Role of nuclear receptor Nur77 during inflammation
Hamers, A.A.J.

Citation for published version (APA):
Hamers, A. A. J. (2015). Role of nuclear receptor Nur77 during inflammation

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE (Digital Academic Repository)

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
General Discussion
General Discussion

Nuclear Receptors (NRs) play key roles in cell differentiation, development and homeostasis and in many diseases such as diabetes, cancer, cardiovascular disease, reproductive abnormalities and obesity. These receptors can be located in the cell nucleus, in the presence or absence of ligand, or in the cytoplasm in the absence of ligand and relocating to the nucleus upon ligand binding. As explained in Chapter 1 NRs typically consist of a variable amino-terminal transactivation domain (AF-1), a highly conserved DNA-binding domain (DBD) and a less conserved carboxyl-terminal ligand-binding domain (LBD). In general, NRs are ligand-activated transcription factors binding directly to DNA thereby regulating expression of specific genes. NRs modulate transcription through repression and activation by binding to sequence-specific promoter elements on their target genes either as monomers, homodimers or heterodimers with the retinoid X receptor (RXR). NR-mediated transrepression involves inhibitory interactions of NRs with other transcription factors. The response of a given receptor to a certain ligand in a specific tissue will also be dictated by the set of proteins with which the receptor can interact, so-called coregulators, hence the receptor can have distinct functions depending on the cell type or tissue. Of the 48 known human NRs, for only 24 receptors an (artificial) ligand has been identified. The receptors for which no ligand has been identified yet, are called orphan receptors. The NR4A receptor subfamily, comprising of Nur77, Nurr1 and NOR-1, belong to this class of NRs (Chapter 1) making them intriguing to investigate.

In this thesis, the involvement of nuclear receptor Nur77 in several inflammatory diseases has been presented. More specifically, Nur77 is studied in atherosclerosis, sepsis, and Inflammatory Bowel Disease and the following cell types; macrophages, endothelial cells, and colon epithelium. Each chapter focuses on a different type of inflammation. This final chapter discusses the distinctive role of Nur77 in acute and chronic inflammation. A brief perspective for future developments will be given, regarding the research in this thesis, as well as in perspective of recent advances in literature.

The main findings from this thesis are:
1. Nur77−/− bone marrow-derived macrophages exhibit changed expression of M2-specific markers and an inflammatory M1-phenotype with enhanced expression of interleukin-12, Interferon-γ, and increased nitric oxide secretion.
2. Myeloid Nur77 deficiency increases atherosclerotic plaque size in Ldlr−/− mice by increasing macrophage, T-cell and smooth muscle cell influx.
3. Nur77 deficiency reduces bacterial influx into the organs via decreased vascular permeability in an E.coli-induced peritonitis mouse model.
4. In humans, Nur77 is present in colon from Crohn’s and Ulcerative colitis patients.
5. Nur77 has an anti-inflammatory role in colon epithelial cells.
6. Nur77 suppresses the inflammatory status of both macrophages and gut epithelial cells, thereby protecting from experimental colitis.
7. Nur77 agonist 6-Mercaptopurine represses inflammation in RAW macrophages and gut epithelial Caco-2 cells, partly through Rac-1 inhibition.

**Nur77 in innate and adaptive immunity**

*Does Nur77 really dampen the innate immune response?*

When thinking of innate immunity, the macrophage immediately comes to mind. This cell is mostly known for its capacity of engulfing bacteria and foreign substances. Macrophages are plastic cells that respond to a plethora of biological stimuli in their microenvironment with dedicated gene expression patterns. In response to LPS, macrophages polarize toward the pro-inflammatory M1 phenotype and IL-4 or IL-13 induce non-classically activated M2 macrophages, yet these phenotypes are the extremes of a complicated spectrum, as schematically illustrated in Figure 1A. It was shown by Tontonoz and coworkers that Nur77 is upregulated in murine RAW macrophages after an LPS stimulus and that this upregulation results in a more pro-inflammatory cellular differentiation. Inconsistently, around the same time it was shown by our research group that Nur77 has an anti-inflammatory role in THP-1 macrophages and by others that it can repress NFκB by binding directly to the p65 NFκB subunit.

