the adaptive immunity. Therefore, the hypothesis was put forward that hapten recognition of MDA by the TcR might mediate the T cell response towards MDA-modified proteins.

4 AIMS OF THE STUDY

(I-II) To elucidate the effects of recognition of lipid peroxidation products by the adaptive immune system, i.e. the collaboration between T- and B-cells in the recognition of aldehyde modified autologous proteins (I) and the molecular recognition of malondialdehyde modified protein through the T cell receptor (II),

(III-IV) To investigate gene expression in atherosclerosis by applying the gene expression array technology in the atherosclerotic Apolipoprotein E knockout mouse model.

(V) To study retinoic acid signaling in atherosclerotic lesions and its effect on the scavenger receptor CD36.

5 METHODOLOGICAL CONSIDERATIONS (PAPER III)

5.1 Animal model and tissue preparations

The apolipoprotein E deficient (ApoE-/-) mouse strain carries a targeted disruption of the ApoE gene and has a C57BL/6 background (C57BL/6J-apoE^{TM1UNC129}) [174-176]. Cholesterol elimination is severely hampered, due to the deficient uptake of VLDL by the LDL receptor, which leads to severe hypercholesterolemia. C57BL/6 control mice fed with normal chow diet have previously been shown to develop plasma cholesterol values of 2-3 mmol/L. In these experiments, ApoE-/- mice increased cholesterol values to about 30 mmol/L after 5 weeks on "Western" diet containing 0.15% cholesterol [21]. Cholesterol levels in ApoE-/- mice fed western diet appear to vary between individual but do not seem to increase with time of diet [177]. ApoE-/-mice on western diet for 10 weeks have previously been shown to develop foam cell lesions and intermediate lesions, whereas ApoE-/- on western diet for 20 weeks have shown intermediate lesions and fibrous plaque lesions [177]. The ApoE-/- mouse has reached broad acceptance as a mouse model for human atherosclerotic disease and is commonly used to study the effects of experimantal intervention on atherosclerosis [178]. Aortas of C57BL/6 mice on normal diet do not develop atherosclerotic lesions and were therefore used as non-atherosclerotic control [179]. Due to the different diets between the two groups certain effects on the gene expression may theoretically be due to components in the western diet e.g. increased cholesterol levels, which are not due to development of atherosclerosis per se. Female mice were used in our study, since female mice have previously been shown to develop increased lesion size at younger age, compared to male mice [158].

In order to get enough mRNA for the analysis by cDNA expression array, we isolated the whole aortas, starting from the beginning of the aortic arch extending to and including the iliac bifurcation. Compared to the isolation of individual atherosclerotic lesions, our procedure reduced the time of the handling of the tissue is, which prevents degradation of the mRNA. Careful rinsing from blood derived cells and adventitial fat tissue was performed. The roots of the aortas were used for immunohistochemical evaluation of the genes, since the extent of atherosclerosis in the roots of the aorta has previously been found to correlate to the disease stage in the aorta [177].

5.2 Signal analysis

For the analysis of mRNA expression with Clontech cDNA membrane arrays, the user can choose between radioactive labeling with ^{32}P and ^{33}P. ^{32}P-lableling is preferred for the analysis and detection of genes expressed in low copies. However, the analysis by the phosphor imager system is hampered by potential overexposure of genes that are highly expressed. Labeling with ^{33}P results in a more linear correlation between the labeled copies and the phosphor imager values, and was therefore applied in our study. The quality of the autoradiographic spots was evaluated and only spots with circle-round demarcation from the background were considered positive. The highest resolution of the phosphor imager software

was applied for the adjustments of the demarcations and the highest value for the individual spots was chosen.

5.3 Statistical considerations

Fifteen aortas from each group were pooled for the hybridization allowing one hybridization per group. Taking into account the number of animals for each group, individual differences were considered acceptably reduced. The samples should therefore mirror the average gene expression of the different groups. Although no statistical evaluation for the technical variation of the hybridization procedure could be performed, other studies have suggested good reproducibility of the commercial filter arrays from Clontech [180]. The overall gene expressions correlated well between the two groups for both 10 (R=0.94) and 20 weeks (R=0.902) of diet, despite the probable changes due to the development of atherosclerosis. This suggested that the hybridizations were reproducible. In addition, the upregulation of genes at both 10 and 20 weeks can serve as duplicates. However, the interpretation of the upregulation of genes at only one of the two time points needs some caution.

5.4 cDNA Array analysis

After the exclusion of genes that were either not expressed or technically unreadable, 370 genes were analyzed. Normalization with the help of house-keeping genes was omitted, since several genes considered to be "house-keeping " genes were changed during disease. The rationale that the overall gene expression might be more robust towards changes induced by the disease motivated the normalization of the individual genes towards the average gene expression. Therefore, the average gene expression of all groups was divided by the average expression of the C57BL/6 group at 10 weeks, and all genes within each group were normalized with the respective ratio.

To follow the changes in gene expression during atherogenesis, ratios between ApoE-/- and C57BL/6 mice were calculated. Values >1 implied an increased expression of the gene in the atherosclerotic lesions and values <1 meant a decreased gene expression. At the very beginning of the development of atherosclerosis, the correlation of the gene expression between C57BL/6 mice and ApoE-/- can hypothetically be assumed to be equal (R=1), implying no differences between the two groups. In order to get one additional point for the analysis of gene expression, the ratios between ApoE-/- and C57BL/6 for all genes were therefore set to 1 for the extrapolated time point 0.

In an attempt to graphical facilitate the expression analysis of the 370 included genes by the visualization of gene expression patterns; we used the web-based program GENECLUSTER that organizes gene expressions into patterns using self-organizing map (SOM) algorithms [181]. Gene with similar expression pattern are identified and collected in clusters. A 4x2 cluster analysis resulting in 8 clusters was chosen, since gene expression following 3 time points offer 7 principal possibilities of gene expression pattern.

5.5 Detection threshold

Of importance for gene expression array analysis is the determination of the detection threshold. Since the analysis of all upregulated genes by quantitative real-time polymerase chain reaction (PCR) was not feasible in terms of costs and tissue material, the quantitative analysis was performed for two genes that showed low expression; iNOS, an atherosclerosis-related gene, and interleukin-15, a novel gene in atherosclerosis. The expression of the latter was also confirmed by immunohistochemistry. Both the gene expressions of both iNOS and

IL-15 could be verified with the sensitive quantitative real-time PCR. This meant that genes expression of gene with similar values possible could be considered exexpressed.

6 RESULTS AND DISCUSSION

6.1 B cell responses towards lipid peroxidation products (I)

B cell responses in atherosclerosis are reflected in the production of IgM and IgG antibodies against epitopes in oxidatively modified LDL [39,40,139]. Since several reactive aldehydes are formed during lipid peroxidation of LDL [51], we studied antibody responses towards several aldehydes in normal C57BL/6 mice. We chose mouse serum albumin (MSA) as model of a self-protein to modify with aldehydes. A commercial preparation of lipid-depleted MSA was modified with saturated and unsaturated aldehydes that contained 4-9 carbon atoms. Antibody production was analyzed by Enzyme-linked immunosorbent assay (ELISA). Immunizations with all aldehyde-modified preparations in adjuvant resulted in both IgM and IgG responses towards the modified MSA, whereas control immunizations with native MSA did not trigger any antibody responses towards MSA. This was expected as selection during B cell development and regulatory control mechanisms of the immune system prevents B cells from starting immune responses to self-proteins [182]. B cells are therefore considered tolerant to self-proteins. Unexpectedly, immunizations with either HNE- or nonanal-modified MSA resulted also in IgG antibody reactivity towards non-modified MSA measured by ELISA. The recognition of non-modified MSA suggested that a break of tolerance had occurred. To further characterize the antibody reactivity against MSA, western blot analysis was performed using denaturing SDS gels, SDS-PAGE. Sera from HNE-MSA-immunized mice showed reactivity against lipid-depleted MSA in ELISAs as well as in denaturing western blots. However, reactivity against freshly isolated MSA was seen in western blot, but not in ELISA. In ELISA, the protein is detected when attached to a plastic surface in native conformation while Western blots from SDS-PAGE gels detect denatured protein. Interstingly, only immunizations with either HNE- or nonanal-modified-MSA could induce the formation of antibodies towards lipid-depleted MSA Both aldehydes contain 9 carbon atoms and are therefore more hydrophobic than the other aldehydes. Due to the covalent addition of hydrophobic adducts, the modification of MSA with these two aldehydes may result in a conformational change of protein structure. As a result cryptic epitopes may be exposed and may interact with the BcR. Thus the pronounced hydrophobic properties of HNE and nonanal may explain why only these two aldehydes resulted in autoantibody production.

The fact that no reactivity to fresh native MSA could be seen by ELISA analysis has two possible explanations. First, clonal deletion has removed all B cells that are reactive to surface-exposed epitopes of circulating native albumin. Therefore, no B cells are available and can be triggered even when T helper cell activity is present. Another possibility is that such B cells are present and are in fact triggered leading to antibody production. However, circulating native albumin is likely to absorb these antibodies, hence they are cleared from plasma and not detectable in the ELISA. In converse, B cells that recognize hidden cryptic epitopes have never encountered their epitopes and are therefore probably not deleted by clonal deletion. However, since hidden cryptic epitopes that are not exposed on the surface of native MSA, no reactivity towards freshly isolated native albumin should be detected in ELISA. Reactivity towards lipid-depleted MSA by sera that are derived from anti-HNE-MSA immunized mized, may therefore indicate that the lipid-depleted MSA is partly denatured exposing cryptic epitopes. However, at all times immunization with the lipid-depleted MSA

did not result in any antibody production against MSA either in ELISA or western blot, clearly showing that such a conformational change was not sufficient to support antibody production. In addition to clonal deletion of B cells, B cell tolerance is maintained by T cell tolerance, i.e. the absence of T helper cells reactive to self antigens [182]. In this study, the introduction of hapten may have led to the presentation of immunogenic epitopes (cryptic, haptenized or both) to T cells. The activated T cell may in turn have provided costimulation and cytokines to induce the antibody production by B cells.

The results suggested that the antibody responses towards the aldehyde modifications on the MSA were T cell dependent. T cell independent antibody responses have also been described towards repetitive antigenic epitopes [146,147]. To study whether the antibody response towards aldehyde-modified MSA was T cell dependent we used nude mice. These mice have a genetic mutation in the gene for the transcription factor Wnt resulting in the absence of thymus and therefore also in the absence of T cell maturation, whereas the development of B cells is unaffected [183,184]. In contrast to antibody responses in C57BL/6 mice, immunization of nude mice with modified-MSA did not result in detectable antibody responses towards the modifications. This shows that antibody responses towards MSA modified with lipid peroxidation-derived aldehydes are indeed T cell dependent.

