Natural Killer T cells and their functions in atherosclerosis and obesity: a therapeutic perspective for cardiovascular diseases

A.M. van de Vrugt[1] and E. Kalkhoven[2]

[1] Biology of Disease Master's Student, Utrecht University, the Netherlands; [2] Department of Metabolic Disorders, University Medical Center Utrecht, Utrecht, the Netherlands

Abstract Atherosclerosis and obesity, both related to the development of cardiovascular diseases (CVDs), are increasing pathologies under the global population. Therefore, both pathologies contribute to the growing problem of CVDs. In both pathologies, chronic inflammation is the key process that exacerbates disease progression. Natural Killer T (NKT) cells are of interest, because these immune cells connect innate and adaptive immunity. Moreover, NKT cells get activated by lipid antigens. These antigens are highly enriched in both atherosclerotic lesions and adipose tissue. An obvious role for these cells in these pathologies has been determined and several pathways involved in these pathologies are known to induce NKT cell activation and modulate NKT cell effector function. However, conflicting results make it hard to address whether the effects achieved by NKT cells are beneficial or pathologic. Therefore, more studies are required that take the role of interfering factors into account, such as the effect of subtypes, environment, and time span. Thereafter, the potential of the NKT cells for therapeutic application in CVD can be determined.

Key words NKT cells ▪ Lipids ▪ Cardiovascular Diseases ▪ Atherosclerosis ▪ Obesity

1. Introduction

Cardiovascular diseases (CVDs) are a major cause of death worldwide[1]. In particular the Western countries are affected by an increase in CVD patients[1]. Atherosclerosis, a chronic inflammatory condition of the vasculature developing during early adulthood, is often the underlying cause of acute cardiovascular syndromes[2]. Together with heart failure, acute cardiovascular syndromes and atherosclerosis fall under the heading of CVDs. Moreover, risk factors contribute to the increase in CVD cases worldwide. An important risk factor for CVD is obesity. Obesity rates dramatically increased during the last years, which led to a simultaneous increase in CVDs[3]. Both pathologies, atherosclerosis and obesity, which are also connected pathologies, contribute to the rising problem of CVDs. In these pathologies innate and adaptive immunity play key roles during their development. Immune cells from both immune systems are linked to CVD development and progression as well. Here, this link is described extensively for a special type of immune cells: Natural Killer T cells (NKT cells).

Abbreviations

α-GalCer	α-galactosylceramide
β-GlcCer	β-glucosylceramide
AAA	Abdominal aortic aneurysms
A-FABP	Adipocyte fatty acid binding protein
Angptl2	Angiopoietin-like protein 2
APC	Antigen presenting cell
AT	Adipose tissue
BAT	Brown AT
VAT	Visceral AT
WAT	White AT
CVD	Cardiovascular disease
DC	Dendritic cell
ECM	Extracellular matrix
FA	Fatty acid
FasL	Fas ligand
GLUT4	Glucose transporter type 4
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HDL	High-density lipoprotein
HFD	High-fat diet
HFHSC	High-fat high-sucrose diet with cholesterol
IFN-γ	Interferon γ
iGb3	Isoglobotrihexosylceramide
IL	Interleukin
Lcn-2	Lipocalin-2
LDL	Low-density lipoprotein
oxLDL	Oxidized LDL
LDLR	LDL receptor
LPS	Lipopolysaccharide
LR	Leptin receptor
LVH	Left ventricular hypertrophy
MCP-1	Monocyte chemoattractant protein-1
MetS	Metabolic syndrome
MMP-9	Matrix metalloproteinase 9
NK cell	Natural Killer cell
NKT cell	Natural Killer T cell
iNKT cell	invariant NKT cell
vNKT cell	variant NKT cell
PAI-1	Plasminogen activator inhibitor-1
PPARγ	Peroxisome proliferator-activated receptor γ
SMC	Smooth muscle cell
VSMC	Vascular SMC
STAT6	Signal transducer and activator of transcription 6
TCR	T cell receptor
TGF-β	Transforming growth factor β
TIMP-1	Tissue inhibitor of metalloproteinase 1
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor α
T reg	Regulatory T cell
TZDs	Thiazolidinediones

1.1 Natural Killer T cells

NKT cells are lymphocytes sharing characteristics of both innate and adaptive immunity, therefore having a function in bridging both immune systems[4]. These cells have described functions in atherosclerosis and obesity, the pathologies involved in CVD development. However, uncertainties and discrepancies need to be deciphered regarding these findings. NKT cells are related to the lineage of Natural Killer (NK) cells and T cells, expressing T cell receptors (TCRs) and NK cell surface molecules[4]. NKT cells possess an invariant TCR of a limited αβ-chain repertoire, allowing NKT cells to specifically recognize lipid antigens[5, 6]. These antigens are often glycolipids, such as synthetic glycosphingolipid α-galactosylceramide(α-GalCer) derived from a sponge and endogenous isoglobotrihexosylceramide (iGb3) often derived from microbes[4, 6]. These antigens are displayed in CD1, a MHC class I-like molecule acting as an antigen-presenting protein[4, 6]. CD1 proteins, CD1a to CD1d, have a different groove, which is deeper, narrower, and more hydrophobic compared to the groove of the two MHC proteins, which allows the specific binding of lipid antigens[4]. CD1d is particularly known for its function in NKT cell recognition[4]. A part of the lipid antigen extends from this CD1d protein, which is recognized by the TCR of the NKT cell, together with a portion of the CD1 molecule[4, 7]. NKT cells can express CD4 or CD8 on their cell surface as well, but double negative NKT cells are also produced[6]. The difference with other αβ T cells is found in their auto reactivity: Activation by antigen presenting cells (APCs) in the absence of exogenous antigens, and their rapid production of both Th1 and Th2 cytokines including IL-2, IL-4, IL-10, IL-13, IL-17, IFN- γ, TGF-β, and TNF-α[6-9]. Which cytokines are released, depends on the local microenvironment, the type of antigen leading

Table 1 NKT-like cells

	CD1d restricted Type 1 NKT cells	CD1d restricted Type 2 NKT cells	CD1a restricted	CD1b restricted	CD1c restricted
TCR-α	$V_\alpha14J_\alpha18$ (M) $V_\alpha24J_\alpha18$ (H)	Diverse, but some $V_\alpha3.2J_\alpha9$, $V_\alpha8$ (M) and some γδ T cells	Diverse and some γδ T cells	Diverse	Diverse and some γδ T cells
TCRβ	$V_\beta8.2$, 7, 2 (M) $V_\beta11$ (H)	Diverse, but some $V_\beta8$ (M)	Diverse	Diverse	Diverse
CD4 and CD8	$CD4^+$ or DN (M) $CD4^+$, DN, $CD8^+$ (H)	$CD4^+$ or DN (M)	$CD4^+$, DN, $CD8^+$	$CD4^+$, DN, $CD8^+$	$CD4^+$, DN, $CD8^+$
α-GalCer reactive	Yes (also analogs such as OCH, C20:2, α-C-GalCer).	No	No	No	No
Other antigens	iGb3, α-GluCer β-GalCer, β-GluCer, α-GalDAG α-Gal-uronosyl-Cer α-Glu-uronosyl-Cer GD3	Sulfatide, lysosulfatide, Lysophosphatidyl-choline, PPBF	Sulfatide, Didehydroxy-mycobactin	Sulfatide, Mycolic acid, Glucosemonomycolate, Diacylated Sulphoglycolipid, PIM, LAM, GM1	Sulfatide, Hexoysl-1-phosphoisoprenoid, Mannosyl-β1-phosphomycoketides

α-C-GalCer, α-GalCer with carbon-based glycosidic linkage; α-GluCer, α-glucosylceramide; α-GalDAG, α-galactosyl-diacylglycerol; α-Gal-uronosyl-Cer, α-galacturonosylceramide; α-glu-uronosyl-Cer, α-glucuronosylceramide; β-GalCer, β-galactosylceramide; β-GluCer, β-glucosylceramide; DN, double negative; GD3, disialo ganglioside; H, human; iGb3, isoglobotrihexosylceramide; LAM, lipoarabinomannan M, mouse; PIM, phosphatidylinositol mannoside; PPBF, phenyl 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonate.

Figure 1: Subpopulations of Natural Killer T cells and Natural Killer T-like cells. The NKT cell pool consists of different subtypes expressing different surface markers and reacting to different antigens. The invariant NKT cells, or type I NKT cells, form the most extensively described NKT cell subpopulation and react with CD1d antigen-presenting proteins. The variant NKT cells, or type II NKT cells, form the second population reacting with CD1d proteins. NKT-like cells are restricted to the other CD1 proteins of the CD1 family, namely CD1a, CD1b, and CD1c.[5]
NKT cell; Natural Killer T cell

to activation, and the NKT cell subpopulation with its associated receptors[9]. The available APCs and antigens that activate the NKT cells differ between organs, resulting in different local effects[10].

1.2 Natural Killer T cell subpopulations, development, and activation

NKT cells are roughly divided into two populations, based on their TCRs: Type I or invariant (iNKT) versus type II or variant (vNKT) NKT cells[5, 6, 11]. An extra population besides these two is reserved for NKT-like cells[5]. Type I NKT cells express an invariant Vα14-Jα18 TCR in mice or Vα24-Jα18 TCR in humans compared to a non-Vα14 TCR or much more diverse TCR in type II NKT cells[6]. A further distinction is made between these two subtypes by the presence or absence of CD4 and CD8 proteins (Figure 1)[5]. The subtypes of NKT cells recognize different antigens, for example α-GalCer is only recognized by iNKT cells[5, 6]. Based on the differences in pro- and anti-inflammatory cytokine profiles, it is expected that the different NKT cell subtypes perform different functions[5, 6]. Furthermore, several subtypes of iNKT cells are already proposed, e.g. a subdivision into Th1-, Th2-, and Th17-like NKT cells, established by their different cytokine profiles and different associated tissues[12]. Identifying all these different subtypes, their functions, and their distribution is of major importance[6].

NKT cell development starts with a process in the thymus driving NKT cell formation from a common ancestor, the $CD4^+CD8^+$ thymocyte[5]. Several transcription factors, including Erg2, NFκB, T-bet, and c-Myc, are known to regulate the different stages of development[5]. For the development of NKT cells, the $CD4^+CD8^+$ thymocyte undergoes random VDJ recombination of the genes involved in TCR assembly[5]. These TCRs are positively selected in the thymus based on their interaction with CD1d[5]. Although less explored, negative selection, which normally occurs to eliminate self-reactive lymphocytes, might shape the NKT cell repertoire even further[5]. Via at least four differentiation stages (stage 0 till 3), based on the expression of markers such as NK1.1, CD24, CD44, the NKT cell pool develops[5]. This does not always occur completely in the thymus, because

immature NKT cells can migrate to the periphery and differentiate into the mature phenotype there[5]. After complete maturation, mature NKT cells can reside in the thymus or leave the thymus to the periphery[5]. In the periphery, the majority of the NKT cells reside in the liver or bone marrow, but they also populate the spleen, lymph nodes, lungs, and the blood circulation[13, 14]. In humans, the omentum is more of a residence for NKT cells instead of the liver in mice[9]. In the different organs different subpopulations might reside, dependent on the organ itself[14]. Finally, the numbers of mature NKT cells differ enormously between mice strains and between human individuals[5, 12].