In Chapter 4 of this thesis we show in RAW macrophages that Nur77 suppresses the inflammatory response of these cells. The discrepancy could be due to the fact that different sets of inflammation-related genes and regulators were investigated. Pei et al. analyzed the mRNA expression of Myristoylated alanine-rich C-kinase substrate, inducible inhibitor of NF-κB (IκB) kinase and NFκB inducing kinase. Our conclusions were based on the observed repression of NFκB activity in RAW cells overexpressing Nur77, corresponding with NFκB repression in a T-cell line. In addition, we have investigated the effect of Nur77-deficiency in primary bone marrow-derived mouse macrophages (BMM; Chapter 2 and 6) and found a pro-inflammatory M1 phenotype in response to LPS in Nur77-deficient cells and a changed expression of M2-phenotypic markers after IL-4 stimulation (Figure 1B). This data was confirmed by two other groups, showing increased TNFα secretion by Nur77-deficient macrophages. Additionally, Hanna et al. observed increased expression of IL-12 and iNOS mRNA and increased p65 phosphorylation after 2 hrs of the TLR4 agonist Kdo Lipid A (KLA) in Nur77−/− mouse peritoneal macrophages compared to WT cells. Whether Nur77 represses NFκB activation by binding directly to p65 or if it positively regulates the repressor IκB-α10 still remains to be elucidated in macrophages. Unexpectedly, when Nur77−/− mouse peritoneal macrophages were stimulated with growth arrested E.coli bacteria they showed a similar inflammatory response as WT cells (Chapter 3). This could be due to the fact that these bacteria not only influence TLR4 signaling, but also activate TLR2, -5 and -9. Since Hedrick’s group has shown an increased expression of pro-inflammatory genes in Nur77−/−.
deficient macrophages in response to a TLR2 or 7 agonist, this discrepancy could be attributed to TLr2 and/or TLr5 signaling. Therefore, it would be interesting to test the response of Nur77-deficient macrophages to different bacteria species and additional pro-inflammatory and TLR-subtype specific stimuli. Recently, we have shown a higher phagocytosis capacity of Nur77-deficient macrophages compared to WT cells regarding heat-inactivated *E. coli*, therefore phagocytosis should be investigated in more detail for multiple bacteria species.

Interestingly, Hanna et al.\textsuperscript{12} discovered that Nur77-/- mice lack the Ly6C\textsuperscript{low} patrolling monocyte subpopulation and that both the Ly6C\textsuperscript{low} and Ly6C\textsuperscript{high} subsets exhibit enhanced NFκB activation. Could this lack of the Ly6C\textsuperscript{low} subpopulation explain why Nur77-deficient macrophages are pro-inflammatory? Recent observations argue against this; Geissmann’s group\textsuperscript{13-15} revealed that Ly6C\textsuperscript{low} monocytes patrol vessels and mark damaged endothelial cells for elimination rather than that these cells differentiate into macrophages. In addition, Hilgendorf et al.\textsuperscript{7} demonstrated that in a model for myocardial infarction Ly6C\textsuperscript{high} monocytes infiltrate the infracted heart where they subsequently differentiate into reparative Ly6C\textsuperscript{low}/F4/80\textsuperscript{high} macrophages.
independent of Nur77. When these Ly6C<sub>low</sub>/F4/80<sub>high</sub> macrophages are deficient for Nur77, however, the inflammatory response is increased resulting in defective healing of the myocardium. Of note, in Chapter 6 we have strong indications that Nur77-deficiency increases collagen deposition and thus favoring reparative macrophage differentiation; meaning a negative role for Nur77 in repair. To further exclude the possibility that Nur77 affects BMM differentiation rather than the inflammatory response of these cells, we performed Nur77 knockdown experiments in fully differentiated WT BMM and demonstrated that also under these conditions IFNγ is upregulated and IL-10 expression is reduced (Figure 1C). Currently, monocyte/macrophage functions are more and more accepted to be different for various organs, making it intriguing to study the behavior of monocyte subsets in other models of injury, such as skin and vessel.