6.2 Recognition of MDA as a hapten by T cells (II)

The presence of IgG antibodies to modified LDL in hypercholesterolemic animals as well as in human plasma and the results showing that induced antibody responses to aldehyde adducts are indeed T cell dependent, strongly suggested the presence of a T cell response to these antigens. The demonstration of T cell reactivity to oxLDL in T cells isolated from human atherosclerotic plaques and in human peripheral blood mononuclear cells provided direct evidence for a T cell response to oxidatively modified LDL [154-156]. However, there are no previous data demonstrating TcR recognition of adducts derived from lipid peroxidation and the molecular basis for T cell recognition has remained unclear.

We therefore performed experiments to determine whether aldehyde-modified MSA could trigger T cell responses in mice. Immunization of C57BL/6 mice with aldehyde modified MSA resulted in weak primary spleen cell responses towards the modified MSA. However, after repeated antigen restimulation of spleen cell cultures, growing cell populations could be detected. Since T cell hybridomas are commonly used to characterize TcR peptide interactions, T cell hybridomas were established by fusion of the spleen cell cultures with the TcR negative thymoma cell line BW5147$\alpha^-\beta^-$ [185]. T cell hybridomas derived from spleen cell cultures restimulated with MDA-MSA were triggered by MDA-MSA, but not by non-modified MSA. To further characterize the mechanism for the T cell responses towards MDA-MSA, two MDA-MSA specific MHC class II (I-A^b) restricted $CD3^+CD4^+CD8^-$ hybridoma were chosen. Both hybridomas expressed TcR V$\beta16^+$ chains, but differed in TcR Vα chain expression. The TcR of hybridoma M20 contains the TcR Vα4 chain whereas M24 expressed the TcR Vα11 chain.

Hapten-recognition, i.e. recognition of non-protein adducts, by T cells has previously been described in detail for trinitrophenylated proteins [167,186]. In this recognition, TcR's directly interact with haptens bound to peptides, which are presented on MHC class I and II molecules (figure 3) [186,187]. T cell hybridomas recognized TNP-modified bovine serum albumin in the presence of APC and TcR recognition of haptens was in one case independent of the carrier peptide sequence [168]. If MDA-MSA recognition were carrier peptide

sequence independent, MDA-modification of naturally processed peptides, i.e. peptides presented without specific antigen stimulation, would result in stimulation of the hybridomas. The M20 hybridoma was indeed triggered by MDA-modified APCs, both modified C57BL/6 spleen cells and modified LB27.4 cells, a B cell lymphoma line. The reactivity increased with an increased number of APCs and was dependent of APCs with the correct MHC set-up (I-A^b), i.e. haplotype. This indicated that the TcR of M20 directly interacted with the MDA modification on peptides already bound to the MHC molecule. Since also MDA-modified glutaraldehyde-fixed APCs triggered M20, processing of MDA-modified surface proteins for presentation appeared unlikely [188]. It is likely that MHC-bound naturally processed peptides were modified and triggered the hybridoma. Unfortunately, very little is known about the nature of naturally processed peptides that are loaded on the MHC class II molecule I-A^b, but a predisposition for peptides containing the amino acid combination N/QxxxxxP has been shown for the peptides eluded from I-A^b molecules [189,190]. Although the nature of the triggering peptide/s remains to be determined, the peptide/s were probably abundant on MHC molecules resulting in the reproducible stimulation of M20 with surface modified APCs. MDA-modified APCs did not trigger M24, indicating that recognition was dependent on the sequence of the carrier peptide. CNBr cleavage of MDA-modified MSA identified the peptide $MSA^{289-322}$ as target peptide for M24. Sequence analysis of the peptide showed a cluster of lysine residues around a central proline in the amino acid chain. Only the modified peptide triggered the hybridoma suggesting recognition of aldehyde adducts by the TcR. However, it was also possible that the reactivity was due to changed peptide conformation rather than direct recognition of the adduct itself.

To further analyze the TcR MDA-peptide interaction, a naturally occurring anti-MDA antibody was established from unimmunized ApoE-/- mice, since interactions between TcR and the hapten/peptide/MHC complex can be analyzed by blocking antibodies [187,191]. The antigen presentation of MDA-MSA to both hybridomas could be blocked by the anti-MDA antibody but not by an isotype matched control antibody. This indicates that the MDA adduct is directed towards to the TcR. Four aldehyde adducts have been identified for the modification of lysines with MDA (figure 2) [192], which may interact with the TcR.

6.3 cDNA array suggesting novel interaction pathways (III)

The recently developed gene expression array technology was used to simultaneously analyze expression of a large number of genes. We applied this technique on the aortas of Apo E-/- mice with normal C57/BL6 mice as controls. Two time points of disease development were chosen for the analysis. The first time point reflecting early and intermediate lesions, and the second time point representing intermediate and advanced lesions [177]. The expression of adhesion molecules on activated endothelial cells and the accumulations of mononuclear leukocyte are established events in atherosclerosis [3,193]. The expression patterns of genes related to these events were therefore evaluated to validate the experimental approach. Expression of the adhesion molecules VCAM-1 and ICAM-1 was increased in ApoE-/- mice compared to C57BL/6 mice after 10 weeks on hypercholesterolemic diet [194,195]. Both genes increased with disease progression. P-selectin, in contrast, showed a slight down-regulation in the ApoE-/- mice at the early time point. Since this gene is important for the development of the disease, this expression of this gene was possibly slightly underestimated As demonstrated for the gene expression of the inducible NO synthetase by quantitative real-time PCR, the quantitation of the ApoE-/- values appeared to be underestimated to some extent. However, gene expression was increased in the ApoE-/- mice for both time points for several genes that are associated with macrophages such as MAC-1, CD14 and CD18. In addition, the presence of T cells was reflected by of CD3 and CD4 genes in the atherosclerotic

mice. Both molecules are markers for T cells and present in the atherosclerotic lesions [13,109]. Thus, several events in atherosclerogenesis were confirmed by the gene expression analysis of the aortas [3,193-195].

6.3.1 CD44 (III and IV)

Results from the gene array showed a strong upregulation of CD44 gene expression in the aortas of atherosclerotic mice and this was confirmed by immunohistochemistry, which revealed CD44 staining throughout the atherosclerotic lesion including the endothelial cell layer. Interestingly, hepatocyte growth factor/scatter factor, which has previously been demonstrated to induce endothelial CD44 expression [196], was also strongly upregulated in ApoE-/- mice at both time points. Furthermore, the inflammatory cytokine IL-1alpha has recently been shown to induce CD44 expression in leukocytes and this activity was mediated by Egr-1 [197]. Egr-1 is a transcription factor, which recently has been associated with atherosclerosis [198]. Egr-1 was strongly expressed in both C57BL/6 and ApoE -/- mice and slightly upregulated (1.3) at 20 weeks of diet. In smooth muscle cells IL1beta has been shown to induce CD44 gene expression [199]. In our array analysis, IL-1beta was increased in Apo E-/- mice with a ratio of 1.6 and 3.2 at 10 and 20 weeks of treatment, respectively.

6.3.2 IL-15 (IV)

IL-15, a potent pro-inflammatory cytokine has recently been shown to increase hyaluronic acid secretion by endothelial cells and to promote the recruitment of inflammatory cells, i.e. macrophages and T cells, by interaction between endothelial hyaluronan and leukocyte CD44 [200,201]. By analysis with both gene array and quantitative real-time PCR, IL-15 gene expression was detected at a relatively low levels, but was upregulated in atherosclerotic mice. To confirm the IL-15 expression at the protein level, the roots of the aorta were analyzed by immunohistochemistry. The specificity of the IL-15 staining was tested by pre-incubation of the antibody with the antigenic peptide before staining of the sections. Immunohistochemistry demonstrated IL-15 protein in endothelial and smooth muscle cells of both mouse strains. However, Apo E-/- lesions showed increased expression of IL-15 in macrophages, which also appeared to be the main source for IL-15 production in the human lesions.

6.4 Vitamin A signaling in Atherosclerosis (III & V)

Gene array analysis revealed an increased expression of cellular retinoic acid binding protein-II (CRABP-II) in aortas of atherosclerotic mice compared to those of control mice. Since expression of CRABP-II is regulated via the retinoic acid receptor (RAR) [202], the detection of CRABP-II in the ApoE-/- mice indicated the presence of the vitamin A signaling pathway in atherosclerosis. Vitamin A is metabolized intracellularly into its active forms all-trans retinoic acid (atRA) and 9-cis retinoic acid through conversion by retinol and alcohol dehydrogenases [74,203]. RAR forms a heterodimer with the RXR and is important for cell regulation during development [204,205]. To analyze if RAR might be present in atherosclerotic lesions, sections of advanced human atherosclerotic lesions were analyzed by immunohistochemistry. Both RAR-α and -γ were seen in macrophages and foam cells, whereas RAR-β could not be detected. An increased expression of RAR-α and -γ genes in atherosclerotic lesions was confirmed by quantitative real-time PCR. Finally, fresh atherosclerotic specimens were incubated and conditioned media were harvested for analysis of ligand activity to RAR applying a RAR-GAL4 reporter assay. In this system, the ligand-

binding domain of RAR is fused with the DNA binding domain of the yeast transcription factor GAL4. Therefore, binding of ligands to RAR mediates GAL4 binding to DNA that can be measured in a luciferase reporter system [206,207]. Ligand activity towards RAR was detectable in the supernatant of cultured human plaques. Although atRA and 9-cis retinoic acids are the principal ligands for RAR [74], the exact nature of the ligand remains to be elucidated.

6.4.1 RAR promotes foam cell formation through induction of CD36 (V)

The demonstration of RAR in foam cells together with data showing that retinoids participate in lipid metabolism [208,209], suggested that RAR might be involved in regulation of scavenger receptor expression. CD36 has been demonstrated to have an important role in lipid metabolism and the development of atherosclerosis [18]. Therefore, we analyzed the effects of atRA on the expression of CD36 in the human monocytic cell line THP-1. AtRA increased the expression of CD36 and the uptake of oxLDL, an effect that could be specifically blocked by the RAR-antagonist CD3106. A synergistic effect on the expression of CD36 in THP-1 cells could be shown for the RAR and PPAR-γ signalling pathways applying specific RAR and PPAR-γ agonists. Although atRA increased the expression of CD36 in freshly isolated monocytes only slightly, addition of atRA together with oxLDL strongly enhanced the expression of CD36 in the monocytes compared to treatment with oxLDL alone. RAR signalling may therefore have an important role in the development of foam cells.