After development, mature NKT cells reside in the thymus or in the periphery waiting to encounter a signal leading to activation. Pathways of direct and indirect activation lead to NKT cell activity, which is dependent on the type of lipid antigen, which can be exogenous or endogenous(figure 2)[4]. For direct activation, APCs present the exogenous microbial antigen in their CD1d protein, which enables the specific TCR of the NKT cell to bind[4, 15]. Indirect activation does not

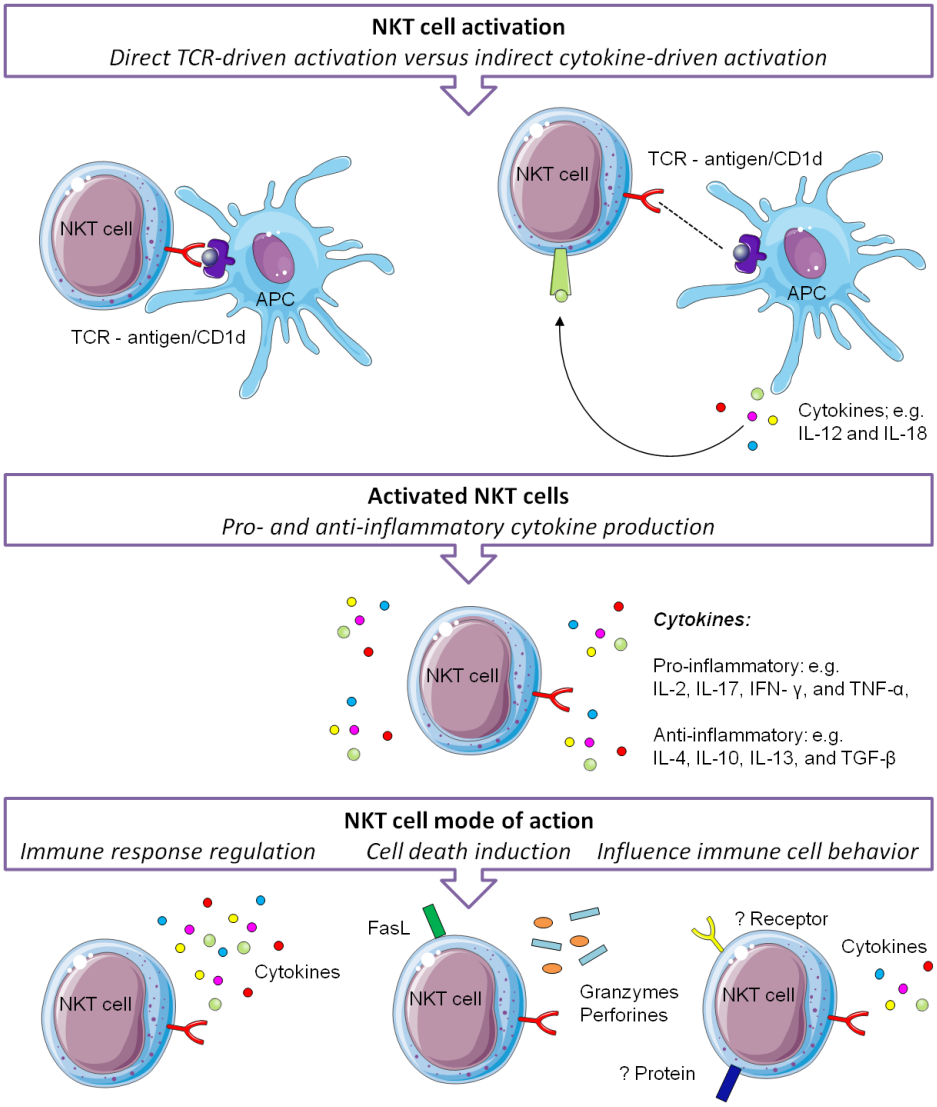

Figure 2: Activation of Natural Killer T cells and their mode of action. NKT cells can be activated directly or indirectly. Direct activation occurs via an interaction with the TCR of the NKT cell and the lipid antigen presented in CD1d on APCs. Indirect activation mainly occurs via cytokine release by the APCs such as IL-12 and IL-18, but a weak interaction between the TCR and antigen/CD1d can be involved as well. After activation of the NKT cells, these cells are capable of producing both pro-inflammatory (including IL-2, IL-17, IFN- γ, and TNF-α) and anti-inflammatory (including IL-4, IL-10, IL-13, and TGF-β) cytokines depending on, among other things, the environment. By production of these cytokines, immune responses can be regulated. But also two other modes of actions can be realized: (1) By expressing FasL, granzymes, and perforines, cell death can be induced in NKT cell targets, and (2) the behavior of other immune cells can be influenced by the release of cytokines or via direct cell-cell contact using cell surface receptors or proteins.

APC, antigen presenting cell; FasL, Fas ligand; IL, interleukin; IFN-γ, Interferon γ; NKT cell, Natural Killer T cell; TCR, T cell receptor; TGF-β, Transforming growth factor β; TNF-α, Tumor necrosis factor α

depend on TCR recognition of the lipid antigen presented by APCs[4, 15]. Instead, indirect activation depends on cytokine release by APCs (e.g. IL-12 and -18 [9]), but the recognition of the endogenous antigen presented in CD1d might strengthen this interaction [4, 15]. After activation, NKT cell activity can be both pro- and anti-inflammatory, which might refer to the different subtypes of the NKT cell pool[4].

After activation, NKT cells regulate the immune response by producing a broad range of cytokines and chemokines, by killing other cells, and by influencing immune cell behavior (figure 2)[5, 7]. IL-4 and IFN-γ are recognized as the major cytokines released by NKT cells[7]. Cell killing is performed by expression of perforin, granzyme B, and Fas ligand (FasL)[7]. Immune cell behavior is either changed by direct cell-cell contact or cytokine production[7, 12]. Therefore, NKT cells modulate immunity, but the NKT cells are not always the direct immune modulators themselves[4, 11]. Furthermore, NKT cells play important roles in tumor immunosurveillance and self-tolerance[4, 5]. However, NKT cell activity can be detrimental as well, as was shown for several diseases including atherosclerosis and obesity[5]. This provided new interests to investigate NKT cell function in the context of these pathologies[5].

1.3 Natural Killer T cells in vasculature and adipose tissue

NKT cells react to a broad range of infectious agents[4]. Especially in the liver, NKT cells patrol for infectious lipid antigens to protect the liver vasculature[12, 13]. There, the NKT cells reside in the sinusoids of the liver together with Kupffer cells[13]. NKT cells reside in other vascular walls as well to respond to infectious agents locally[13]. In mouse and human adipose tissue (AT) NKT cells are also highly enriched[12]. Moreover, their numbers decreased during obesity and recovered after a period of weight loss, suggesting a protective NKT cell phenotype in a lean state[12, 16].

NKT cell deficiency or dysfunction is involved in several diseases, such as in chronic inflammation, which is the key process occuring in atherosclerosis and obesity[12]. Also vascular diseases have been related to NKT cells[17-19]. The involvement of NKT cells in such diseases is demonstrated by several studies. In this review we searched for, among other things, similarities between atherosclerosis and obesity at the level of NKT cells. Atherosclerosis and obesity, related to vasculature and adipose tissue, respectively, are pathologies that share connections and both relate to CVDs. We attempted to translate the (immunological) findings between atherosclerosis, obesity, and CVD with the ultimate objective to use NKT cells for therapeutic purposes to fight CVDs. iNKT cells are the most abundantly described population of all NKT cells, therefore most insights are gained using these cells. Please note that in this review NKT cells will refer to iNKT cells unless stated otherwise.

2. Natural Killer T cells and atherosclerosis

Atherosclerosis is often the underlying cause of CVDs. Atherosclerosis is now recognized as a chronic inflammatory disease, involving both innate and adaptive immunity (box 1)[20]. Therefore, NKT cells might have an important function in the atherosclerotic process. How NKT cells are activated and the amount of lipid antigens present in atherosclerotic plaques, were of direct interest to explore the role of NKT cells in atherosclerosis[21]. Moreover, a pro-inflammatory NKT cell phenotype might aggravate atherosclerotic progression, but an anti-inflammatory NKT cell phenotype might ameliorate the atherosclerotic process. Therefore, many studies were performed to elucidate the role of NKT cells in atherosclerosis.

2.1 Natural Killer T cell mice models and atherosclerosis

To elucidate whether NKT cells are enhancers or attenuators of atherosclerosis, mice studies were performed. Several mice models were used, often on an apoE knockout or LDL receptor (LDLR)

knockout background[8, 11]. Different high-fat diets (HFDs) of varying duration were given to these mice to induce and study atherosclerosis[8, 11].

Three major strategies were applied to investigate the role of NKT cells in atherosclerosis, namely removal, activation, or addition of NKT cells[8, 11]. To remove the NKT cell population in a mice model, the CD1d knockout strategy can be used. CD1d knockout mice do not possess any NKT cell population, because CD1d is needed for the development of the NKT cell pool in the thymus[8]. CD1d expression was observed on both human and mouse APCs in atherosclerotic plaques[8]. Some factors, also present in atherosclerotic lesions, can increase this CD1d expression by APCs, such as oxidized low-density lipoprotein (oxLDL)[8]. Furthermore, a different mice model type can be used that eliminates the iNKT cells specifically; Jα18 knockout mice[11].

α-GalCer treatment was used in several studies to activate NKT cells in mice and to determine the effect on atherosclerosis, e.g. at the level of the aortic root[11]. Indeed, activation led to increased atherosclerotic lesions in apoE knockout mice in several studies[11]. Not only lesion area increased, also the plaque phenotype changed towards a less collagen-rich and more vulnerable phenotype[22]. In the case of CD1d knockout mice, no differences were found compared to control mice when treated with α-GalCer, which shows the importance of NKT cells in the atherosclerotic process[11]. Other studies that did not use a NKT cell activator, showed similar results: CD1d knockout mice on an apoE knockout or LDLR knockout background had less severe atherosclerotic lesions, indicating that NKT cells are “bad players” in the atherosclerotic process[8, 11]. Studies using Jα18 knockout mice showed also a decrease in atherosclerosis, indicating that the iNKT cell population is pro-atherogenic[21].

Finally, Vα14 transgenic mice were used for NKT cell addition, because these mice possess an overload of NKT cells. Based on the previous mentioned results, an aggravated outcome would be expected. Indeed, atherosclerotic lesions were exaggerated, although the effect obtained was site-specific[11]. Furthermore, adoptive transfer of various subsets of NKT cells showed that CD4+ NKT cells are responsible for the pro-atherogenic effect[21].

However, not all studies pointed in the same direction as described here. A minority of the studies indicated the NKT cells as “neutral” or “good players” in atherogenesis[8, 11]. Therefore, based on the majority of the studies and their results, the conclusion was drawn that NKT cells are in general of a pro-atherogenic phenotype[8, 11, 21].

2.2 Possible mechanisms leading to a pro-atherogenic phenotype of Natural Killer T cells

Different mechanisms could explain the suspected pro-atherogenic phenotype of NKT cells. Several NKT cell activation pathways involved in atherosclerosis are described. As a first mechanism, Toll-like receptors (TLRs), which are pattern recognition receptors, have shown to be important in NKT cell activation. TLRs are present on APCs and these cells get activated after pattern recognition by these receptors[11]. TLR activation can lead to enhanced production of endogenous lipid antigens or specific cytokines (e.g. IFN-γ), leading to increased NKT cell activation via the indirect pathway[11]. Several TLRs are important in atherosclerosis, especially TLR2 and TLR4[24]. For example, TLR4 activation leads to the polarization of NKT cells towards an inflammatory phenotype, which also occurs in response to lipid-rich dendritic cells (DCs) compared to the tolerogenic NKT cell phenotype induced by lipid-poor DCs[8]. Activation of TLR4 on macrophages by modified LDL led to enhanced oxLDL uptake and cytokine production by the macrophage itself, which in turn might influence NKT cell activation[11]. Other self antigens are expressed in the plaque as well, such as β-glucosylceramide (β-GlcCer), which led to atherosclerotic progression[8]. The increased levels of this self antigen presented by DCs in the plaque after TLR4 activation, suggest an agonistic effect of

Box 1: Atherosclerosis and inflammation

Atherosclerosis is an inflammatory disease of the vessel wall, starting at early adulthood. The vessel wall consists of three layers, the tunica intima, the tunica media, and the tunica adventitia (figure box 1a). Lipids accumulate in the inner layer of the vessel wall, the tunica intima[20]. First the endothelial cell layer gets activated and as a consequence leukocytes are attracted into the vessel wall[20]. These group of leukocytes includes monocytes and T cells, which are able to induce inflammation by the production of cytokines and chemokines[20]. Attracted monocytes maturate into macrophages[20]. Macrophages can take up the lipids in the form of oxLDL, which results in their transformation to foam cells (figure box 1b)[20]. As a next step, smooth muscle cells (SMCs) migrate from the tunica media towards the tunica intima, where they proliferate and produce extracellular matrix (ECM) products such as collagen (figure box 1c)[20]. These ECM products form a cap, that protects the pro-thrombotic inside of the plaque from the flowing blood stream[20]. However, this fibrous cap can burst, which can result in thrombus formation and vessel occlusion with concomitant systemic consequences (figure box 1d)[20]. Additionally, both foam cells and SMCs can die in the atherosclerotic plaque[20]. This creates an extracellular lipid-core and cholesterol crystals can be deposited[20]. Advanced plaques can have a microvasculature, extension of the vasa vasorum, of their own as well[20].