There are strong indications for Nur77 playing a role in other innate immune cells such as dendritic cells (DCs), neutrophils and eosinophils. DCs are antigen-presenting cells that are crucial in the innate immune compartment. Recently, Karthaus et al. have shown that Nur77 mRNA is present in both immature conventional and plasmacytoid DCs and that its expression is downregulated upon TLR7/8 or TLR9-induced maturation of these cells. The functional role of Nur77 in DC maturation and function remains to be elucidated. In these studies Nur77<sup>−/−</sup> mice will be instrumental. Neutrophils are the first cells to respond to foreign invaders and our preliminary data show that Nur77 is slightly downregulated in human neutrophils after LPS, Granulocyte macrophage colony-stimulating factor (GM-CSF) and mycoplasma lipopeptide (MALP) stimulation (Figure 2). The NR4A subfamily members Nurr1 and NOR-1 seem more relevant in neutrophils, since Nurr1 is downregulated and NOR-1 expression is highly increased after LPS, IL-4, GM-CSF and MALP stimulation. Nur77 is also expressed in peripheral blood eosinophils derived from patients with atopic dermatitis. So far, Nur77 has been shown to regulate apoptosis and thus

![Figure 2. NR4A expression in activated human neutrophils.](image-url)

Neutrophils were isolated from blood by buffy coat, seeded and stimulated for 6 hrs with LPS, IL-4, GM-CSF, or MALP. RNA was isolated, cDNA was made and qRT-PCR was performed for Nur77, Nurr1, and NOR-1. Input was corrected for 36B4 (housekeeping gene). C = control, AU = arbitrary units, LPS = lipopolysaccharide, IL = interleukin, GM-CSF = granulocyte macrophage colony-stimulating factor, MALP = mycoplasma lipopeptide, n=1. cDNA was obtained from Dr Timo van den Berg.
many questions remain for eosinophils such as; “Does Nur77 repress inflammation in these cells or modulate migration?” Since eosinophils are crucial in development of allergic diseases such as asthma and atopic dermatitis and are involved in eradicating nematodes/worms these diseases may be worth investigating in Nur77−/− mice. In short, it is too simplified to say that Nur77 dampens the innate immune response, since there are many different cell types with unique functions involved in this intricate defense system, and these cells are active at specific stages of infection and/or respond different to each foreign invader. Based on our results and recent literature we may however, definitely conclude that Nur77 has an anti-inflammatory role in the TLR4-activated macrophage and is essential for Ly6C<sup>low</sup> monocyte development.

**Nur77 represses the adaptive immune response**

Nur77 in adaptive immunity was first described in 1994 by Woronicz et al. showing that Nur77 expression is correlated with T-cell receptor (TCR)-mediated apoptosis in thymocytes, which is necessary to eliminate autoreactive T-cells; the so called negative selection, to safeguard tolerance to self-antigens and to protect against autoimmune attack. Winoto demonstrated in mice that overexpression of a dominant-negative variant of Nur77 in developing thymocytes results in a defect in negative selection. The mere fact that Nur77−/− mice do not show a T-cell phenotype, suggests redundancy within the Nr4A family for this function. The relative contribution of all family members remains to be defined. The underlying mechanism of Nur77-mediated apoptosis involves translocation of Nur77 from the nucleus to mitochondria and its association with Bcl2, an anti-apoptotic molecule, which is subsequently converted into a killer molecule. Other mechanisms described in literature concern Nur77 being involved in modulation of the Fas/Fas ligand pathway and deviation of self-reactive TCR to an alternative lineage, such as forkhead box P3 (Foxp3)<sup>+</sup> regulatory T-cells (Treg), by which self-reactivity is withdrawn. Fassett et al. reported Nur77 to both regulate negative selection and the Treg pool in the thymus. Nur77 was shown to also induce apoptosis in peripheral T-cells, and increase Treg numbers. Recently, ectopic expression of Nur77 was found to induce Foxp3 expression and suppress effector cytokine expression in T-cell receptor stimulated CD4<sup>+</sup> T-cells, providing evidence that Nur77 is crucial in regulating the Th1/Treg balance.