7 IMPLICATIONS

7.1 Leukocyte recruitment

LDL extravasation, reflecting plasma LDL levels, and retention in the subendothelial space are considered prerequisites for atherosclerosis [5,6,43]. Several lines of evidence, starting with the finding of oxLDL in atherosclerotic lesions, has led to the identification of oxLDL as an important factor in the pathogenesis of atherosclerosis [4,8]. The activation of the transcription factor NF-κB by oxLDL has been studied in great detail and NF-κB appears to mediate several important effects of oxLDL [210,211]. Interestingly, NF-κB regulates the expression of several molecules that are involved in recruitment of inflammatory cells to sites of tissue damage [11]. OxLDL has been demonstrated to affect both rolling and adhesion of leukocytes to endothelial cells [81,212,213]. Expression of P-selectin and VCAM-I, potentially induced by oxLDL, has been shown to be important for the development of atherosclerosis [77-80]. An alternative mechanism behind rolling of leukocytes has recently been described [214]. Interaction of CD44 on activated leukocytes with hyaluronate on the endothelium mediates rolling under shear stress conditions. This interaction is followed by a VCAM-I/VLA4 mediated adhesion to the endothelium, whereas ICAM-1/LFA-1 interaction appeared not necessary [215]. Also extravasation of activated T cells to sites of inflammation was dependent on the interaction between CD44 and hyaluronate [216]. The expression of hyaluronate on the endothelium therefore seems central for the recruitment of memory T cells to sites of inflammation. IL-15 has previously been demonstrated to induce the expression of hyaluronate in vascular endothlial cells and thus mediate CD44 dependent extravasation of activated T cell [201].

In our study, we could demonstrate IL-15 expression in endothelial cells of both unaffected and atherosclerotic (IV). It is therefore appealing to speculate that IL-15 may contribute to the recruitment of activated T cells to atherosclerotic lesions. Considering the effects of oxLDL on the activation of the NF-κB signaling pathway, oxLDL may enhance transcription of IL-15, which has an NF-κB binding site in the promoter region [217]. IL-15 secretion by endothelial cells may then induce the secretion of hyaluronate in an autocrine manner, ultimately leading to increased rolling of leukocytes. However, regulation of IL-15 occurs at several levels, including posttranscriptionally [218]. An increased IL-15 transcription in the endothelium may therefore not necessarily result in an enhanced IL-15 secretion. Since the endothelium in healthy as well as atherosclerotic arteries contained IL-15, the pathophysiologically most important source of IL-15 in atherogenesis may be lesional macrophgages. OxLDL may induce production of IL-15 in macrophages, which appeared to be the main source for IL-15 in advanced human lesions (IV and [219]). This may lead to continued T cell recruitment throughout atherogenesis.

Recruitment of monocytes to sites of inflammation and atherosclerosis is also mediated by chemokines. MCP-1 has convincingly been shown to participate in the development of atherosclerosis [14]. OxLDL has been demonstrated to induce the expression of MCP-1 in both endothelial cells and smooth muscle cells [61]. Interestingly, both atRA and 9-cis

retinoic acid have previously been shown to induce MCP-1 expression in monocytic cell lines [220]. The presence of oxLDL and retinoids may therefore favor the MCP-1 mediated recruitment of monocytes to the intima during atherosgenesis.

7.2 Regulation of foam cell formation

Expression of scavenger receptors Sr-A and CD36 increases during monocyte-macrophage differentiation [221,222], a process thst is likely to occur in the intima. Scavenger receptor mediated uptake of oxLDL by macrophages leads to the accumulation of cholesterol ester and foam cell formation [91,92,94]. OxLDL has recently been shown to enhance macrophage differentiation and to increase its own uptake through upregulation of CD36 [69,70]. OxLDL-derived 9-HODE and 13-HODE were identified as ligands to PPAR-γ, which binds to the promoter region of CD36. OxLDL was demonstrated to both upregulate PPAR-γ and promote monocyte differentiation [70,223]. By this mechanism oxLDL can be considered a self-enhancer of its own uptake. This has been proposed to enhance foam cell formation. In our study (V), atRA was demonstrated to induce expression of CD36 via the RAR. RAR and PPAR-γ promote foam cell formation in vitro by increasing the uptake of oxLDL via CD36. Since RAR-α and -γ proteins were identified in macrophages, a cooperative effect of PPAR-γ and RAR signaling on the expression of CD36 and increased oxLDL uptake appears likely to take place in the intima thus promoting foam cell formation in vivo.

However, both uptake of oxLDL and the reverse cholesterol transport from macrophages are decisive for foam cell formation [224]. Interestingly, the ATP-binding cassette transporter (ABC1) has recently been shown to regulate the reverse cholesterol transport out of cells resulting in foam cell throughout the body in Tangier disease [225-227]. Therefore, the efflux of cholesterol is an important regulatory mechanism to prevent foam cell formation. PPAR-γ upregulates the expression of liver X receptor-alpha, inducing ABC-1 in macrophages [228]. Thus, PPAR-γ induces both the CD36 mediated uptake of oxLDL and the ABC mediated efflux of free cholesterol in macrophages in vitro. In vivo, difficiency of PPAR-γ in macrophages increased foam cell formation [228]. The effect of PPAR-γ on cholesterol efflux appears therefore more important than its effect on cholesterol uptake. 9-cis retinoic acid and specific ligands for RXR ligands, so-called rexinoids, increase the expression of ABC1 in macrophages through activation of the LXR-α/RXR heterodimer and the expression of CD36 [229]. Thus, the net-effect of the nuclear receptors on the mechanisms that regulate oxLDL uptake and cholesterol reflux is therfore decisive for foam cell development.

IFN-γ inhibits ABC1 gene expression, thereby promoting foam cell formation [230]. IFN-γ has been demonstrated to inhibit the expression of both Sr-A and CD36 [231,232]. Interestingly, IFN-γ induced uptake of oxLDL in the monocytic cell line THP-1 promoting foam cell formation (Wuttge et al manuscript). This effect could be assocated by an increased transcription of the recently cloned scavenger receptor SR-PSOX [97]. Hence, by deviating the balance towards increased uptake of oxLDL together with decreased reverse transport of free cholesterol IFN-γ may be an important mediator of foam cell formation and therefore a proatherosclerotic cytokine.

7.3 MDA, an initiator of cellular immunity in atherosclerosis

The interaction between macrophages and T cells is probably the most important event for the cell-mediated immunity in atherosclerosis, since TcR interaction with a triggering antigen that is presented on MHC molecules by APCs is the key step in T cell activation [114]. Early

characterization of human atherosclerosis demonstrated the presence of activated T cells in these lesions [19,20,111]. Several candidate antigens for T cells in atherosclerosis have been identified, including oxLDL, heat shock proteins 60/65, β(2)glycoprotein I and *Chlamydia pneumoniae* [154,156,233-236]. Lack of knowledge of the molecular recognition of oxLDL by T cells, led us to analyze if lipid peroxidation-derived aldehydes may form neoepitopes that can be recognized by T cells. We demonstrated that MDA-adducts directly interacted with TcR, i.e. they were recognized as haptens (II). One feature of hapten recognition by T cells is the recognition of chemical modification of complete cells [237]. One T cell hybridoma, M20, recognized MDA-modified APC and fullfilled this criterion. It is interesting to speculate that MDA may escape from LDL, due to the hydrophilic property of MDA [52]. Free MDA may now interact with any kind of positively charged chemical groups. As result, MDA may directly bind to peptides that are loaded on either MHC class I or class II molecules, which may have some interesting implications for atherosclerosis. Firstly, modification of MHC class I or II molecules may lead to the activation of not only CD4+ but also CD8+ T cells, which normally react to intracellularly derived antigens. Secondly, the surface modification of peptides bound to MHC molecules may explain why atherosclerosis is wide spread in the population and why convincing MHC allele restriction have not been found yet [1]. In comparison, diabetes is an autoimmune disease with low prevalence (0.2-0.4% in the united states) that has been associated to certain MHC class II alleles [238,239]. In the described mechanism (II), neoepitopes would be added to peptides already MHC bound on the surface. Restrictions of peptide binding to certain MHC molecules would therefore become of redundant.

In addition, despite the many different possible antigens in the atherosclerotic plaque, a limited number of epitopes may dominate the immune response as suggested by the clonal dominance of a few clonal TcR subsets in atherosclerotic lesions of ApoE-/- mice [240]. Interestingly, the TcR Vβ16 chain was one of the preferentially used TCR Vβ chains in this study. Since the two T cells hybridomas analyzed in this thesis expressed TcR Vβ16 chains, it is intriguing to speculate that T cells recognizing MDA-modified epitopes may commonly share the TCR Vβ16-chain and that MDA adducts may represent a dominating T cell epitope in atherosclerosis.

7.4 Impact of T cell immunity on atherogenesis

In order to characterize the role of the adaptive immunity in the pathogenesis of atherosclerosis, several experiments with mice deficient in T and B cells have been performed. Early experiments with high cholesterol diets in rag knockout mice, suggested a minor impact of the adaptive immune system on atherosclerosis [241,242]. However, recent studies were done with ApoE-/- mice crossed with either rag 1-/-, rag 2-/- or SCID on normal chow diet. Immune deficient mice developed lesions that were only 19-46% of those in fully immunocompetent mice [243-245]. Thus, these studies show that the adaptive immune system exerts a proatherosclerotic effect. The role of either T cells in general or of different T cell antigens in particular on atherogenesis has been further investigated by others. T cell deficient nude mice and CD4+ ablated C57BL/6 on high cholesterol diet showed decreased fatty streak formation [246]. Furthermore, T- and B cell deficient SCID and ApoE double knockout mice that were reconstituted with CD4+ cells showed increased lesions compared to control ApoE-/- SCID mice [245]. These findings suggest that CD4+ T cells have a proatherogenic. Early studies of mice defective in MHC class I signaling showed increased fatty streak formation that may suggest a protective role of CD8+ T cells [247]. However, despite the presence of CD8+ T cells in atherosclerosis [13,109,111,248], only one study has addressed the function

of CD8+ T cells in atherosclerosis. CD8+ T cell attack on autoantigen bearing cells in arteries of ApoE-/- mice was recently shown to accelerate atherosclerosis.