There are basically two plaque phenotypes: the vulnerable and the stable plaque. The vulnerable plaque has a large lipid core, high numbers of immune cells, and a thin fibrous cap[23]. A stable plaque has a small lipid core, lower numbers of immune cells, and a thick fibrous cap[23]. The vulnerable plaque is more prone to rupture and therefore, more dangerous in terms of clinical outcomes[23].

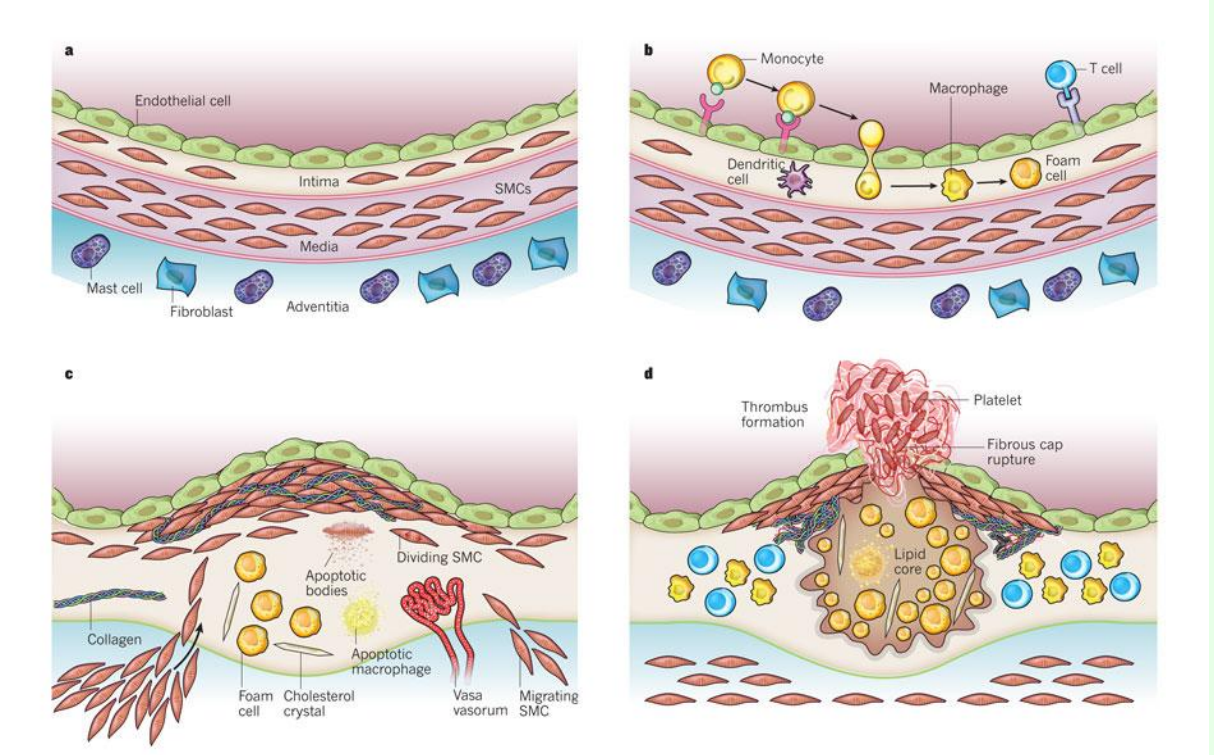

Figure box 1: The different stages of the atherosclerotic process. a) The vessel wall is divided into three layers: the tunica intima, the tunica media, and the tunica adventitia. The endothelial cell layer is part of the tunica intima. b) Monocytes are attracted into the tunica intima and differentiate into macrophages. After lipid uptake, macrophages transform into foam cells. T cells and other immune cells are attracted as well into the tunica intima. c) SMCs migrate from the tunica media to the tunica intima. There they produce ECM matrix molecules including collagen. Cells go into apoptosis locally, cholesterol crystals arise, and a microvasculature is attracted into the forming plaque. d) Many immune cells are attracted into the plaque and a lipid core is formed. A fibrous cap protects the inside of the plaque from the blood flow. However, when the plaque ruptures, the inside is exposed and a thrombus will form.[20]
ECM, extracellular matrix; SMC, smooth muscle cell

β-GlcCer in NKT cell activation and thus exacerbation of atherosclerotic lesions[8]. Besides, microbial infections have shown to enhance atherogenesis in both animals and humans[25, 26]. Lipopolysaccharide (LPS) was found to increase the size and vulnerability of atherosclerotic lesions via activation of TLR4[25]. Andoh *et al.* showed that this LPS mediated aggravation of atherosclerosis in size and vulnerability is mediated by NKT cells by having an effect on NK cells[25]. NKT cells activate NK cells that perform the effect on atherosclerosis[25]. Thus, increased TLR4 and CD1d expression on APCs present in the plaque, that occurs in the presence of oxLDL, might lead to an increased NKT cell-atherogenesis axis. Therefore, not only lipids induce atherosclerosis via this pathway, also exposure to high levels of pathogens or pathogen components (e.g. received via the colon) should be controlled to prevent atherosclerotic progression[25].

Moreover, LDL itself was found to activate NKT cells and also improved apoE-mediated antigen presentation[8, 11, 21]. ApoE can bind to the lipid antigen and these apoE-antigen complexes are delivered to APCs via LDLR mediated uptake in both humans and mice[8, 11, 21]. These pathways could increase NKT cell activation and therefore, might aggravate atherosclerotic outcome. Additionally, CD1d is expressed by vascular smooth muscle cells (VSMCs)[8]. Although no evidence is found yet for the expression of CD1d on VSMCs at the site of the plaque, this might form another NKT cell activation pathway[8]. An overview of the NKT cell activation pathways involved in atherosclerosis is depicted in figure 3.

After activation, NKT cells are capable of producing granulocyte-macrophage colony-stimulating factor (GM-CSF), resulting in an increased production of monocytes and increased differentiation of monocytes into DCs[8, 11]. Obviously, more circulating monocytes could lead to increased foam cell levels in the atherosclerotic lesions. Also the function, maturation, and proliferation of T cells, B cells, DCs, and NK cells could be affected by NKT cells and their cytokine production[11].

2.3 Additional evidence for the involvement of Natural Killer T cells in the atherosclerotic process

More interactions between NKT cells and atherosclerosis were demonstrated by other studies. Using atherosclerotic plaques derived from abdominal aortic aneurysms (AAA), which are often caused by atherosclerosis, an interaction between VSMCs and NKT cells was indicated[17]. VSMCs support NKT cell growth, but NKT cells increase apoptotic cell death of VSMCs[17]. This might result in worse atherosclerotic lesions due to an increased vulnerability of the plaques as a consequence of reduced ECM production and a richer lipid core[17]. Consequently, plaque rupture might occur, especially since NKT cells reside in the shoulder region of a plaque[12, 17]. Furthermore, in a model of arterial injury, the lipid antigens presented via the CD1d-NKT cell pathway showed to be involved in neointima formation, a common feature of atherosclerosis whereby the tunica intima thickens as a response to vascular injury[27].

NKT cells are suggested to play roles in both the initiation and progression of atherosclerotic lesions, but their effect is more pronounced during the early phase and only transient during the progression of atherosclerosis[8, 11, 21]. Anergy, the acquired tolerance of the immune system against antigens, might develop after prolonged stimulation with lipid antigens, as in chronic dyslipidemia[8, 11, 21]. This suggests a prominent role for NKT cells in especially the development of early lesions[8, 11, 21].

2.4 Natural Killer T cells: Observations and biomarker potential in human atherosclerosis

NKT cells are present in human plaques, which was also confirmed for human AAAs[8, 17]. CD1d is expressed in human plaques as well, which indicates that the NKT cells present can be

activated[8]. However, CD1d expression was only observed in neovascularized plaques of symptomatic patients (and not in asymptomatic patients), but the total number of circulating NKT cells in these patients was reduced[8]. Furthermore, the location of these NKT cells in plaques was taken into account. NKT cells reside in particular at the shoulder region[21]. This might refer to a dangerous function of NKT cells, because the shoulder region is often prone to rupture. In general, these observations are comparable to the experimental data obtained from mice: NKT cells are bad for the atherosclerotic patient.

Unfortunately, nothing is noted about the sensitivity of plaque-derived NKT cells compared to circulatory NKT cells under different pathologic conditions[8]. It would be interesting to see whether the NKT cell sensitivity correlates with the stage of the atherosclerotic lesion, so this could predict clinical outcome in patients that underwent endarterectomie[8]. The use of NKT cells as a biomarker was already described for patients with angina pectoris by Andoh *et al.* Circulating NKT cell numbers decreased in patients with angina pectoris[28]. Therefore, lower numbers of NKT cells in peripheral blood are related to coronary artery disease[28]. This was only indicated for this specific pathological setting, more studies are needed to get specific information from these cells in relation to the disease and the status of that disease.

3. Natural Killer T cells and obesity

Obesity is a risk factor for many diseases, such as type II diabetes, cancer, and CVDs[9, 29]. In obesity, the amount of AT increases with concomitant

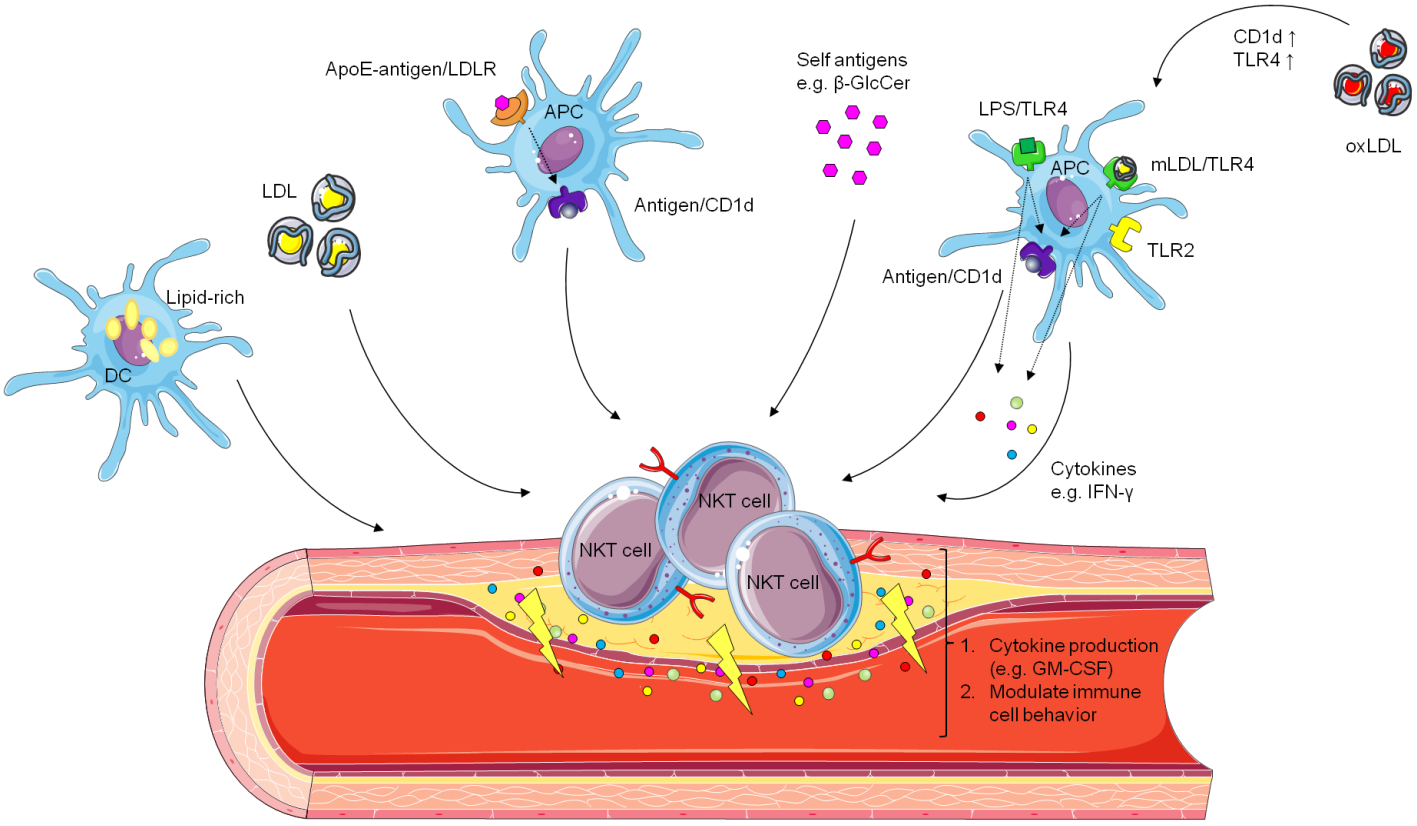

Figure 3: Activation of Natural Killer T cells in atherosclerosis. In a plaque, NKT cells get activated via several pathways. Lipid-rich DCs lead to the polarization of NKT cells towards an inflammatory phenotype. LDL and self antigens, e.g. β-GlcCer, present in the plaque can induce NKT cell activation. On APCs, such as macrophages, different receptors are expressed including the LDLR. The LDLR binds apoE, which can target lipid antigens. Thereby, an uptake circle is created, which might result in antigen presentation towards the NKT cells. TLRs (e.g. TLR2 and TLR4) are expressed as well by APCs. These TLRs can react to LPS, but also to modified LDL (e.g. oxLDL). This creates a loop towards NKT cell activation via increased antigen presentation (TCR-driven activation) or cytokine release (cytokine-driven activation). Furthermore, in presence of oxLDL, CD1d and TLR4 expression will be increased on APCs.