T cell function in atherosclerosis has further been investigated by interfering with different T cell cytokines. Abolishing IFN-γ signaling reduced lesion size, whereas administration of IFN-g enhanced the development of atherosclerosis [127,128]. Interestingly, specific inhibition of Th1 differentiation by pentoxyfyllin in ApoE-/- mice reduced lesion development and therefore suggests a role for CD4+ mediated IFN-γ production [249]. These studies imply that the proinflammatory Th1 cytokine IFN-γ exerts proatherosclerotic activity. In support of this, studies of different congenic mouse strains showed that Th1-prone strains are particularly susceptible to development of atherosclerosis [179,250]. The abundant presence of CD44 thoughout the lesion (IV) may influence the local cytokine milieu, since CD44 has been shown to bind IFN-γ on smooth mucle cells [251]. Interestingly, binding of IFN-γ to CD44 enhanced the activity of IFN-γ on these cells, probably by increasing the availability of IFN-γ for the IFN-γ receptor. The presence of CD44 throughout the lesion could support a Th1 milieu. On the other hand, retinoids may down-regulate the IFN-γ production in atherosclerotic lesions by inhibiting the CD28 pathway in T cells favoring a Th2 milieu [252]. Taken together, IFN-γ-producing CD4+ T cells may mediate proatherosclerotic effects of the adaptive immune system.

Recent studies examining T cells and antibody responses towards HSP65 and β(2)glycoprotein I [253,254] further support this conclusion. Adoptive transfer of either HSP65 or β(2)glycoprotein I-primed T cells as well as transfer of anti-HSP65 IgG accelerated the development of atherosclerosis. In contrast immunization with either oxLDL or MDA-LDL reduces lesion development in atherosclerotic animal models [169-173]. Whether this effect is T cell mediated or B cell mediated is still controversial [171,173]. However, MDA-adduct specific T cells may have a possible regulating role by influencing both T and B cell activation induced by MDA-LDL [255].

8 CONCLUSIONS

This study has addressed three important mechanisms in the atherogenesis, leukocyte recruitment, foam cell formation and T cell activation. The identification of IL-15 in atherosclerosis suggests a novel mechanism for the recruitment of activated CD44+ T cells to sites of atherosclerotic lesions. The CD44+ mediated T cell recruitment will be of interest to address in future studies to characterize important T cell recruitment mechanism in atherosclerosis [256]. The RAR signaling pathway has been identified in atherosclerotic lesion and may be involved for the regulation of the scavenger receptor CD36 and foam cell formation. Also effects of IFN-γ are of decisive importance for foam cell formation and development of atherosclerosis in general. The demonstration of the molecular recognition of MDA-adducts by CD4+ T cells provides therefore an important tool to specifically address the impact of MDA-adduct activated, antigen specific T cells on atherogenesis.

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10 REFERENCES

1. Braunwald E: **Shattuck lecture-cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities**. *N Engl J Med* 1997, **337**:1360-1369.
2. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W: **Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions**. *J Clin Invest* 1997, **100**:2680-2690.
3. Ross R: **Atherosclerosis-an inflammatory disease**. *N Engl J Med* 1999, **340**:115-126.
4. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL: **Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity [see comments]. [Review] [94 refs]**. *N Engl J Med* 1989, **320**:915-924.
5. Camejo G: **The interaction of lipids and lipoproteins with the intercellular matrix of arterial tissue: its possible role in atherogenesis**. *Adv Lipid Res* 1982, **19**:1-53.
6. Frank JS, Fogelman AM: **Ultrastructure of the intima in WHHL and cholesterol-fed rabbit aortas prepared by ultra-rapid freezing and freeze-etching**. *J Lipid Res* 1989, **30**:967-978.
7. Tamminen M, Mottino G, Qiao JH, Breslow JL, Frank JS: **Ultrastructure of early lipid accumulation in ApoE-deficient mice**. *Arterioscler Thromb Vasc Biol* 1999, **19**:847-853.
8. Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D: **Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man**. *J Clin Invest* 1989, **84**:1086-1095.
9. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, et al.: **The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture**. *Arterioscler Thromb Vasc Biol* 1996, **16**:831-842.
10. Maziere C, Auclair M, Djavaheri MM, Packer L, Maziere JC: **Oxidized low density lipoprotein induces activation of the transcription factor NF kappa B in fibroblasts, endothelial and smooth muscle cells**. *Biochemistry & Molecular Biology International* 1996, **39**:1201-1207.
11. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T: **Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers**. *Faseb J* 1995, **9**:899-909.
12. Walpola PL, Gotlieb AI, Cybulsky MI, Langille BL: **Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress :published erratum appears in Arterioscler Thromb Vasc Biol 1995 Mar;15(3):429:**. *Arterioscler Thromb Vasc Biol* 1995, **15**:2-10.
13. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK: **Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque**. *Arteriosclerosis* 1986, **6**:131-138.
14. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ: **Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice**. *Mol Cell* 1998, **2**:275-281.
15. Smith JD, Trogan E, Ginsberg M, Grigaux C, Tian J, Miyata M: **Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E**. *Proc Natl Acad Sci U S A* 1995, **92**:8264-8268.
16. Yamada Y, Doi T, Hamakubo T, Kodama T: **Scavenger receptor family proteins: roles for atherosclerosis, host defence and disorders of the central nervous system**. *Cell Mol Life Sci* 1998, **54**:628-640.
17. Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, Ueda O, Sakaguchi H, Higashi T, Suzuki T, et al.: **A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection**. *Nature* 1997, **386**:292-296.

18. Febbraio M, Podrez EA, Smith JD, Hajjar DP, Hazen SL, Hoff HF, Sharma K, Silverstein RL: **Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice**. *J Clin Invest* 2000, **105**:1049-1056.
19. Hansson GK, Holm J, Jonasson L: **Detection of activated T lymphocytes in the human atherosclerotic plaque**. *Am J Pathol* 1989, **135**:169-175.
20. Jonasson L, Holm J, Skalli O, Gabbiani G, Hansson GK: **Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis**. *Journal of Clinical Investigation* 1985, **76**:125-131.
21. Zhou X, Paulsson G, Stemme S, Hansson GK: **Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice**. *J Clin Invest* 1998, **101**:1717-1725.
22. Frostegard J, Ulfgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, Hansson GK: **Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines**. *Atherosclerosis* 1999, **145**:33-43.
23. Autio I, Jaakkola O, Solakivi T, Nikkari T: **Oxidized low-density lipoprotein is chemotactic for arterial smooth muscle cells in culture**. *FEBS Lett* 1990, **277**:247-249.
24. Nomoto A, Mutoh S, Hagihara H, Yamaguchi I: **Smooth muscle cell migration induced by inflammatory cell products and its inhibition by a potent calcium antagonist, nilvadipine**. *Atherosclerosis* 1988, **72**:213-219.
25. Schwartz SM, deBlois D, O'Brien ER: **The intima. Soil for atherosclerosis and restenosis**. *Circ Res* 1995, **77**:445-465.
26. Newby AC, Zaltsman AB: **Fibrous cap formation or destruction--the critical importance of vascular smooth muscle cell proliferation, migration and matrix formation**. *Cardiovasc Res* 1999, **41**:345-360.
27. Gutstein DE, Fuster V: **Pathophysiology and clinical significance of atherosclerotic plaque rupture**. *Cardiovasc Res* 1999, **41**:323-333.
28. van der Wal AC, Becker AE, van der Loos CM, Das PK: **Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology**. *Circulation* 1994, **89**:36-44.
29. Geng YJ, Wu Q, Muszynski M, Hansson GK, Libby P: **Apoptosis of vascular smooth muscle cells induced by in vitro stimulation with interferon-gamma, tumor necrosis factor-alpha, and interleukin-1 beta**. *Arterioscler Thromb Vasc Biol* 1996, **16**:19-27.
30. Schonbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP, Libby P: **Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture?** *Circ Res* 1997, **81**:448-454.
31. Mach F, Schonbeck U, Bonnefoy JY, Pober JS, Libby P: **Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor**. *Circulation* 1997, **96**:396-399.
32. Mach F, Schonbeck U, Fabunmi RP, Murphy C, Atkinson E, Bonnefoy JY, Graber P, Libby P: **T lymphocytes induce endothelial cell matrix metalloproteinase expression by a CD40L-dependent mechanism: implications for tubule formation**. *Am J Pathol* 1999, **154**:229-238.
33. Carmeliet P: **Proteinases in cardiovascular aneurysms and rupture: targets for therapy?** *J Clin Invest* 2000, **105**:1519-1520.
34. Holvoet P, Collen D: **Thrombosis and atherosclerosis**. *Curr Opin Lipidol* 1997, **8**:320-328.
35. Myant NB: *Cholesterol metabolism, LDL and the LDL receptor*. San Diego: Academic Press; 1990: 99-111.
36. Gotto AM, Jr.: *Plasma Lipoproteins*. Amsterdam, New York, and Oxford: Elsevier; 1987.
37. Esterbauer H, Jurgens G, Quehenberger O, Koller E: **Autoxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes**. *J Lipid Res* 1987, **28**:495-509.
38. Yang CY, Gu ZW, Weng SA, Kim TW, Chen SH, Pownall HJ, Sharp PM, Liu SW, Li WH, Gotto AJ, et al.: **Structure of apolipoprotein B-100 of human low density lipoproteins**. *Arteriosclerosis* 1989, **9**:96-108.
39. Palinski W, Rosenfeld ME, Yla HS, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL: **Low density lipoprotein undergoes oxidative modification in vivo**. *Proc Natl Acad Sci U S A* 1989, **86**:1372-1376.

40. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL: **ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum**. *Arterioscler Thromb* 1994, **14**:605-616.
41. Vasile E, Simionescu M, Simionescu N: **Visualization of the binding, endocytosis, and transcytosis of low-density lipoprotein in the arterial endothelium in situ**. *J Cell Biol* 1983, **96**:1677-1689.
42. Camejo G, Hurt-Camejo E, Wiklund O, Bondjers G: **Association of apo B lipoproteins with arterial proteoglycans: pathological significance and molecular basis**. *Atherosclerosis* 1998, **139**:205-222.
43. Boren J, Olin K, Lee I, Chait A, Wight TN, Innerarity TL: **Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding**. *J Clin Invest* 1998, **101**:2658-2664.
44. Kuhlencordt PJ, Chen J, Han F, Astern J, Huang PL: **Genetic deficiency of inducible nitric oxide synthase reduces atherosclerosis and lowers plasma lipid peroxides in apolipoprotein E-knockout mice**. *Circulation* 2001, **103**:3099-3104.
45. Detmers PA, Hernandez M, Mudgett J, Hassing H, Burton C, Mundt S, Chun S, Fletcher D, Card DJ, Lisnock J, et al.: **Deficiency in inducible nitric oxide synthase results in reduced atherosclerosis in apolipoprotein E-deficient mice**. *J Immunol* 2000, **165**:3430-3435.
46. Cyrus T, Pratico D, Zhao L, Witztum JL, Rader DJ, Rokach J, FitzGerald GA, Funk CD: **Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein e-deficient mice**. *Circulation* 2001, **103**:2277-2282.
47. Brennan ML, Anderson MM, Shih DM, Qu XD, Wang X, Mehta AC, Lim LL, Shi W, Hazen SL, Jacob JS, et al.: **Increased atherosclerosis in myeloperoxidase-deficient mice**. *J Clin Invest* 2001, **107**:419-430.
48. Parthasarathy S: **Mechanism(s) of cell-mediated oxidation of low density lipoprotein.** In *Free Radicals in the Environment, Medicine and Toxicology*. Edited by Nohl H, Esterbauer H, Evans CR: Richelieu Press; 1994:163-179.,
49. Heinecke JW: **Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis**. *Atherosclerosis* 1998, **141**:1-15.
50. Halliwell B, Chirico S: **Lipid peroxidation: its mechanism, measurement, and significance. [Review] [103 refs]**. *Am J Clin Nutr* 1993.
51. Esterbauer H, Gebicki J, Puhl H, Jurgens G: **The role of lipid peroxidation and antioxidants in oxidative modification of LDL. [Review]**. *Free Radic Biol Med* 1992, **13**:341-390.
52. Esterbauer H, Ramos P: **Chemistry and pathophysiology of oxidation of LDL. [Review]**. *Rev Physiol Biochem Pharmacol* 1996, **127**:31-64.
53. Esterbauer H, Schaur RJ, Zollner H: **Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. [Review]**. *Free Radic Biol Med* 1991, **11**:81-128.
54. Uchida K: **Role of reactive aldehyde in cardiovascular diseases**. *Free Radic Biol Med* 2000, **28**:1685-1696.
55. Uchida K, Stadtman ER: **Modification of histidine residues in proteins by reaction with 4-hydroxynonenal**. *Proceedings of the National Academy of Sciences of the United States of America* 1992, **89**:4544-4548.
56. Uchida K, Stadtman ER: **Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction**. *Journal of Biological Chemistry* 1993, **268**:6388-6393.
57. Schuh J, Fairclough GF, Jr., Haschemeyer RH: **Oxygen-mediated heterogeneity of apo-low-density lipoprotein**. *Proc Natl Acad Sci U S A* 1978, **75**:3173-3177.
58. Hoff HF, O'Neil J: **Lesion-derived low density lipoprotein and oxidized low density lipoprotein share a lability for aggregation, leading to enhanced macrophage degradation**. *Arterioscler Thromb* 1991, **11**:1209-1222.
59. Hoff HF, Whitaker TE, O'Neil J: **Oxidation of low density lipoprotein leads to particle aggregation and altered macrophage recognition**. *J Biol Chem* 1992, **267**:602-609.
60. Breuer O, Bjorkhem I: **Simultaneous quantification of several cholesterol autoxidation and monohydroxylation products by isotope-dilution mass spectrometry**. *Steroids* 1990, **55**:185-192.

61. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, Gerrity R, Schwartz CJ, Fogelman AM: **Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells**. *Proc Natl Acad Sci U S A* 1990, **87**:5134-5138.
62. Vora DK, Fang ZT, Liva SM, Tyner TR, Parhami F, Watson AD, Drake TA, Territo MC, Berliner JA: **Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression**. *Circ Res* 1997, **80**:810-818.
63. Esterbauer H: **Cytotoxicity and genotoxicity of lipid-oxidation products. [Review]**. *Am J Clin Nutr* 1993.
64. Parthasarathy S, Barnett J: **Phospholipase A2 activity of low density lipoprotein: evidence for an intrinsic phospholipase A2 activity of apoprotein B-100**. *Proceedings of the National Academy of Sciences of the United States of America* 1990, **87**:9741-9745.
65. Sartipy P, Johansen B, Gasvik K, Hurt-Camejo E: **Molecular basis for the association of group IIA phospholipase A(2) and decorin in human atherosclerotic lesions**. *Circ Res* 2000, **86**:707-714.
66. Murohara T, Scalia R, Lefer AM: **Lysophosphatidylcholine promotes P-selectin expression in platelets and endothelial cells. Possible involvement of protein kinase C activation and its inhibition by nitric oxide donors**. *Circ Res* 1996, **78**:780-789.
67. Kume N, Cybulsky MI, Gimbrone MA, Jr.: **Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells**. *J Clin Invest* 1992, **90**:1138-1144.
68. Sugiyama S, Kugiyama K, Ogata N, Doi H, Ota Y, Ohgushi M, Matsumura T, Oka H, Yasue H: **Biphasic regulation of transcription factor nuclear factor-kappaB activity in human endothelial cells by lysophosphatidylcholine through protein kinase C-mediated pathway**. *Arterioscler Thromb Vasc Biol* 1998, **18**:568-576.
69. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM: **Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma**. *Cell* 1998, **93**:229-240.
70. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM: **PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL [In Process Citation]**. *Cell* 1998, **93**:241-252.
71. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, et al.: **The nuclear receptor superfamily: the second decade**. *Cell* 1995, **83**:835-839.
72. Giguere V: **Orphan nuclear receptors: From gene to function**. *Endocrine Reviews* 1999, **20**:689-725.
73. Glass CK: **Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers**. *Endocr Rev* 1994, **15**:391-407.
74. Giguere V: **Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling**. *Endocr Rev* 1994, **15**:61-79.
75. McEver RP: **Selectins**. *Curr Opin Immunol* 1994, **6**:75-84.
76. Cybulsky MI, Gimbrone MJ: **Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis**. *Science* 1991, **251**:788-791.
77. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD: **The combined role of P- and E-selectins in atherosclerosis**. *J Clin Invest* 1998, **102**:145-152.
78. Dong ZM, Brown AA, Wagner DD: **Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice**. *Circulation* 2000, **101**:2290-2295.
79. Shih PT, Brennan ML, Vora DK, Territo MC, Strahl D, Elices MJ, Lusis AJ, Berliner JA: **Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet**. *Circ Res* 1999, **84**:345-351.
80. Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, Beaudet AL: **P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice**. *J Exp Med* 2000, **191**:189-194.
81. Bourdillon MC, Poston RN, Covacho C, Chignier E, Bricca G, McGregor JL: **ICAM-1 deficiency reduces atherosclerotic lesions in double-knockout mice (ApoE(-/-)/ICAM-1(-/-)) fed a fat or a chow diet**. *Arterioscler Thromb Vasc Biol* 2000, **20**:2630-2635.
82. Seifert PS, Hugo F, Hansson GK, Bhakdi S: **Prelesional complement activation in experimental atherosclerosis. Terminal**

C5b-9 complement deposition coincides with cholesterol accumulation in the aortic intima of hypercholesterolemic rabbits. *Lab Invest* 1989, **60**:747-754.
83. Torzewski J, Oldroyd R, Lachmann P, Fitzsimmons C, Proudfoot D, Bowyer D: **Complement-induced release of monocyte chemotactic protein-1 from human smooth muscle cells. A possible initiating event in atherosclerotic lesion formation**. *Arterioscler Thromb Vasc Biol* 1996, **16**:673-677.
84. Han KH, Tangirala RK, Green SR, Quehenberger O: **Chemokine receptor CCR2 expression and monocyte chemoattractant protein- 1-mediated chemotaxis in human monocytes : A regulatory role for plasma LDL**. *Arterioscler Thromb Vasc Biol* 1998, **18**:1983-1991.
85. Boring L, Gosling J, Cleary M, Charo IF: **Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis**. *Nature* 1998, **394**:894-897.
86. Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA: **A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice**. *J Clin Invest* 1998, **101**:353-363.
87. Gerrity RG: **The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions**. *Am J Pathol* 1981, **103**:181-190.
88. Medzhitov R, Janeway C, Jr.: **Innate immune recognition: mechanisms and pathways**. *Immunol Rev* 2000, **173**:89-97.
89. Akira S, Takeda K, Kaisho T: **Toll-like receptors: critical proteins linking innate and acquired immunity**. *Nat Immunol* 2001, **2**:675-680.
90. Pearson AM: **Scavenger receptors in innate immunity**. *Curr Opin Immunol* 1996, **8**:20-28.
91. Goldstein JL, Ho YK, Basu SK, Brown MS: **Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition**. *Proc Natl Acad Sci U S A* 1979, **76**:333-337.
92. Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D: **Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL. Correlation with changes in receptor-mediated catabolism**. *Arteriosclerosis* 1987, **7**:135-143.
93. Kodama T, Reddy P, Kishimoto C, Krieger M: **Purification and characterization of a bovine acetyl low density lipoprotein receptor**. *Proc Natl Acad Sci U S A* 1988, **85**:9238-9242.
94. Steinbrecher UP: **Receptors for oxidized low density lipoprotein**. *Biochim Biophys Acta* 1999, **1436**:279-298.
95. Abraham R, Choudhury A, Basu SK, Bal V, Rath S: **Disruption of T cell tolerance by directing a self antigen to macrophage-specific scavenger receptors**. *J Immunol* 1997, **158**:4029-4035.
96. Nicoletti A, Caligiuri G, Tornberg I, Kodama T, Stemme S, Hansson GK: **The macrophage scavenger receptor type A directs modified proteins to antigen presentation**. *Eur J Immunol* 1999, **29**:512-521.
97. Shimaoka T, Kume N, Minami M, Hayashida K, Kataoka H, Kita T, Yonehara S: **Molecular cloning of a novel scavenger receptor for oxidized low density lipoprotein, SR-PSOX, on macrophages [In Process Citation]**. *J Biol Chem* 2000, **275**:40663-40666.
98. Unanue ER: **Antigen-presenting function of the macrophage**. *Annu Rev Immunol* 1984, **2**:395-428.
99. Lanzavecchia A: **Mechanisms of antigen uptake for presentation**. *Curr Opin Immunol* 1996, **8**:348-354.
100. Villadangos JA, Ploegh HL: **Proteolysis in MHC class II antigen presentation: who's in charge?** *Immunity* 2000, **12**:233-239.
101. Riese RJ, Chapman HA: **Cathepsins and compartmentalization in antigen presentation**. *Curr Opin Immunol* 2000, **12**:107-113.
102. Bakke O, Nordeng TW: **Intracellular traffic to compartments for MHC class II peptide loading: signals for endosomal and polarized sorting**. *Immunological Reviews* 1999, **172**:171-187.
103. Babbitt BP, Allen PM, Matsueda G, Haber E, Unanue ER: **Binding of immunogenic peptides to Ia histocompatibility molecules**. *Nature* 1985, **317**:359-361.
104. Monaco JJ: **Structure and function of genes in the MHC class II region**. *Curr Opin Immunol* 1993, **5**:17-20.
105. Margulies DH: **The Major Histocompatibility Complex**. In *Fundamental Immunology*, edn fourth. Edited by Paul WE: Lippincott-Raven; 1999:263-285.,
106. Monaco JJ: **Pathways for the processing and presentation of**

antigens to T cells. *J Leukoc Biol* 1995, **57**:543-547.
107. Jensen PE, Weber DA, Thayer WP, Westerman LE, Dao CT: **Peptide exchange in MHC molecules**. *Immunol Rev* 1999, **172**:229-238.
108. Salomon B, Bluestone JA: **Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation**. *Annu Rev Immunol* 2001, **19**:225-252.
109. Zhou X, Stemme S, Hansson GK: **Evidence for a local immune response in atherosclerosis. CD4+ T cells infiltrate lesions of apolipoprotein-E-deficient mice**. *Am J Pathol* 1996, **149**:359-366.
110. Munro JM, van der Walt JD, Munro CS, Chalmers JA, Cox EL: **An immunohistochemical analysis of human aortic fatty streaks**. *Hum Pathol* 1987, **18**:375-380.
111. Stemme S, Holm J, Hansson GK: **T lymphocytes in human atherosclerotic plaques are memory cells expressing CD45RO and the integrin VLA-1**. *Arterioscler Thromb* 1992, **12**:206-211.
112. Nemazee D: **Receptor selection in B and T lymphocytes**. *Annu Rev Immunol* 2000, **18**:19-51.
113. Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA: **An alpha beta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex**. *Science* 1996, **274**:209-219.
114. Davis MM, Boniface JJ, Reich Z, Lyons D, Hampl J, Arden B, Chien Y: **Ligand recognition by alpha beta T cell receptors**. *Annu Rev Immunol* 1998, **16**:523-544.
115. Rothenberg EV: **The development of functionally responsive T cells**. *Adv Immunol* 1992, **51**:85-214.
116. Carter LL, Zhang X, Dubey C, Rogers P, Tsui L, Swain SL: **Regulation of T cell subsets from naive to memory**. *J Immunother* 1998, **21**:181-187.
117. Swain SL, Bradley LM: **Helper T cell memory: more questions than answers**. *Semin Immunol* 1992, **4**:59-68.
118. Sprent J, Surh CD: **Generation and maintenance of memory T cells**. *Curr Opin Immunol* 2001, **13**:248-254.
119. Lanzavecchia A, Sallusto F: **Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells**. *Science* 2000, **290**:92-97.
120. Mosmann TR, Sad S: **The expanding universe of T-cell subsets: Th1, Th2 and more**. *Immunol Today* 1996, **17**:138-146.
121. Steimle V, Siegrist CA, Mottet A, Lisowska-Grospierre B, Mach B: **Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA**. *Science* 1994, **265**:106-109.
122. Hansson GK, Jonasson L, Holm J, Clowes MM, Clowes AW: **Gamma-interferon regulates vascular smooth muscle proliferation and Ia antigen expression in vivo and in vitro**. *Circ Res* 1988, **63**:712-719.
123. Hansson GK, Hellstrand M, Rymo L, Rubbia L, Gabbiani G: **Interferon gamma inhibits both proliferation and expression of differentiation-specific alpha-smooth muscle actin in arterial smooth muscle cells**. *J Exp Med* 1989, **170**:1595-1608.
124. Hansson GK, Holm J: **Interferon-gamma inhibits arterial stenosis after injury**. *Circulation* 1991, **84**:1266-1272.
125. Peilot H, Rosengren B, Bondjers G, Hurt-Camejo E: **Interferon-gamma induces secretory group IIA phospholipase A2 in human arterial smooth muscle cells. Involvement of cell differentiation, STAT-3 activation, and modulation by other cytokines**. *J Biol Chem* 2000, **275**:22895-22904.
126. Mach F, Schonbeck U, Sukhova GK, Bourcier T, Bonnefoy JY, Pober JS, Libby P: **Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis**. *Proceedings of the National Academy of Sciences of the United States of America* 1997, **94**:1931-1936.
127. Gupta S, Pablo AM, Jiang X, Wang N, Tall AR, Schindler C: **IFN-gamma potentiates atherosclerosis in ApoE knock-out mice**. *J Clin Invest* 1997, **99**:2752-2761.
128. Whitman SC, Ravisankar P, Elam H, Daugherty A: **Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein E-/- mice**. *Am J Pathol* 2000, **157**:1819-1824.
129. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D, Glass CK: **Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase**. *Nature* 1999, **400**:378-382.
130. Bogdan C, Vodovotz Y, Paik J, Xie QW, Nathan C: **Mechanism of suppression of nitric oxide synthase expression by interleukin-4 in primary mouse**

macrophages. *Journal of Leukocyte Biology* 1994, **55**:227-233.
131. Niiro H, Otsuka T, Tanabe T, Hara S, Kuga S, Nemoto Y, Tanaka Y, Nakashima H, Kitajima S, Abe M, et al.: **Inhibition by interleukin-10 of inducible cyclooxygenase expression in lipopolysaccharide-stimulated monocytes: its underlying mechanism in comparison with interleukin-4**. *Blood* 1995, **85**:3736-3745.
132. George J, Mulkins M, Shaish A, Casey S, Schatzman R, Sigal E, Harats D: **Interleukin (IL)-4 deficiency does not influence fatty streak formation in C57BL/6 mice**. *Atherosclerosis* 2000, **153**:403-411.
133. Mach F, Schonbeck U, Sukhova GK, Atkinson E, Libby P: **Reduction of atherosclerosis in mice by inhibition of CD40 signalling**. *Nature* 1998, **394**:200-203.
134. Geng YJ, Henderson LE, Levesque EB, Muszynski M, Libby P: **Fas is expressed in human atherosclerotic intima and promotes apoptosis of cytokine-primed human vascular smooth muscle cells**. *Arterioscler Thromb Vasc Biol* 1997, **17**:2200-2208.
135. Henderson EL, Geng YJ, Sukhova GK, Whittemore AD, Knox J, Libby P: **Death of smooth muscle cells and expression of mediators of apoptosis by T lymphocytes in human abdominal aortic aneurysms**. *Circulation* 1999, **99**:96-104.
136. Zhou X, Hansson GK: **Detection of B cells and proinflammatory cytokines in atherosclerotic plaques of hypercholesterolaemic apolipoprotein E knockout mice**. *Scand J Immunol* 1999, **50**:25-30.
137. Sohma Y, Sasano H, Shiga R, Saeki S, Suzuki T, Nagura H, Nose M, Yamamoto T: **Accumulation of plasma cells in atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits**. *Proc Natl Acad Sci U S A* 1995, **92**:4937-4941.
138. Ramshaw AL, Parums DV: **Immunohistochemical characterization of inflammatory cells associated with advanced atherosclerosis**. *Histopathology* 1990, **17**:543-552.
139. Salonen JT, Yla-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssonen K, Palinski W, Witztum JL: **Autoantibody against oxidised LDL and progression of carotid atherosclerosis [see comments]**. *Lancet* 1992, **339**:883-887.
140. Heyman B: **Regulation of antibody responses via antibodies, complement, and Fc receptors**. *Annu Rev Immunol* 2000, **18**:709-737.
141. Baumgarth N: **A two-phase model of B-cell activation**. *Immunol Rev* 2000, **176**:171-180.
142. Parker DC: **The functions of antigen recognition in T cell-dependent B cell activation. [Review]**. *Seminars in Immunology* 1993, **5**:413-420.
143. Bishop GA, Hostager BS: **B lymphocyte activation by contact-mediated interactions with T lymphocytes**. *Curr Opin Immunol* 2001, **13**:278-285.
144. Purkerson J, Isakson P: **A two-signal model for regulation of immunoglobulin isotype switching**. *Faseb J* 1992, **6**:3245-3252.
145. Snapper CM, Marcu KB, Zelazowski P: **The immunoglobulin class switch: beyond "accessibility"**. *Immunity* 1997, **6**:217-223.
146. Vos Q, Lees A, Wu ZQ, Snapper CM, Mond JJ: **B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms**. *Immunol Rev* 2000, **176**:154-170.
147. Mond JJ, Vos Q, Lees A, Snapper CM: **T cell independent antigens**. *Curr Opin Immunol* 1995, **7**:349-354.
148. Goodnow CC, Cyster JG, Hartley SB, Bell SE, Cooke MP, Healy JI, Akkaraju S, Rathmell JC, Pogue SL, Shokat KP: **Self-tolerance checkpoints in B lymphocyte development**. *Adv Immunol* 1995, **59**:279-368.
149. Palinski W, Horkko S, Miller E, Steinbrecher UP, Powell HC, Curtiss LK, Witztum JL: **Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma**. *J Clin Invest* 1996, **98**:800-814.
150. Landsteiner K, Scheer Jvd: **Serological studies on azoproteins. Antigens containing azo components with aliphatic side chains.** *J Exp Med* 1934, **59**:751-768.
151. Monroe JG, Lowy A, Granstein RD, Greene MI: **Studies of immune responsiveness and unresponsiveness to the p-azobenzenearsonate (ABA) hapten**. *Immunol Rev* 1984, **80**:103-131.
152. Katz DH, Benacerraf B: **The regulatory influence of activated T cells on B cell**