The activation of NKT cells result in cytokine release, other mode of actions might be performed, and the behavior of other immune cells, e.g. NK cells, can be influenced by the NKT cells. This probably increases the pro-inflammatory processes occurring in the atherosclerotic plaque.

β-GlcCer, β-glucosylceramide; APC, antigen presenting cell; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN-γ, Interferon γ; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPS, lipopolysaccharide; NK cell, Natural Killer cell; NKT cell, Natural Killer T cell; oxLDL, oxidized LDL; TLR, Toll-like receptor

changes in both immune and metabolic pathways, called immunometabolism[9, 30]. Both processes are integrated in AT, mostly visceral AT (VAT)[9]. Immune cells are present in AT and are scarcely distributed between the adipocytes[9, 30]. Normally, AT is a dynamic organ that adapts to physiological changes in food intake, increased energy demand, or starvation, but long-term changes can lead to both disrupted immune and metabolic pathways[9, 30]. Insulin resistance and metabolic dysregulation can occur as a consequence of this disbalance, resulting in an increased risk for obesity-related diseases (box 2)[9].

Obesity is associated with a state of chronic low-grade inflammation, so-called metainflammation[30, 31]. This inflammatory response occurs in metabolically active tissues such as AT, whereby immune cells infiltrate[30, 31]. The number of infiltrating immune cells, their phenotype, and cytokine expression profile shifts during obesity[9]. As an important player in obesity, the role of macrophages is extensively described[9, 30]. However, the role of other immune cells that regulate immunometabolism in AT are not well characterized yet[9]. Still, little is known about the role of NKT cells in AT inflammation and obesity-related diseases[9, 31]. The ability of NKT cells to release both pro- and anti-inflammatory cytokines might aggravate or improve AT inflammation.

3.1 Natural Killer T cells in AT during obesity

The distribution of NKT cells is not equal between the different organs, but NKT cells are normally abundantly enriched in AT[34]. 10-20% of the T cell pool in AT is populated by the NKT cells and compared with liver and spleen, most NKT cells are DN (CD4- CD8-), possess reduced expression of NK1.1, and produce both Th1 and Th2 cytokines[9, 10, 16, 35]. Without external stimuli the AT resident NKT cells are more biased towards a Th2 cytokine profile, with increased expression of IL-4 and IL-10 and reduced expression of IFN-γ, suggesting an anti-inflammatory function of NKT cells in AT [9, 16, 30, 35]. This anti-inflammatory phenotype is also more pronounced in AT compared to spleen and liver[16].

Recruitment of NKT cells to AT might be achieved via interactions with MCP-1/CCR2, CXCR2, and CXCR6[35]. MCP-1 is a chemokine that attracts besides macrophages NKT cells as well and CCR2 is the receptor for this chemokine expressed by NKT cells[35]. Several studies observed a drop in NKT cell number during obesity in, among other things, peripheral blood, the liver, and AT, which could indicate that NKT cells are protectors of obesity-related diseases[9, 16, 30, 34, 36]. This drop seems rather contradictory, because MCP-1 levels are increased in AT during obesity. However, weight loss was able to restore NKT cell numbers, indicating that NKT cell defects are reversible, which is beneficial from a therapeutic perspective[16]. Additionally, Huh *et al.* demonstrated that the decreased ratio of NKT cells/lymphocytes is HFD dependent[34]. This decrease in NKT cell number after the consumption of a HFD, is not caused by a defect in NKT cell development, because the drop in NKT cells was restricted to the AT[34]. But, as a consequence of a HFD, NKT cells could go into apoptosis[34]. This reduction in NKT cell numbers occurs before CD8+ T cells and macrophages infiltrate the AT[9, 30]. Ji *et al.* indicated that this infiltration might be NKT cell dependent, which could indicate a pro-inflammatory function of the NKT cells[36]. Next to decreased cell numbers, a HFD increased NKT cell activation, which was assessed by the increased expression of activation markers[9, 30]. This occurred in response to a lipid overload, but no exact mechanisms are known yet involved in this process[9]. Based on these findings, NKT cells are probably one of the first responders in AT during obesity, as a consequence of a nutrition/lipid overload[9, 30].

3.2 Natural Killer T cell mice models and obesity

Similar mice models were used as described before applying the three strategies of removal, activation,

Box 2: Obesity and AT inflammation

There are two types of AT in mammals: white (WAT) and brown adipose tissue (BAT). BAT has a different role than WAT, as WAT stores energy and acts as an endocrine organ by secreting adipokines and BAT is important for heat generation[29, 32]. In obesity, the amount of AT increases and a state of chronic low-grade inflammation prevails (figure box 2)[33]. Not only adipocyte numbers increase, also the adipocytes themselves increase in size: hypertrophy[9]. This hypertrophy is induced by the lipid excess that has to be stored in WAT and causes changes in the adipocyte cell characteristics[9]. Immune cells, such as macrophages, T cells, and B cells, infiltrate the AT during obesity, where they release different pro- and anti-inflammatory cytokines[9]. More of these immune cells are attracted as a response to the storage of lipids in adipocytes, because this results in the activation of metabolic and stress signaling pathways, negatively regulating insulin sensitivity[16]. For example, adipocytes secrete monocyte chemoattractant protein-1 (MCP-1), which is a factor that attracts more M1-type macrophages into the AT[9, 33]. The macrophages in obese subjects possess a pro-inflammatory phenotype (M1), which secretes TNF-α and IL-6, and the adipocytes express a disturbed adipokine profile (figure box 2B)[9, 33]. In lean subjects, macrophages mainly produce anti-inflammatory cytokines such as IL-10 (M2-type macrophages) and a certain level of healthy adipokines are expressed by the adipocytes (figure box 2A)[9, 33]. Pro-inflammatory cytokines such as TNF-α are able to aggravate insulin resistance by affecting insulin receptor signaling in AT after, among other things, binding to cytokine receptors on adipocytes[9, 33]. Next to macrophages, significant numbers of CD8+ T cells infiltrate the AT during obesity[9, 33]. Another event occurring, the levels of leptin, an adipokine which regulates food intake and expenditure of energy, increase and as a result, leptin resistance can occur leading to increased food intake[9, 33]. Therefore, the changes that occur locally in the AT environment during obesity, result in systemic effects as well. Moreover, when adipocytes reach their full capacity, the overflow of lipids might cause damage to other organs, resulting in high glucose levels, high fatty acid (FA) levels, and whole body insulin resistance[16].

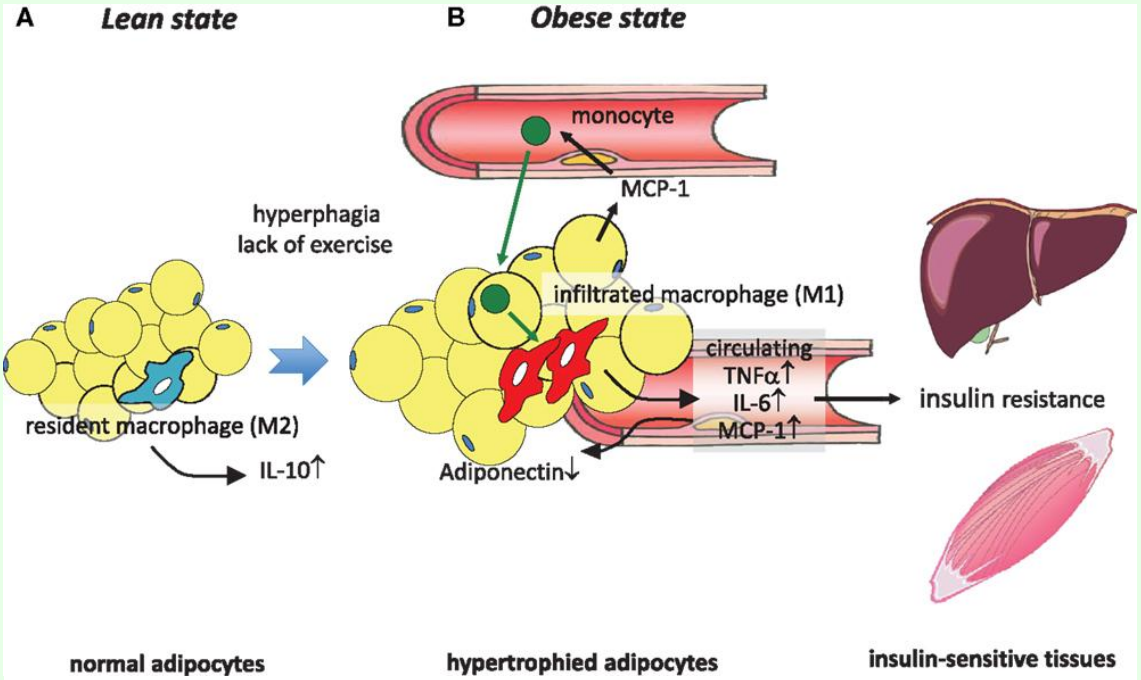

Figure box 2: The immunological and metabolic changes during obesity. A) During a lean state, the adipocytes have a normal size. The resident macrophages are of a M2 phenotype and produce the anti-inflammatory cytokine IL-10. B) During an obese state, caused by e.g. hyperphagia and/or a lack of exercise, the adipocytes become hypertrophied. MCP-1 is secreted by the adipocytes and is released into the circulation to attract monocytes towards the AT. These monocytes differentiate into the inflammatory M1-type macrophages in the AT. These macrophages secrete many inflammatory cytokines including TNF-α, IL-6, and MCP-1. This contributes to lower secreted levels of the adipokine adiponectin. Furthermore, these inflammatory cytokines contribute to insulin resistance in liver and skeletal muscle.[33]

AT, adipose tissue; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF-α, Tumor necrosis factor α

or addition of NKT cells. Furthermore, these mice received different types of diets, generally a normal low fat diet (LFD) or a HFD. However, there are many differences between the contents of the different HFDs. In general, human and murine NKT cell responses as a consequence of obesity and weight loss were indicated as similar[16]. This makes it easier to translate the data obtained from mice to humans.

Under conditions of a LFD, NKT cells have a protective function in obesity[16, 35]. Rakhshandehroo *et al.* suggested that this might be caused by the LFD-associated lipid pool in AT, that result in the basal activation of NKT cells to maintain AT homeostasis[9]. However, the results whether NKT cells are beneficial or harmful in obesity after HFD-feeding were less consistent[9, 30]. The type of diet is thus of interest, which is also reflected by a finding of Schipper *et al.* that showed that different diets lead to different AT phenotypes[35]. However, the type of diet is not only a matter of concern, differences in the duration of a diet are of major importance as well[9]. During a short period of HFD-feeding (<8 weeks) NKT cells act protective in metabolic regulation[9]. However, most studies focus on the role of NKT cells during the long-term effects of obesity[34]. During a longer HFD-feeding period, the results varied more from a protective effect, to a pathogenic effect, or no effect at all[30]. Therefore, the long-term effects accomplished by NKT cells in obesity might be different than their effects achieved on the short-term. On the long-term, the effects induced by the NKT cells on AT during the early phases of obesity might be masked and meanwhile compensatory mechanisms could be induced to maintain homeostasis[34]. Moreover, the diminished effect of NKT cells after a long-term HFD might be described by the decreased numbers and activity of NKT cells due to anergy[10, 35]. Constant stimulation due to overnutrition may desensitize the NKT cells[10, 35]. However, Ji *et al.* showed that the agonistic activation of NKT cells also induced long-term improvement of metabolic regulation, indicating the discrepancies between the studies again[36]. Furthermore, presentation of different lipid antigens at different time points during obesity, which results in a Th1 or Th2 response, might explain the different effects on the short- and long-term[9, 34]. During the early phase, the NKT cell effect is probably directed towards an anti-inflammatory phenotype[9, 10, 34]. As a proof of this principle, Huh *et al.* showed that HFD fed NKT cell deficient mice are more susceptible to obesity and glucose intolerance than wild type mice[34]. Adiponectin levels decreased, glucose transporter type 4 (GLUT4) expression decreased, and pro-inflammatory genes increased[34]. These results were confirmed by a study of Schipper *et al.* that showed an increase in adipocyte dysfunction in the absence of NKT cells, reflected by decreased levels of insulin-sensitizing adipokine adiponectin and increased levels of the insulin-desensitizing adipokine leptin[35]. Adipocyte dysfunction is besides AT inflammation also a key player in the development of insulin resistance[35]. Based on these results, NKT cells might be positive regulators of both AT inflammation and metabolic parameters.

In accordance with these results, NKT cell transfer showed increased levels of adiponectin, decreased levels of leptin, and increased IL-10 levels[16]. IL-10 counteracts the effect of TNF-α in obesity, resulting in improved insulin sensitivity and an improved adipocyte phenotype[16]. Furthermore, NKT cell activation via α-GalCer treatment led to a HFD dependent effect, which included improved glucose tolerance and insulin sensitivity in models of short-term, long-term, and chronic HFD models[10, 16, 36]. Therefore, a strong agonist such as α-GalCer, which activates AT resident NKT cells, might provide beneficial effects in obesity by acting on AT inflammation and glucose tolerance[36]. These beneficial effects are mediated by the production of IL-4 and IL-10[16]. α-GalCer activation also causes weight loss, adipocyte hypertrophy, improved fat metabolic parameters, and induced proliferation of NKT cells[16]. NKT cells mediate their effect either directly by their own actions or indirectly by controlling the functions of other cells[16]. Therefore,

NKT cells are likely not the only immune cells involved in the beneficial metabolic effects mediated by NKT cells[16].

The role of NKT cells in AT and their contribution to a systemic effect was challenged by a different study. Strodthoff *et al.* concluded that NKT cells did not had an effect on glucose clearance, but manipulated lipid metabolism in liver and AT[31]. They did not find substantial numbers of NKT cells in AT during both obese and lean conditions[31]. Therefore, they concluded that the NKT cell effect is mainly mediated by the liver[31]. The overall effects of NKT cells were found to be pathogenic in this study as depletion of NKT cells improved adipocyte function, decreased inflammation, and improved metabolic regulation[31]. However, the study of Schipper *et al.* presented a prominent role for AT instead of the prominent role described by Strodthoff *et al.* for the liver[35]. Schipper *et al.* did not observed any liver abnormalities and found large NKT cell numbers in AT, suggesting a pronounced function for AT resident NKT cells in obesity[35]. This is in accordance with many other studies, which also found large NKT cell numbers in AT[16, 34-36].

Despite all the performed studies, nothing could be concluded about the NKT cell effect in obesity using these models[9, 30]. However, it is quite clear that NKT cells do play a key role in obesity and obesity-related diseases. Their effect seems rather protective than harmful, especially in an acute setting of obesity.

3.3 Receptors and cell surface proteins expressed by adipocytes, APCs, and Natural Killer T cells

CD1d is not only expressed on the plasma membranes of professional APCs, also human and murine adipocytes express CD1d on their cell surface[9, 34, 35]. Therefore, adipocytes may sense the nutritional changes during obesity and pass this information on to the NKT cells via a direct CD1d-TCR interaction[34, 35]. Interestingly, peroxisome proliferator-activated receptor γ (PPARγ), a very important transcription factor expressed by adipocytes, showed to be involved in increasing the CD1d expression on adipocytes and APCs, thereby regulating NKT cell activity[8, 34]. Comparable to the decrease in NKT cells, CD1d and PPARγ expression decreased during obesity [34].

Next to CD1d, adipocytes express other cell surface proteins as well on their plasma membranes, including multiple cytokine receptors and TLRs such as TLR2 and TLR4[9]. HFD- fed TLR2 knockout or TLR4 knockout mice are resistant to insulin resistance, indicating a metabolic regulator function for these TLRs[9]. TLRs can function as danger sensors for inflammatory signals[9]. Interestingly, FAs or in general nutrient lipids might activate TLRs (TLR2 and TLR4) on adipocytes or APCs and initiate an inflammatory response by secretion of cytokines or processing of these lipids into lipid antigens that can be presented in CD1d[9, 30]. Thereby, FAs might provide a danger signal to the adipocytes during obesity when FA levels are increased, which resulted in increased lipolysis and decreased insulin sensitivity[9]. It would be interesting to identify whether FAs can regulate the activation NKT cells via this TLR-mediated adipocyte pathway. Also changes in gut microbiota and gut permeability due to a HFD might result in NKT cell activation via TLR activation on adipocytes or APCs and therefore, might impact NKT cell number and function[9, 30]. Wu *et al.* referred to a reverse correlation as well, whereby NKT cells influence colonization in the gut instead of the gut microbiota that influences NKT cell activation[30].

As indicated, TLRs are expressed by APCs as well[9]. β-GlcCer, a self antigen, accumulates in APCs after TLR4 activation by LPS[9]. This might result in lipid presentation to NKT cells and thus activation of NKT cells. There are controlling mechanisms known, but how these mechanisms prevent autoimmunity as a consequence of constant activation via self antigens, is unknown[9].

Besides TLRs, other receptors can facilitate NKT cell activation too. As described for atherosclerosis, the LDLR can mediate uptake of antigens that can

be loaded onto CD1d[9]. ApoE is the regulator that binds to the receptor and the antigen, thereby mediating uptake[9]. Scavenger Receptors (SRs) such as SRA, SRB1, and CD36 showed their involvement as well in targeting lipids for CD1d presentation and regulating NKT cell function[9]. Because adipocytes express both the LDLR and SRs, a possible AT resident NKT cell activation pathway could be reflected by lipoprotein uptake[9]. Indirect activation of AT resident NKT cells might occur as well depending on the direct environment created by, among others, the adipocytes[9].

Furthermore, the NKT cells themselves express a variety of receptors. A functional leptin receptor (LR) is expressed by activated NKT cells[37]. Leptin can bind to this receptor. Thereby, it inhibits NKT cell proliferation and NKT cell cytokine production[37]. *In vivo* inhibition of LR signaling led to altered fat pad features and insulin resistance[37]. The effect of this pathway is NKT cell dependent[37]. This leptin/NKT cell LR pathway indicated a new link in the communication between NKT cells and AT to keep metabolic homeostatis[37]. Although on the longer term, leptin resistance might induce a disbalance in this mechanism.

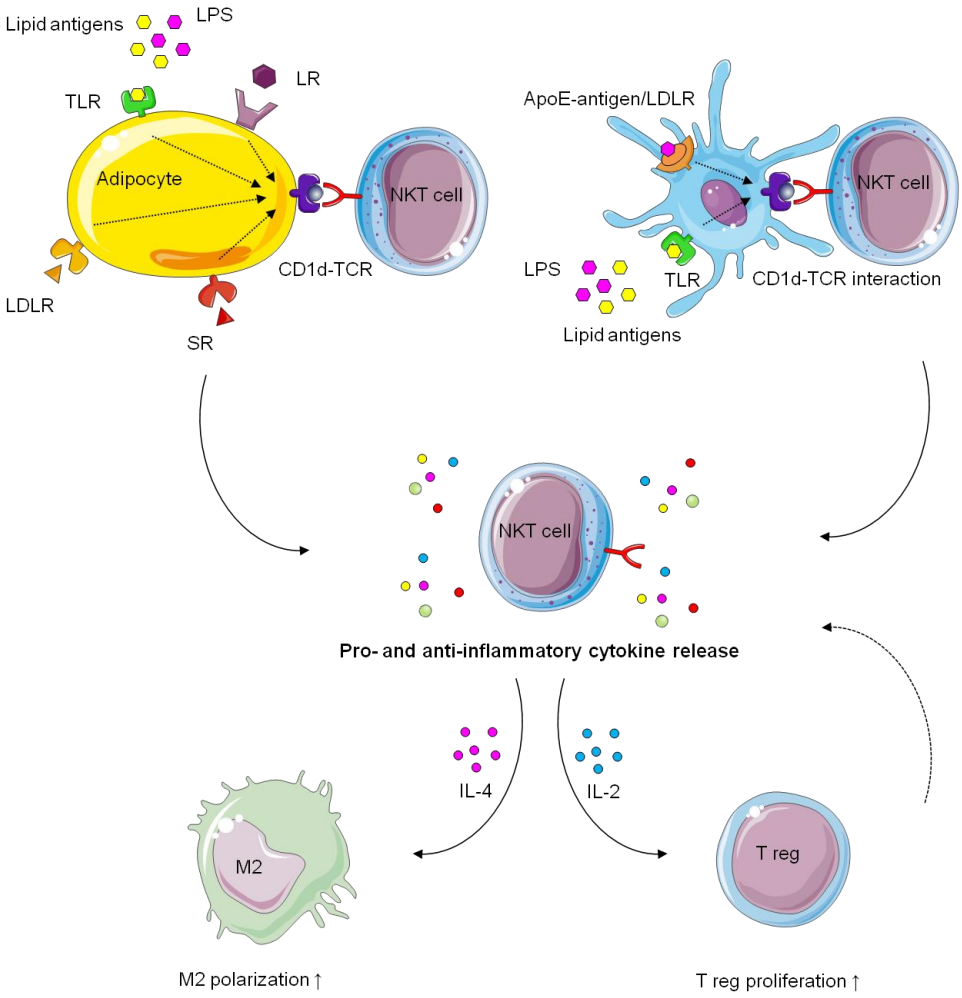

Figure 4: Natural Killer T cells in obesity. NKT cells can not only be activated via classical APCs, also adipocytes can act as APCs via their CD1d expression. This antigen presentation via adipocytes can be enhanced via the action of several receptors; TLRs, LDLRs, SRs, and LRs. TLRs react to LPS and to lipid antigens, which might act as danger signals derived from the dietary status. LPS and lipid antigens can increase the classical APC presentation as well via interaction with TLRs. LDLRs and SRs react to LDL particles. These receptors might be important for apoE-mediated uptake of lipid antigens, which can lead to presentation towards the NKT cells. The LR reacts with leptin and is likely involved in the regulation of NKT cell function as well.

The increased antigen presentation results in increased NKT cell activation and thus cytokine release by these cells. IL-4 can promote M2 macrophage polarization. These macrophages decrease the inflammatory status of the AT. Therefore, NKT cells have an effector function in modulating the behavior of other immune cells. Furthermore, they are able to increase T reg proliferation via secretion of IL-2. T regs act as anti-inflammatory immune cells and also influence NKT cell behavior.

APC, antigen presenting cell; AT, adipose tissue; IL, interleukin; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPS, lipopolysaccharide; LR, leptin receptor; NKT cell, Natural Killer T cell; SR, scavenger receptor; TLR, Toll-like receptor; T reg, regulatory T cell

3.4 Natural Killer T cells: The interplay with macrophages and regulatory T cells in obesity

NKT cells interact with other immune cells including macrophages and regulatory T cells (T regs) during obesity. M1-type macrophages are important players in AT inflammation and the initiation of insulin resistance[9]. This effect is partially mediated by the secretion of pro-inflammatory cytokines[9]. Macrophages interact with other immune cells as well such as CD4+ and CD8+ T cells, which increases the inflammatory status of AT[9]. Next to these inflammatory immune cells, also anti-inflammatory cells interact in the process of AT inflammation, including T regs, macrophages (M2), and NKT cells[9].