responses to antigen. *Adv Immunol* 1972, **15**:1-94.
153. Yla-Herttuala S, Palinski W, Butler SW, Picard S, Steinberg D, Witztum JL: **Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL**. *Arterioscler Thromb* 1994, **14**:32-40.
154. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK: **T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein**. *Proc Natl Acad Sci U S A* 1995, **92**:3893-3897.
155. Frostegard J, Wu R, Giscombe R, Holm G, Lefvert AK, Nilsson J: **Induction of T-cell activation by oxidized low density lipoprotein**. *Arteriosclerosis & Thrombosis* 1992, **12**:461-467.
156. Wu R, Giscombe R, Holm G, Lefvert AK: **Induction of human cytotoxic T lymphocytes by oxidized low density lipoproteins**. *Scand J Immunol* 1996, **43**:381-384.
157. Caligiuri G, Paulsson G, Nicoletti A, Maseri A, Hansson GK: **Evidence for antigen-driven T-cell response in unstable angina**. *Circulation* 2000, **102**:1114-1119.
158. Caligiuri G, Nicoletti A, Zhou X, Tornberg I, Hansson GK: **Effects of sex and age on atherosclerosis and autoimmunity in apoE-deficient mice**. *Atherosclerosis* 1999, **145**:301-308.
159. Brown MS, Basu SK, Falck JR, Ho YK, Goldstein JL: **The scavenger cell pathway for lipoprotein degradation: specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages**. *J Supramol Struct* 1980, **13**:67-81.
160. Haberland ME, Fogelman AM, Edwards PA: **Specificity of receptor-mediated recognition of malondialdehyde-modified low density lipoproteins**. *Proc Natl Acad Sci U S A* 1982, **79**:1712-1716.
161. Abraham R, Singh N, Mukhopadhyay A, Basu SK, Bal V, Rath S: **Modulation of immunogenicity and antigenicity of proteins by maleylation to target scavenger receptors on macrophages**. *J Immunol* 1995, **154**:1-8.
162. Amigorena S, Bonnerot C: **Fc receptors for IgG and antigen presentation on MHC class I and class II molecules**. *Semin Immunol* 1999, **11**:385-390.
163. Lougheed M, Zhang HF, Steinbrecher UP: **Oxidized low density lipoprotein is resistant to cathepsins and accumulates within macrophages**. *J Biol Chem* 1991, **266**:14519-14525.
164. Hoppe G, O'Neil J, Hoff HF: **Inactivation of lysosomal proteases by oxidized low density lipoprotein is partially responsible for its poor degradation by mouse peritoneal macrophages**. *Journal of Clinical Investigation* 1994, **94**:1506-1512.
165. ONeil J, Hoppe G, Sayre LM, Hoff HF: **Inactivation of cathepsin B by oxidized LDL involves complex formation induced by binding of putative reactive sites exposed at low pH to thiols on the enzyme**. *FREE RADICAL BIOLOGY AND MEDICINE* 1997, **23**:215-225.
166. Kastrup IB, Andersen MH, Elliott T, Haurum JS: **MHC-restricted T cell responses against posttranslationally modified peptide antigens**. *Adv Immunol* 2001, **78**:267-289.
167. Weltzien HU, Moulon C, Martin S, Padovan E, Hartmann U, Kohler J: **T cell immune responses to haptens. Structural models for allergic and autoimmune reactions**. *Toxicology* 1996, **107**:141-151.
168. Kohler J, Hartmann U, Grimm R, Pflugfelder U, Weltzien HU: **Carrier-independent hapten recognition and promiscuous MHC restriction by CD4 T cells induced by trinitrophenylated peptides**. *J Immunol* 1997, **158**:591-597.
169. Palinski W, Miller E, Witztum JL: **Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis**. *Proc Natl Acad Sci U S A* 1995, **92**:821-825.
170. Ameli S, Hultgardh NA, Regnstrom J, Calara F, Yano J, Cercek B, Shah PK, Nilsson J: **Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits**. *Arterioscler Thromb Vasc Biol* 1996, **16**:1074-1079.
171. Freigang S, Horkko S, Miller E, Witztum JL, Palinski W: **Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes**. *Arterioscler Thromb Vasc Biol* 1998, **18**:1972-1982.
172. George J, Afek A, Gilburd B, Levkovitz H, Shaish A, Goldberg I, Kopolovic Y, Wick G, Shoenfeld Y, Harats D: **Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density**

lipoprotein suppresses early atherogenesis. *Atherosclerosis* 1998, **138**:147-152.
173. Zhou X, Caligiuri G, Hamsten A, Lefvert AK, Hansson GK: **LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis**. *Arterioscler Thromb Vasc Biol* 2001, **21**:108-114.
174. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL: **Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells**. *Cell* 1992, **71**:343-353.
175. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N: **Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells**. *Proc Natl Acad Sci U S A* 1992, **89**:4471-4475.
176. Zhang SH, Reddick RL, Piedrahita JA, Maeda N: **Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E**. *Science* 1992, **258**:468-471.
177. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R: **ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree**. *Arterioscler Thromb* 1994, **14**:133-140.
178. Smith JD, Breslow JL: **The emergence of mouse models of atherosclerosis and their relevance to clinical research**. *J Intern Med* 1997, **242**:99-109.
179. Paigen B, Morrow A, Brandon C, Mitchell D, Holmes P: **Variation in susceptibility to atherosclerosis among inbred strains of mice**. *Atherosclerosis* 1985, **57**:65-73.
180. Smid-Koopman E, Blok LJ, Chadha-Ajwani S, Helmerhorst TJ, Brinkmann AO, Huikeshoven FJ: **Gene expression profiles of human endometrial cancer samples using a cDNA-expression array technique: assessment of an analysis method**. *Br J Cancer* 2000, **83**:246-251.
181. Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, Dmitrovsky E, Lander ES, Golub TR: **Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation**. *Proc Natl Acad Sci U S A* 1999, **96**:2907-2912.
182. Cornall RJ, Goodnow CC, Cyster JG: **The regulation of self-reactive B cells**. *Curr Opin Immunol* 1995, **7**:804-811.
183. Pantelouris EM: **Absence of thymus in a mouse mutant**. *Nature* 1968, **217**:370-371.
184. Segre JA, Nemhauser JL, Taylor BA, Nadeau JH, Lander ES: **Positional cloning of the nude locus: genetic, physical, and transcription maps of the region and mutations in the mouse and rat**. *Genomics* 1995, **28**:549-559.
185. Kappler JW, Skidmore B, White J, Marrack P: **Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition**. *J Exp Med* 1981, **153**:1198-1214.
186. Ortmann B, Martin S, von BA, Schiltz E, Hosch:utzky H, Weltzien HU: **Synthetic peptides anchor T cell-specific TNP epitopes to MHC antigens**. *J Immunol* 1992, **148**:1445-1450.
187. Kohler J, Martin S, Pflugfelder U, Ruh H, Vollmer J, Weltzien HU: **Cross-reactive trinitrophenylated peptides as antigens for class II major histocompatibility complex-restricted T cells and inducers of contact sensitivity in mice. Limited T cell receptor repertoire**. *Eur J Immunol* 1995, **25**:92-101.
188. Thomas DW: **Hapten-specific T lymphocyte activation by glutaraldehyde-treated macrophages: an argument against antigen processing by macrophages**. *J Immunol* 1978, **121**:1760-1766.
189. Rudensky A, Preston HP, Hong SC, Barlow A, Janeway CJ: **Sequence analysis of peptides bound to MHC class II molecules [see comments]**. *Nature* 1991, **353**:622-627.
190. Rudensky A, Preston HP, al RB, Rothbard J, Janeway CJ: **Truncation variants of peptides isolated from MHC class II molecules suggest sequence motifs**. *Nature* 1992, **359**:429-431.
191. Shevach EM, Chan C, Clement LT: **Nature of the antigenic complex recognized by T lymphocytes. VIII. Specific inhibition of the stimulatory capacity of antigen-pulsed hapten-modified peritoneal exudate cells by anti-hapten antibody**. *Eur J Immunol* 1982, **12**:819-824.
192. Uchida K, Sakai K, Itakura K, Osawa T, Toyokuni S: **Protein modification by lipid peroxidation products: formation of malondialdehyde-derived N(epsilon)-(2-propenol)lysine in proteins**. *Arch Biochem Biophys* 1997, **346**:45-52.
193. Hansson GK: **Cell-mediated immunity in atherosclerosis**. *Curr Opin Lipidol* 1997, **8**:301-311.

194. Davies MJ, Gordon JL, Gearing AJ, Pigott R, Woolf N, Katz D, Kyriakopoulos A: **The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis**. *J Pathol* 1993, **171**:223-229.
195. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R: **Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse**. *Arterioscler Thromb Vasc Biol* 1998, **18**:842-851.
196. Hiscox S, Jiang WG: **Regulation of endothelial CD44 expression and endothelium-tumour cell interactions by hepatocyte growth factor/scatter factor**. *Biochem Biophys Res Commun* 1997, **233**:1-5.
197. Fitzgerald KA, O'Neill LA: **Characterization of CD44 induction by IL-1: a critical role for Egr-1**. *J Immunol* 1999, **162**:4920-4927.
198. McCaffrey TA, Fu C, Du B, Eksinar S, Kent KC, Bush H, Jr., Kreiger K, Rosengart T, Cybulsky MI, Silverman ES, et al.: **High-level expression of Egr-1 and Egr-1-inducible genes in mouse and human atherosclerosis**. *J Clin Invest* 2000, **105**:653-662.
199. Foster LC, Arkonac BM, Sibinga NE, Shi C, Perrella MA, Haber E: **Regulation of CD44 gene expression by the proinflammatory cytokine interleukin-1beta in vascular smooth muscle cells**. *J Biol Chem* 1998, **273**:20341-20346.
200. Oppenheimer-Marks N, Brezinschek RI, Mohamadzadeh M, Vita R, Lipsky PE: **Interleukin 15 is produced by endothelial cells and increases the transendothelial migration of T cells In vitro and in the SCID mouse- human rheumatoid arthritis model In vivo**. *J Clin Invest* 1998, **101**:1261-1272.
201. Estess P, Nandi A, Mohamadzadeh M, Siegelman MH: **Interleukin 15 induces endothelial hyaluronan expression in vitro and promotes activated T cell extravasation through a CD44-dependent pathway in vivo**. *J Exp Med* 1999, **190**:9-19.
202. Niforas P, Chu MD, Bird P: **A retinoic acid/cAMP-responsive enhancer containing a cAMP responsive element is required for the activation of the mouse thrombomodulin-encoding gene in differentiating F9 cells**. *Gene* 1996, **176**:139-147.
203. Duester G: **Involvement of alcohol dehydrogenase, short-chain dehydrogenase/reductase, aldehyde dehydrogenase, and cytochrome P450 in the control of retinoid signaling by activation of retinoic acid synthesis**. *Biochemistry* 1996, **35**:12221-12227.
204. Mangelsdorf DJ: **Vitamin A receptors**. *Nutr Rev* 1994, **52**:S32-44.
205. Sporn MB, Roberts AB, Goodman DS: **The retinoids, 2nd ed.** *New York:Raven Press* 1994.
206. Botling J, Castro DS, Oberg F, Nilsson K, Perlmann T: **Retinoic acid receptor/retinoid X receptor heterodimers can be activated through both subunits providing a basis for synergistic transactivation and cellular differentiation**. *J Biol Chem* 1997, **272**:9443-9449.
207. Toresson H, Mata de Urquiza A, Fagerstrom C, Perlmann T, Campbell K: **Retinoids are produced by glia in the lateral ganglionic eminence and regulate striatal neuron differentiation**. *Development* 1999, **126**:1317-1326.
208. Standeven AM, Beard RL, Johnson AT, Boehm MF, Escobar M, Heyman RA, Chandraratna RA: **Retinoid-induced hypertriglyceridemia in rats is mediated by retinoic acid receptors**. *Fundam Appl Toxicol* 1996, **33**:264-271.
209. Davies PJ, Berry SA, Shipley GL, Eckel RH, Hennuyer N, Crombie DL, Ogilvie KM, Peinado-Onsurbe J, Fievet C, Leibowitz MD, et al.: **Metabolic effects of rexinoids: tissue-specific regulation of lipoprotein lipase activity**. *Mol Pharmacol* 2001, **59**:170-176.
210. Andalibi A, Liao F, Imes S, Fogelman AM, Lusis AJ: **Oxidized lipoproteins influence gene expression by causing oxidative stress and activating the transcription factor NF-kappa B**. *Biochem Soc Trans* 1993, **21**:651-655.
211. Thurberg BL, Collins T: **The nuclear factor-kappa B/inhibitor of kappa B autoregulatory system and atherosclerosis**. *Curr Opin Lipidol* 1998, **9**:387-396.
212. Khan BV, Parthasarathy SS, Alexander RW, Medford RM: **Modified low density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells**. *J Clin Invest* 1995, **95**:1262-1270.
213. Amberger A, Maczek C, Jurgens G, Michaelis D, Schett G, Trieb K, Eberl T, Jindal S, Xu Q, Wick G: **Co-expression of ICAM-1, VCAM-1, ELAM-1 and Hsp60 in human arterial and venous endothelial cells in response to cytokines and**