There are two types of macrophages; M1-type and M2-type macrophages. M1 macrophages are pro-inflammatory macrophages induced by Th1 cytokines, such as IFN-γ and TNF-α[38]. M2 macrophages are induced by Th2 cytokines, such as IL-4, IL-10, and IL-13, which lead to a macrophage phenotype that is anti-inflammatory and focuses on tissue repair[38]. Dependent on the microenvironment at the local tissue site, macrophages polarize to a certain phenotype[38]. In the AT of lean subjects, monocytes differentiate mostly towards the M2 phenotype and in the AT of obese subjects mostly towards M1[9, 34]. However, activation of NKT cells by α-GalCer promotes M2 polarization in AT during obesity by stimulation of the IL-4-STAT6 (signal transducer and activator of transcription 6) axis[36]. This observation is supported by NKT cell knockout models, which had increased macrophage numbers and an increased M1/M2 ratio in AT[9, 34]. NKT cells purified form AT of short-term HFD fed mice produced increased levels of IL-4, which is a M2 polarization signal[10]. Already after 4 days of HFD-feeding, macrophages in AT showed increased M2 polarization and Arg1, a key marker of M2 polarization, protein expression[10]. This effect was NKT cell and HFD dependent[10]. The M2 polarization signal IL-4 might be directly produced by the NKT cells, but indirect production by other cell types is also possible[10]. This NKT cell-mediated effect is possibly an acute immune reaction upon the short-term HFD to prepare the system for diet-induced challenges for a longer term[10]. Additionally, a strong negative correlation was found between the amount of M1 macrophages or total number of macrophage infiltration and NKT cell presence in AT[16]. However, not all studies identified this positive effect achieved by the NKT cells on macrophage infiltration and polarization, inverse correlations were found as well[9].

A second interplay is described for NKT cells and T regs. T regs are anti-inflammatory T cells present in, among other things, AT[9, 35]. During obesity, T reg numbers decreased in both human and mice AT, which is similar to the decrease in NKT cells during obesity[9]. However, after NKT cell depletion, T reg numbers increased[35]. This might indicate a mechanism that prevents further metabolic impairment and decreased insulin sensitivity in the absence of NKT cells[35]. T regs interact with NKT cells via direct cell-cell contact[9]. Furthermore, NKT cells produce IL-2, which stimulates the proliferation of T regs[9]. In turn, T regs can regulate NKT cell function by suppression of their proliferation, their Th1 and Th2 cytokine secretion, and their cytotoxic activity[9].

These data underpins the interplay between different types of immune cells, namely the interplay between NKT cells and macrophages or T regs. These interplays create a network, which collectively results in the induction or inhibition of AT inflammation and insulin resistance. Figure 4 gives an overview of some of the discussed mechanisms.

4. Links between atherosclerosis and obesity: A possible role for Natural Killer T cells

Individual findings concerning atherosclerosis or obesity show the involvement of both pathologies in the development of CVDs. These pathologies are linked to each other as well. For example, in both atherosclerosis and obesity, chronic inflammation is the key process occurring. Both pathologies might

share mechanisms involved in the development of CVDs.

Several circulatory markers such as matrix metalloproteinase 9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), plasminogen activator inhibitor-1 (PAI-1), IL-18, and IL-6, are associated with CVDs[39]. These factors are expressed in AT as well and increased levels were observed in CVD patients[39]. This indicated that the overall inflammatory activity in AT is significantly associated with CVD, forming a pathogenic link between obesity and CVD[39].

Furthermore, other studies indicated a link between obesity-related diseases, such as metabolic syndrome (MetS) and atherosclerosis. MetS is often a consequence of obesity, and consists of at least three of the following five factors; characteristics of hypertension, high triglyceride levels, high fasting blood glucose, low high-density lipoprotein (HDL) plasma levels, and abdominal obesity[40]. Many of these factors, as a consequence of obesity, have been related to atherosclerotic parameters[41, 42]. It must be pointed out that there are differences between men and women during development of MetS and thus cardiovascular health[43]. Other CVD precursors such as cardiac steatosis (lipid accumulation in the heart that correspond with increased CVD risk) correlate with MetS parameters as well[44]. Especially, the amount of VAT correlates with cardiac steatosis[44]. A study performed with obese women also indicated a higher prevalence of left ventricular hypertrophy (LVH) under obese subjects[45].

AT secretes many adipokines that play a role in the pathogenesis of CVDs[39]. Moreover, adipokines form direct links between obesity-related diseases and atherosclerosis. Pro-inflammatory adipokines (e.g. TNF-α) are harmful and anti-inflammatory adipokines (e.g. adiponectin) are beneficial in the development of atherosclerosis[46]. For example, angiopoietin-like protein 2 (Angptl2) is a pro-inflammatory adipokine which promotes and accelerates AT inflammation and insulin resistance in obesity, but also aggravates the atherosclerotic process[46]. Likely, a balance is needed between pro- and anti-inflammatory adipokines to prevent CVD development as a consequence of the deterioration of the atherosclerotic process[46]. Furthermore, the two adipokines lipocalin-2 (lcn-2) and adipocyte fatty acid binding protein (A-FABP) are known for their function to modulate vascular function and therefore, influence atherosclerotic progression[47]. Interestingly, adipokines specifically released by perivascular AT might influence atherosclerosis in the vessel wall directly[46, 48]. The local release of adipokines from perivascular AT could have pro- or anti-inflammatory effects[46]. The quantity of perivascular AT is associated with the presence of atherosclerosis, but an independent role for perivascular AT in the development of atherosclerosis still needs to be proven, as the amount of perivascular AT is associated with the amount of visceral AT[48]. Obesity itself also led to increased cytokine expression and macrophage infiltration in perivascular AT[48]. In conclusion, these studies indicate an association between atherosclerosis, obesity, and CVD.

4.1 Natural Killer T cells as a link between atherosclerosis and obesity

As indicated by several studies, NKT cells play a role in the pathogenesis of atherosclerosis and obesity. However, only one study by Subramanian *et al.* looked at the effect achieved by the NKT cells in both pathologies at the same time. In this study Vα14Jα18 TCR transgenic mice on a LDLR knockout background were used to study the effects of NKT cells on metabolic abnormalities and atherosclerosis[49]. Vα14Jα18 TCR transgenic mice possess large NKT cell numbers to amplify the natural response of NKT cells *in vivo*[49]. The mice were fed with a HFHSC (high-fat high-sucrose diet with cholesterol) diet[49]. They gained weight, which led to a dysregulated metabolism, including changed lipoprotein profiles, higher fasting glucose, and insulin resistance[49]. More macrophages of the M1 phenotype indicated the ongoing process of AT inflammation[49]. At the atherosclerotic level, aortic

atherosclerosis and inflammation was worsened[49]. So, both metabolic and atherosclerotic parameters were deteriorated in the presence of large circulating NKT cell numbers[49]. However, when these mice were fed with a Western-type diet (milk fat and cholesterol), no increase in atherosclerotic lesions or increase in body weight was measured[49]. This indicates a strong interaction between the type of diet and the effect achieved by the NKT cells on the aggravation of atherosclerosis, probably mediated by the enhanced levels of circulating lipids[49]. The findings that NKT cells are "bad players" in metabolic regulation are not supported by most studies described before (chapter 3.2). However, here a LDLR knockout mouse model was used and the specificity of the diet, could explain the difference[49].

The study by Subramanian *et al.* was the only study performed to investigate atherosclerosis and obesity at the same time point. However, other links between the involvement of NKT cells and both pathologies can be found at different, maybe less obvious, levels as well. Chemokines play important roles in the pathogenesis of atherosclerosis and obesity. Therefore, a possible link between the involved processes might be found in chemokine expression. CXCR6 is the chemokine receptor for chemokine CXCL16 and is expressed by NKT cells[50]. The role of the CXCL16/CXR6 interaction in atherosclerosis was already known[50]. Also in patients with MetS, increased levels of CXCL16/CXCR6 were associated with atherosclerotic parameters[50]. The increased numbers of CXCR6+ NKT cells and CXCR6+ T cells were correlated to atherosclerotic parameters, which might indicate an important function for these cell types in atherogenesis in patients with MetS[50]. Thereby, chemokine CXCL16 could serve as a risk factor for and participant in atherosclerosis[50]. We could speculate that this chemokine serves as a risk factor for AT inflammation and obesity as well, concerning the origin of the MetS.

Additionally, a study by Madonna *et al.* pointed to another link between atherosclerosis and obesity at the level of NKT cells. In this study was stated that CD1d could mediate NFκB activation and diastolic dysfunction of the hearts of obese mice[51]. Therefore, the regulation of CD1d can influence NFκB activation and thereby also cardiac dysfunction[51]. CD1d expression increases on APCs in atherosclerotic lesions due to increased oxLDL levels[22], which might indicate a mechanism leading to CVD during obesity. This CD1d mediated mechanism might point to the involvement of NKT cells, because increased expression could activate more NKT cells, leading to worsened atherogenesis. However, during obesity CD1d expression on adipocytes was decreased, indicating that adipocytes and possibly also their interacting AT resident NKT cells might not be involved in this NFκB activating pathway[51]. Moreover, another lesson can be learned from CD1d mediated effects concerning CVDs. PPARγ agonists, thiazolidinediones (TZDs), used in the past to treat obese patients, induced a risk for myocardial infarction[52]. This effect is in contrast with TZD function, as TZDs improve adipocyte function and metabolic regulation and therefore were expected to lower the risk for CVDs[52]. The regulation of PPARγ by these drugs, that regulates CD1d expression on adipocytes and APCs, might explain this[8, 34]. As most studies declare that NKT cells are pro-atherogenic, activation of NKT cells by a higher level of CD1d, might explain these controversial results.

Finally, another connection between atherosclerosis and obesity might be found in MMP expression. MMPs are involved in the demolition of the collagen cap of an atherosclerotic plaque. Weiss *et al.* suggested to inhibit AT-derived MMP-9, as MMP-9 from AT is correlated with CVD[39]. Others indicated the advantages of MMP-9 inhibition in atherosclerosis as well[39]. NKT cells could contribute to the pool of MMPs[53], eventually having an effect on CVDs by destabilizing the fibrous cap of an atherosclerotic lesion. Manipulation of NKT cell MMP expression may contribute to improved stabilized plaques and eventually less CVDs.

4.2 Natural Killer T cells in atherosclerosis and obesity: Interfering factors

Because of the participation of NKT cells in atherosclerosis and obesity, and correlations between these pathologies, it would be instructive to indicate mechanisms recruited in both pathologies. Subramanian *et al.* was the first group that looked at both processes at the same time, which might suggest a similar mechanism occurring in both pathologies. In terms of activation, in both pathologies receptors on APCs and on NKT cells are likely activated by lipid antigens. The lipid antigens discovered in relation to atherosclerosis could be usable as well to activate similar receptors related to obesity, and vice versa, but tissue specificity needs to be addressed. Also, the effects obtained in atherosclerotic studies are not compatible with all obesity-related results. For example, how is it possible that NKT cells induce M2 polarization in obesity, but more M1-type macrophages are obtained in the atherosclerotic lesions? The degree of inflammation is possibly a big interfering factor, as inflammatory processes in atherosclerosis might be more intensive than the low-grade chronic inflammation in AT inflammation during obesity. The cytokine milieu might be too intensive to be overruled by NKT cell cytokine expression. Additionally, in obesity T regs might contribute to an anti-inflammatory environment.

Furthermore, the NKT cell pool is roughly divided into two populations: iNKT and vNKT cells. Most studies are performed regarding these iNKT cells, but it would be interesting to study the vNKT subset as well for their specific functions[11]. For example, the balance between iNKT/vNKT cells might result in a different lipid and lipoprotein metabolism, which could affect the atherosclerotic process as well[11]. Unfortunately, no specific knockout models are available to study the vNKT cell population separately[11]. However, there are more subtypes described besides these two based on their cell surface marker expression (figure 1). iNKT cells possess a heterogeneous phenotype with differences in expression of other surface markers such as CD4 and NK markers next to their specific TCR[7]. These markers might influence the NKT cell effector function[7], so it is highly likely that these subtypes have different functions. The exact subpopulations might differ between the studies discussed here. This could partially explain why the results of the individual studies differ and therefore, remained inconclusive. As an example, the type of NKT cell chosen for adoptive therapy might be the wrong one to perceive beneficial effects. It is of interest to identify and test all subtypes, starting with the general iNKT and vNKT subtypes, and find those populations that are both atheroprotective and beneficial in obesity[8]. However, it might be hard to find these populations as all those markers make it hard to isolate the specific subtypes and to determine any subtypes at all. That is also why the role of vNKT cells is not really addressed yet, due to insufficient models. New studies need to be performed in a controlled matter, whereby all experimental factors are similar and also the populations discussed per study are similar to determine whether the total NKT cell effect is beneficial or pathogenic. Differentiation of the iNKT population into the vNKT population might be beneficial for both diseases, but we need more studies to confirm the role of the different subpopulations in obesity and atherosclerosis[10]. It might be possible to stimulate the differentiation of those beneficial populations in the thymus, the organ where the NKT cells get their phenotypic characteristics.

In addition, the microenvironment such as the cytokine milieu in the specific organ/tissue where the NKT cells reside, influences locally NKT cell behavior[7]. This contributes to the difficulties experienced with predicting the function of NKT cells in an organ[7]. For example, the effects of NKT cells in the liver and AT during obesity were shown to differ from each other[36]. The NKT cells are more biased towards a Th2 cytokine profile in AT[36]. This might be caused by differences in microenvironment between the tissues, the presence of tissue-specific APCs, and the presence

of tissue-specific subpopulations in the different organs, such as the liver and AT[36]. This might result in tissue-specific effects achieved by the NKT cells and we need to acknowledge these differences when we address the NKT cell effects in various tissues[36].

Time span might also be very important for NKT cell function, as in later stages of constant NKT cell stimulation, NKT cells can go into anergy; the unresponsiveness of NKT cells towards antigens. The activity of the NKT cells at various stages during the development of atherosclerosis and obesity needs to be taken into account for future studies[49]. Not only NKT cells can be influenced by time span, the polarization of macrophages in AT (and in the vessel wall) might be influenced as well[49]. Therefore, a time-dependent evolution of macrophage subtypes might occur, since these cells may change their expression patterns during HFD-feeding[49]. Furthermore, the effect achieved by various other immune cells with a role in atherosclerosis and obesity might be dependent on time span. As a consequence, the crosstalk between the involved immune cells may differ in time, which influences disease outcome. Peralbo *et al.* underlined the importance of time span for NKT cell function even further, as their study showed that the composition of the NKT cell subpopulations changed during age[54].

Because of the discrepancies in results obtained for both atherosclerosis and obesity studies, the differences in experimental setting need to be addressed including genetic background, time frame, used NKT cell subtypes, diet duration, protocol for NKT cell activation, endogenous microbiota in animal facilities, male/female subjects, and the use of littermates[9, 16, 30]. Due to these interfering factors, the role of NKT cells remained inconclusive. Moreover, the type and the composition of the diet might be the biggest interfering factor. Distinct HFDs content different lipid antigens, which might lead to divergent NKT

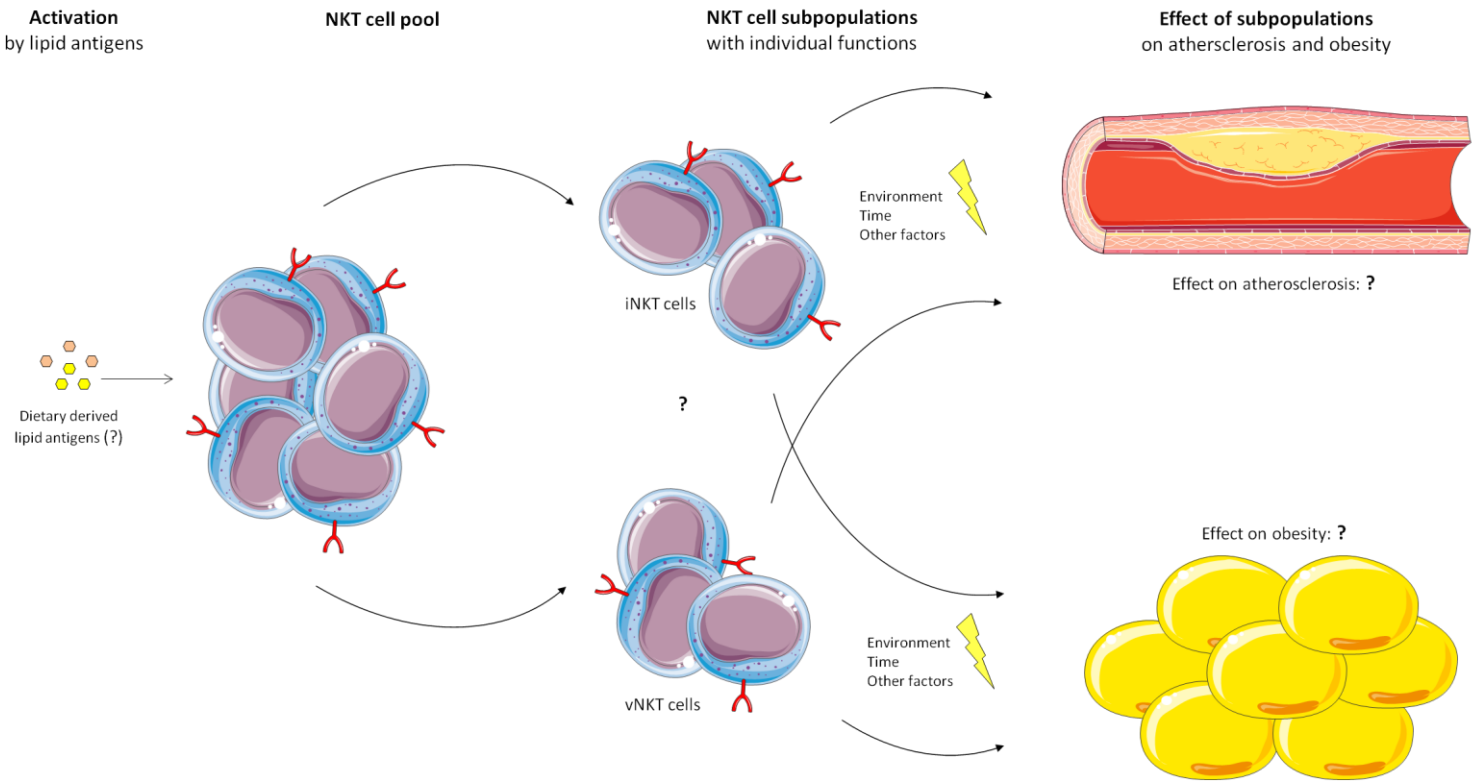

Figure 5: Natural Killer T cells in atherosclerosis and obesity. It is highly likely that the NKT cell pool gets activated as a consequence of dietary-derived lipid antigens, as different diets seem to have impact on the effect accomplished by NKT cells. The NKT cell pool is divided in subpopulations. There are more subpopulations than the depicted iNKT and vNKT cells, but further distinction is still under investigation. Each subtype of NKT cells could have a different effector function, which has a different consequence for atherosclerosis or obesity-related processes. Not only the subtype determines the outcome, also the local environment (e.g. the presence of tissue-specific APCs and the local cytokines) influences this. Furthermore, NKT cells might change their responses during time, resulting in different functions of the NKT cells and different interactions with other immune cells. Other factors, even still unknown factors, could influence NKT cell function leading to different overall effects on the pathologies of atherosclerosis and obesity.

iNKT cell, invariant Natural Killer T cell; vNKT cell, variant Natural Killer T cell

cell responses and a final beneficial or pathogenic effect. To exclude such interfering factors and the differences in diets, the composition and duration of the diet need to be standardized and the experiments need to be performed in the same laboratory[49]. It might be possible that the type or origin of the dietary factors and self antigens, might determine the outcome whether the effect of NKT cells is beneficial or harmful[35]. Unfortunately, the self antigens and their origin/composition, that can be processed by APCs or adipocytes and presented to NKT cells to activate these immune cells via either direct or indirect pathways, are not known yet[10, 49]. A dietary origin of these antigens is highly likely, because of the distinct diets with different lipid and FA content that influenced the study outcome[10, 49]. It would be helpful to identify these antigens to intervene with them as a basis for therapeutics. Figure 5 highlights the points discussed to determine the NKT cell effect in atherosclerosis and obesity.

4.3 Natural Killer T cells: A therapeutic perspective

In general, the risk of getting atherosclerotic lesions and obesity-related diseases could be lowered by changing the life style including increasing exercise, consuming a healthy diet, and quit smoking. The findings related to NKT cell function may provide new therapeutic strategies regarding treatment of atherosclerosis and obesity. Unfortunately, no consistent evidence was found in relation to the pathogenic or beneficial role of NKT cells in both pathologies. Therefore, we do not know for sure at this point whether it would be beneficial to have more or less NKT cells and active or less active NKT cells. However, a significant role for this cell type can be attributed and therefore, defining the therapeutic application is of great importance. Individual associations between the pathologies can be made as well. Therefore, it might be expected that both diseases react similar to an excess or shortage of NKT cells. This cannot be excluded, however it seems that NKT cells are beneficial for obesity in an acute setting, but aggravate the atherosclerotic process. This could indicate that we need local therapy with different effects to reach beneficial effects in both pathologies. The lapse of time and local environment need to be taken into account, because this determines when the NKT cells need to be activated or inhibited.

New therapies need to be designed to prevent atherosclerotic progression and induction of obesity-related diseases by controlling NKT cell activity in the vessel wall or AT[9]. NKT cells might be targeted using TCR antagonists, lipid antigens, blocking or activating CD1d antibodies, or NKT cell-depleting antibodies[30]. The other receptors involved in NKT cell activation such as TLRs and SRs may contribute to new therapeutic perspectives as well to improve the effectiveness of these therapies. These receptors might even be systemically modified, if the modification is useful for inhibition of both atherosclerosis and obesity-related diseases. Also DCs as presenters for NKT cells could be treated with glycolipids in the presence of TLR antagonists to perceive a tolerant NKT cell for that specific antigen[8]. Furthermore, several studies indicated the use of NKT activating glycolipids in a clinical setting to treat obesity-related diseases, type II diabetes and MetS[16, 36]. The use of α-GalCer, a glycolipid which is safe and well-tolerated in humans, is already employed in cancer treatment[16, 36]. However, as indicated by the studies that addressed the role of NKT cells in atherosclerosis and obesity, systemic activation of NKT cells likely provides side effects as the effects for both pathologies might differ. Therefore, finding a target with similar effects in both pathologies is of interest. So, whether treatments that increase or activate NKT cells systemically are beneficial need to be determined. In addition, designing a NKT cell activation therapy could be difficult in terms of anergy, which will be induced after a longer period of stimulation. This time frame when anergy sets in needs to be investigated. Probably, a local therapy suits the requirements better, but this is also more

difficult to achieve. Designing target-specific inhibitors or activators to target the vessel wall or AT specifically remains difficult, because we need to identify targets that are specifically expressed by these tissues. Subsequently, drugs that bind to these targets can be manufactured and as a last step, (new) strategies, preferably non-invasive techniques, are needed to deliver these drugs locally. Therefore, many things can still be learned regarding these research areas.

5. Conclusion

Chronic inflammation is the key process occurring in atherosclerosis and obesity, which are both pathologies related to the development of CVDs. NKT cells are active participants in this process. However, conflicting results make it hard to address whether the effects achieved by NKT cells are beneficial or pathologic in these pathologies. The effects concerning atherosclerosis are tending towards a pro-atherogenic phenotype, while they likely protect against metabolic dysregulation in an acute setting of obesity. More studies are needed concerning, among other things, the different NKT cell subpopulations, the local environment, and time span, to address the NKT cell effect in different tissues. Modulation of the NKT cell effect can be valuable for the treatment of atherosclerosis and obesity-related diseases, thereby lowering the risk of CVDs.

6. Acknowledgements

I thank dr. E. Kalkhoven for his assistance, his guidance, and for the suggestions he made during the writing of my Master thesis. I thank dr. S.C.A. de Jager for her help and her performance as my second reviewer.

7. References

1. World Health Organization. Updated March 2013. Consulted on: 07/01/2014 http://www.who.int/mediacentre/factsheets/fs317/en/index.html.

2. American Heart Association. Updated December 2013. Consulted on: 07/01/2014 http://www.heart.org/HEARTORG/Conditions/Cholesterol/WhyCholesterolMatters/Atherosclerosis_UCM_305564_Article.jsp.

3. World Health Organization. Updated March 2013. Consulted on: 07/01/2014 http://www.who.int/mediacentre/factsheets/fs311/en/index.html.

4. Tupin, E., Kinjo, Y. & Kronenberg, M. The unique role of natural killer T cells in the response to microorganisms. *Nat. Rev. Microbiol.* **5**, 405-417 (2007).

5. Godfrey, D. I., Stankovic, S. & Baxter, A. G. Raising the NKT cell family. *Nat. Immunol.* **11**, 197-206 (2010).

6. Seino, K. & Taniguchi, M. Functionally distinct NKT cell subsets and subtypes. *J. Exp. Med.* **202**, 1623-1626 (2005).