oxidized low-density lipoproteins. *Cell Stress Chaperones* 1997, **2**:94-103.
214. DeGrendele HC, Estess P, Picker LJ, Siegelman MH: **CD44 and its ligand hyaluronate mediate rolling under physiologic flow: a novel lymphocyte-endothelial cell primary adhesion pathway**. *J Exp Med* 1996, **183**:1119-1130.
215. Siegelman MH, Stanescu D, Estess P: **The CD44-initiated pathway of T-cell extravasation uses VLA-4 but not LFA-1 for firm adhesion**. *J Clin Invest* 2000, **105**:683-691.
216. DeGrendele HC, Estess P, Siegelman MH: **Requirement for CD44 in activated T cell extravasation into an inflammatory site**. *Science* 1997, **278**:672-675.
217. Washizu J, Nishimura H, Nakamura N, Nimura Y, Yoshikai Y: **The NF-kappaB binding site is essential for transcriptional activation of the IL-15 gene**. *Immunogenetics* 1998, **48**:1-7.
218. Waldmann TA, Tagaya Y: **The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens**. *Annu Rev Immunol* 1999, **17**:19-49.
219. Houtkamp MA, van Der Wal AC, de Boer OJ, van Der Loos CM, de Boer PA, Moorman AF, Becker AE: **Interleukin-15 expression in atherosclerotic plaques: an alternative pathway for T-cell activation in atherosclerosis?** *Arterioscler Thromb Vasc Biol* 2001, **21**:1208-1213.
220. Zhu LY, Bisgaier CL, Aviram M, Newton RS: **9-cis retinoic acid induces monocyte chemoattractant protein-1 secretion in human monocytic THP-1 cells**. *Arteriosclerosis Thrombosis and Vascular Biology* 1999, **19**:2105-2111.
221. Geng Y, Kodama T, Hansson GK: **Differential expression of scavenger receptor isoforms during monocyte-macrophage differentiation and foam cell formation**. *Arterioscler Thromb* 1994, **14**:798-806.
222. Huh HY, Pearce SF, Yesner LM, Schindler JL, Silverstein RL: **Regulated expression of CD36 during monocyte-to-macrophage differentiation: Potential role of CD36 in foam cell formation**. *Blood* 1996, **87**:2020-2028.
223. Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W, Glass CK: **Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein**. *Proc Natl Acad Sci U S A* 1998, **95**:7614-7619.
224. von Eckardstein A: **Cholesterol efflux from macrophages and other cells**. *Curr Opin Lipidol* 1996, **7**:308-319.
225. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, et al.: **Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1**. *Nat Genet* 1999, **22**:352-355.
226. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, et al.: **The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease**. *Nat Genet* 1999, **22**:347-351.
227. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, et al.: **Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency**. *Nat Genet* 1999, **22**:336-345.
228. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, et al.: **A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis**. *Mol Cell* 2001, **7**:161-171.
229. Costet P, Luo Y, Wang N, Tall AR: **Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor**. *J Biol Chem* 2000, **275**:28240-28245.
230. Panousis CG, Zuckerman SH: **Interferon-gamma induces downregulation of Tangier disease gene (ATP-binding-cassette transporter 1) in macrophage-derived foam cells**. *Arterioscler Thromb Vasc Biol* 2000, **20**:1565-1571.
231. Geng YJ, Hansson GK: **Interferon-gamma inhibits scavenger receptor expression and foam cell formation in human monocyte-derived macrophages**. *J Clin Invest* 1992, **89**:1322-1330.
232. Nakagawa T, Nozaki S, Nishida M, Yakub JM, Tomiyama Y, Nakata A, Matsumoto K, Funahashi T, Kameda-Takemura K, Kurata Y, et al.: **Oxidized LDL increases and interferon-gamma decreases expression of CD36 in human monocyte-derived macrophages**.

Arterioscler Thromb Vasc Biol 1998, **18**:1350-1357.
233. Xu Q, Kleindienst R, Waitz W, Dietrich H, Wick G: **Increased expression of heat shock protein 65 coincides with a population of infiltrating T lymphocytes in atherosclerotic lesions of rabbits specifically responding to heat shock protein 65**. *J Clin Invest* 1993, **91**:2693-2702.
234. Kleindienst R, Xu Q, Willeit J, Waldenberger FR, Weimann S, Wick G: **Immunology of atherosclerosis. Demonstration of heat shock protein 60 expression and T lymphocytes bearing alpha/beta or gamma/delta receptor in human atherosclerotic lesions**. *Am J Pathol* 1993, **142**:1927-1937.
235. George J, Harats D, Gilburd B, Afek A, Shaish A, Kopolovic J, Shoenfeld Y: **Adoptive transfer of beta(2)-glycoprotein I-reactive lymphocytes enhances early atherosclerosis in LDL receptor-deficient mice**. *Circulation* 2000, **102**:1822-1827.
236. Mosorin M, Surcel HM, Laurila A, Lehtinen M, Karttunen R, Juvonen J, Paavonen J, Morrison RP, Saikku P, Juvonen T: **Detection of Chlamydia pneumoniae-reactive T lymphocytes in human atherosclerotic plaques of carotid artery**. *Arterioscler Thromb Vasc Biol* 2000, **20**:1061-1067.
237. Martin S, Weltzien HU: **T cell recognition of haptens, a molecular view**. *Int Arch Allergy Immunol* 1994, **104**:10-16.
238. Jacobson DL, Gange SJ, Rose NR, Graham NM: **Epidemiology and estimated population burden of selected autoimmune diseases in the United States**. *Clin Immunol Immunopathol* 1997, **84**:223-243.
239. Nepom GT, Kwok WW: **Molecular basis for HLA-DQ associations with IDDM**. *Diabetes* 1998, **47**:1177-1184.
240. Paulsson G, Zhou X, Tornquist E, Hansson GK: **Oligoclonal T cell expansions in atherosclerotic lesions of apolipoprotein E-deficient mice**. *Arterioscler Thromb Vasc Biol* 2000, **20**:10-17.
241. Daugherty A, Pure E, Delfel BD, Chen S, Leferovich J, Roselaar SE, Rader DJ: **The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E-/- mice**. *J Clin Invest* 1997, **100**:1575-1580.
242. Dansky HM, Charlton SA, Harper MM, Smith JD: **T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse**. *Proc Natl Acad Sci U S A* 1997, **94**:4642-4646.
243. Song L, Leung C, Schindler C: **Lymphocytes are important in early atherosclerosis**. *J Clin Invest* 2001, **108**:251-259.
244. Reardon CA, Blachowicz L, White T, Cabana V, Wang Y, Lukens J, Bluestone J, Getz GS: **Effect of immune deficiency on lipoproteins and atherosclerosis in male apolipoprotein E-deficient mice**. *Arterioscler Thromb Vasc Biol* 2001, **21**:1011-1016.
245. Zhou X, Nicoletti A, Elhage R, Hansson GK: **Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice**. *Circulation* 2000, **102**:2919-2922.
246. Emeson EE, Shen ML, Bell CG, Qureshi A: **Inhibition of atherosclerosis in CD4 T-cell-ablated and nude (nu/nu) C57BL/6 hyperlipidemic mice**. *Am J Pathol* 1996, **149**:675-685.
247. Fyfe AI, Qiao JH, Lusis AJ: **Immune-deficient mice develop typical atherosclerotic fatty streaks when fed an atherogenic diet**. *J Clin Invest* 1994, **94**:2516-2520.
248. Boyle JJ: **Association of coronary plaque rupture and atherosclerotic inflammation**. *J Pathol* 1997, **181**:93-99.
249. Laurat E, Poirier B, Tupin E, Caligiuri G, Hansson GK, Bariety J, Nicoletti A: **In vivo downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice**. *Circulation* 2001, **104**:197-202.
250. Huber SA, Sakkinen P, David C, Newell MK, Tracy RP: **T helper-cell phenotype regulates atherosclerosis in mice under conditions of mild hypercholesterolemia**. *Circulation* 2001, **103**:2610-2616.
251. Hurt-Camejo E, Rosengren B, Sartipy P, Elfsberg K, Camejo G, Svensson L: **CD44, a cell surface chondroitin sulfate proteoglycan, mediates binding of interferon-gamma and some of its biological effects on human vascular smooth muscle cells**. *J Biol Chem* 1999, **274**:18957-18964.
252. Cantorna MT, Nashold FE, Chun TY, Hayes CE: **Vitamin A down-regulation of IFN-gamma synthesis in cloned mouse Th1 lymphocytes depends on the CD28 costimulatory pathway**. *J Immunol* 1996, **156**:2674-2679.

253. George J, Afek A, Gilburd B, Shoenfeld Y, Harats D: **Cellular and humoral immune responses to heat shock protein 65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice**. *J Am Coll Cardiol* 2001, **38**:900-905.
254. Shoenfeld Y, Harats D, George J: **Heat shock protein 60/65, beta 2-glycoprotein I and oxidized LDL as players in murine atherosclerosis**. *J Autoimmun* 2000, **15**:199-202.
255. Shevach EM: **Regulatory T cells in autoimmmunity**. *Annu Rev Immunol* 2000, **18**:423-449.
256. Brocke S, Piercy C, Steinman L, Weissman IL, Veromaa T: **Antibodies to CD44 and integrin alpha4, but not L-selectin, prevent central nervous system inflammation and experimental encephalomyelitis by blocking secondary leukocyte recruitment**. *Proc Natl Acad Sci U S A* 1999, **96**:6896-6901.