7. Matsuda, J. L., Mallevaey, T., Scott-Browne, J. & Gapin, L. CD1d-restricted iNKT cells, the 'Swiss-Army knife' of the immune system. *Curr. Opin. Immunol.* **20**, 358-368 (2008).

8. Bondarenko, S., Catapano, A. L. & Norata, G. D. The CD1d-Natural Killer T Cell Axis in Atherosclerosis. *J. Innate Immun.* (2013).

9. Rakhshandehroo, M., Kalkhoven, E. & Boes, M. Invariant natural killer T cells in adipose tissue: novel regulators of immune-mediated metabolic disease. *Cell Mol. Life Sci.* (2013).

10. Ji, Y. *et al*. Short term high fat diet challenge promotes alternative macrophage polarization in adipose tissue via natural killer T cells and interleukin-4. *J. Biol. Chem.* **287**, 24378-24386 (2012).

11. Getz, G. S., Vanderlaan, P. A. & Reardon, C. A. Natural killer T cells in lipoprotein metabolism and atherosclerosis. *Thromb. Haemost.* **106**, 814-819 (2011).

12. Brennan, P. J., Brigl, M. & Brenner, M. B. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nat. Rev. Immunol.* **13**, 101-117 (2013).

13. Hickey, M. J. & Kubes, P. Intravascular immunity: the host-pathogen encounter in blood vessels. *Nat. Rev. Immunol.* **9**, 364-375 (2009).

14. Tarazona, R. *et al*. Human NKT cells in health and disease. **22**, 359-370 (2003).

15. Van Kaer, L. NKT cells: T lymphocytes with innate effector functions. *Curr. Opin. Immunol.* **19**, 354-364 (2007).

16. Lynch, L. *et al*. Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* **37**, 574-587 (2012).

17. Chan, W. L. *et al*. Atherosclerotic abdominal aortic aneurysm and the interaction between autologous human plaque-derived vascular smooth muscle cells, type 1 NKT, and helper T cells. *Circ. Res.* **96**, 675-683 (2005).

18. Papakosta, D., Manika, K., Kyriazis, G., Kontakiotis, T. & Zarogoulidis, K. NK cell populations in collagen vascular disease. *Clin. Exp. Rheumatol.* **30**, 693-699 (2012).

19. Tupin, E. *et al*. CD1d-dependent activation of NKT cells aggravates atherosclerosis. *J. Exp. Med.* **199**, 417-422 (2004).

20. Libby, P., Ridker, P. M. & Hansson, G. K. Progress and challenges in translating the biology of atherosclerosis. *Nature* **473**, 317-325 (2011).

21. Braun, N. A., Covarrubias, R. & Major, A. S. Natural killer T cells and atherosclerosis: form and function meet pathogenesis. *J. Innate Immun.* **2**, 316-324 (2010).

22. Nakai, Y. *et al*. Natural killer T cells accelerate atherogenesis in mice. *Blood* **104**, 2051-2059 (2004).

23. W van Lammeren, G. *et al*. Atherosclerotic plaque biomarkers: beyond the horizon of the vulnerable plaque. *Curr. Cardiol. Rev.* **7**, 22-27 (2011).

24. Cole, J. E., Kassiteridi, C. & Monaco, C. Toll-like receptors in atherosclerosis: a 'Pandora's box' of advances and controversies. *Trends Pharmacol. Sci.* **34**, 629-636 (2013).

25. Andoh, Y. *et al*. Natural killer T cells are required for lipopolysaccharide-mediated enhancement of atherosclerosis in apolipoprotein E-deficient mice. *Immunobiology* **218**, 561-569 (2013).

26. Hansson, G. K., Robertson, A. K. & Soderberg-Naucler, C. Inflammation and atherosclerosis. *Annu. Rev. Pathol.* **1**, 297-329 (2006).

27. Strom, A. *et al*. Involvement of the CD1d-natural killer T cell pathway in neointima formation after vascular injury. *Circ. Res.* **101**, e83-9 (2007).

28. Andoh, Y. *et al*. Lower prevalence of circulating natural killer T cells in patients with angina: a potential novel marker for coronary artery disease. *Coron. Artery Dis.* **17**, 523-528 (2006).

29. Trayhurn, P. Adipose tissue in obesity--an inflammatory issue. *Endocrinology* **146**, 1003-1005 (2005).

30. Wu, L. & Van Kaer, L. Contribution of lipid-reactive natural killer T cells to obesity-associated

inflammation and insulin resistance. *Adipocyte* **2**, 12-16 (2013).

31. Strodthoff, D. *et al*. Lack of invariant natural killer T cells affects lipid metabolism in adipose tissue of diet-induced obese mice. *Arterioscler. Thromb. Vasc. Biol.* **33**, 1189-1196 (2013).

32. Saely, C. H., Geiger, K. & Drexel, H. Brown versus white adipose tissue: a mini-review. *Gerontology* **58**, 15-23 (2012).

33. Tateya, S., Kim, F. & Tamori, Y. Recent advances in obesity-induced inflammation and insulin resistance. *Front. Endocrinol. (Lausanne)* **4**, 93 (2013).

34. Huh, J. Y. *et al*. A novel function of adipocytes in lipid antigen presentation to iNKT cells. *Mol. Cell. Biol.* **33**, 328-339 (2013).

35. Schipper, H. S. *et al*. Natural killer T cells in adipose tissue prevent insulin resistance. *J. Clin. Invest.* **122**, 3343-3354 (2012).

36. Ji, Y. *et al*. Activation of natural killer T cells promotes M2 Macrophage polarization in adipose tissue and improves systemic glucose tolerance via interleukin-4 (IL-4)/STAT6 protein signaling axis in obesity. *J. Biol. Chem.* **287**, 13561-13571 (2012).

37. Venken, K. *et al*. A bidirectional crosstalk between iNKT cells and adipocytes mediated by leptin modulates susceptibility for T cell mediated hepatitis. *J. Hepatol.* (2013).

38. Sica, A. & Mantovani, A. Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* **122**, 787-795 (2012).

39. Weiss, T. W., Seljeflot, I., Hjerkinn, E. M. & Arnesen, H. Adipose tissue pro-inflammatory gene expression is associated with cardiovascular disease. *Int. J. Clin. Pract.* **65**, 939-944 (2011).

40. National Heart, Lung, and Blood Institute. Consulted on: 07/01/2014 http://www.nhlbi.nih.gov/health/health-topics/topics/ms/.

41. Viscogliosi, G., Andreozzi, P. & Marigliano, V. Associations of plasma glucose levels and traits of metabolic syndrome with carotid intima media thickness in nondiabetic elderly subjects: are they mediated by insulin resistance? *Metab. Syndr. Relat. Disord.* **11**, 41-45 (2013).

42. Ryu, J., Yong, H. S., Huh, S., Kang, E. Y. & Woo, O. H. Relation of coronary atherosclerosis and metabolic syndrome in asymptomatic subjects: evaluation with coronary CT angiography. *Int. J. Cardiovasc. Imaging* (2013).

43. Pradhan, A. D. Sex Differences in the Metabolic Syndrome: Implications for Cardiovascular Health in Women. *Clin. Chem.* (2013).

44. Graner, M. *et al*. Cardiac steatosis associates with visceral obesity in nondiabetic obese men. *J. Clin. Endocrinol. Metab.* **98**, 1189-1197 (2013).

45. Salvetti, G. *et al*. Prevalence of left ventricular hypertrophy and determinants of left ventricular mass in obese women. *High. Blood Press Cardiovasc. Prev.* **19**, 33-39 (2012).

46. Tian, Z. *et al*. Perivascular adipose tissue-secreted angiopoietin-like protein 2 (Angptl2) accelerates neointimal hyperplasia after endovascular injury. *J. Mol. Cell. Cardiol.* **57**, 1-12 (2013).

47. Wu, G. *et al*. Mechanism and clinical evidence of lipocalin-2 and adipocyte fatty acid-binding protein linking obesity and atherosclerosis. *Diabetes Metab. Res. Rev.* (2013).

48. Verhagen, S. N. & Visseren, F. L. Perivascular adipose tissue as a cause of atherosclerosis. *Atherosclerosis* **214**, 3-10 (2011).

49. Subramanian, S. *et al*. Increased levels of invariant natural killer T lymphocytes worsen metabolic abnormalities and atherosclerosis in obese mice. *J. Lipid Res.* **54**, 2831-2841 (2013).

50. Lv, Y., Hou, X., Ti, Y. & Bu, P. Associations of CXCL16/CXCR6 with carotid atherosclerosis in patients with metabolic syndrome. *Clin. Nutr.* **32**, 849-854 (2013).

51. Madonna, R., Wu, H., Shelat, H. & Geng, Y. J. CD1d-associated expression of NF-kB and cardiac dysfunction in diabetic and obese mice. *Int. J. Immunopathol. Pharmacol.* **26**, 59-73 (2013).

52. Ahmadian, M. *et al*. PPARgamma signaling and metabolism: the good, the bad and the future. *Nat. Med.* **19**, 557-566 (2013).

53. Edsparr, K., Basse, P. H., Goldfarb, R. H. & Albertsson, P. Matrix metalloproteinases in cytotoxic lymphocytes impact on tumour infiltration and immunomodulation. *Cancer. Microenviron* **4**, 351-360 (2011).

54. Peralbo, E., Alonso, C. & Solana, R. Invariant NKT and NKT-like lymphocytes: two different T cell subsets that are differentially affected by ageing. *Exp. Gerontol.* **42**, 703-708 (2007).

Natural Killer T cellen en hun functies in atherosclerose en obesitas: een therapeutisch perspectief voor cardiovasculaire ziekten

A.M. van de Vrugt[1] & E. Kalkhoven[2]

[1] Biology of Disease master student, Universiteit Utrecht, Nederland; [2] Afdeling Metabole Ziekten, Universitair Medisch Centrum Utrecht, Utrecht, Nederland

Aderverkalking of atherosclerose en obesitas zijn ziekten die beiden gerelateerd zijn aan de ontwikkeling van hart- en vaatziekten, ook wel bekend als cardiovasculaire ziekten. Bij atherosclerose hoopt vet zich op in de wanden van de bloedvaten en bij obesitas neemt de algehele vetmassa drastisch toe. De aantallen patiënten die lijden aan atherosclerose en/of obesitas nemen in sterke mate toe. Daardoor dragen deze ziekten bij aan het groeiende probleem van hart- en vaatziekten, één van de belangrijkste doodsoorzaken wereldwijd.

In de ontwikkeling van atherosclerose en obesitas vervult het immuunsysteem een belangrijke rol. Het immuunsysteem met de daarbij behorende immuuncellen kan worden opgedeeld in twee grote takken; een aangeboren of aspecifieke tak en een verworven of adaptieve tak. Beide takken van het immuunsysteem spelen een rol in de ontwikkeling van atherosclerose en obesitas. Dit is onder andere af te leiden aan de aanwezigheid van grote aantallen immuuncellen in de vaatwand en in het vetweefsel. Er vindt een continu (chronisch) ontstekingsproces plaats, wat de staat van beide ziekten in de loop der tijd verergert. Een speciaal type immuuncellen genaamd Natural Killer T (NKT) cellen bevindt zich ook in de vaatwand en in het vetweefsel. Deze cellen dragen eveneens bij aan de progressie van atherosclerose en obesitas. NKT cellen zijn bijzondere immuuncellen, omdat ze een rol spelen in beide takken van het immuunsysteem en deze met elkaar verbindt.

NKT cellen worden geactiveerd door stofjes bestaande uit vet, ook wel lipide antigenen genoemd. Deze antigenen zijn rijkelijk aanwezig in de vaatwand en in het vetweefsel. Door deze activatie stap kunnen de NKT cellen een functie gaan uitvoeren, zoals het uitscheiden van bepaalde stoffen en het communiceren met andere immuuncellen. Verschillende processen die leiden tot NKT cel activatie of het aanpassen van de NKT cel functie zijn bekend voor zowel atherosclerose als obesitas. Hieruit blijkt dat er een duidelijke rol is weggelegd voor deze cellen in beide ziektebeelden. Echter, tegenstrijdige resultaten maken het moeilijk om te bepalen of het effect van de NKT cellen voordelig of nadelig is voor de ontwikkeling van atherosclerose en obesitas. Dat is waarom er meer studies nodig zijn die ook de rol van storende factoren in ogenschouw nemen, zoals het effect van de NKT cel subtypes, het lokale milieu, en de tijdsduur. Daarna kan de potentie van NKT cellen voor de behandeling van hart- en vaatziekten bepaald worden.