Making use of a reporter mouse in which green fluorescent protein (GFP) expression is placed under the control of the Nur77 regulatory region, robust induction was observed after B-cell receptor (BCR) activation, revealing that Nur77 is involved in splenic B-cell development. This data suggest functional involvement of Nur77 in B-cell development and thus leaving much questions for further investigations and one may even speculate that Nur77 is necessary in regulatory B-cell function.

**Does Nur77 attenuate acute inflammation or merely aggravate the damaging consequences?**

Given the anti-inflammatory function of Nur77 in macrophages after LPS stimulation, it is very tempting to hypothesize that this receptor has a protective role to deleterious
processes caused by acute inflammation. One should however, realize that multiple cells are involved in a dramatic disease such as sepsis. In Chapter 3, we sought to investigate the response of the Nur77−/− mice to E. coli-induced peritonitis. We found that Nur77-deficiency had no effect on innate defence to E. coli peritonitis and sepsis. However, Uhrin et al., using different sepsis models, have shown increased cytokine release in Nur77−/− mice after a high dose LPS challenge and increased mortality. Also in a cecal ligation and puncture (CLP) model accelerated morbidity was observed Nur77-deficient mice. The CLP model is a polymicrobial sepsis model where normal bacteria from the gut flora leak into the abdomen and bloodstream. These normal gut bacteria are eradicated quickly by the innate defense system through phagocytosis, complement killing and the inflammatory reaction. Also, regarding the discrepancies between the data from our study and the CLP model, studies on the response of WT and Nur77−/− mice to different bacterial infections (especially the ones colonizing the colon) such as Enterococcus faecalis, Lactobacillus or Bacteroides strains may resolve this issue. The E. coli strain we have utilized (O18:K1) is resistant to the isolated processes and can only be killed by activated macrophages in the presence of complement, it is therefore extremely hard to eradicate by the immune system. This may at least partially explain why we did not see a difference in immune response between WT and Nur77−/− mice. Non-immune cells, such as endothelial cells, also play a vital role in inflammation. Nur77 expression has been shown to increase in endothelial cells upon several stimuli and its expression in these cells plays a role in atherosclerosis and angiogenesis. In the sepsis model described in Chapter 3, we observed lower bacterial influx into the distant organs and attributed this to diminished permeability of the endothelial cell layer in Nur77−/− mice. When overexpressing Nur77 in HUVEC the expression of junction proteins is reduced and permeability of the monolayer increases, which is in line with previous observations. Goddard et al. described that vascular permeability preceding implantation in the uterus is controlled by the progesterone receptor involving Nur77 activation.

Could this mechanism also be involved in our sepsis model? Since the decreased permeability after Nur77 overexpression was already observed in unstimulated HUVECs, this may be unlikely. Finally, in Chapter 6 we demonstrate that Nur77−/− BMM have a higher E. coli phagocytosis capacity, which may also contribute to the lower number of bacteria that we observed in the lungs and liver of Nur77−/− mice. I propose that the discrepancies are merely attributed to the different bacterial species/stimuli involved, since the role of Nur77 in innate immune cells depends on the stimulus. Since the above mentioned studies are the only sepsis models tested in Nur77−/− mice, I am indeed curious to see how these mice respond to bacterial pneumonia or even a Rhodococcus aurantiacus granulomatous infection or fungal infection.

In Chapter 4 experimental colitis was studied in two different acute models: dextran sodium sulphate (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) and the overall observation was that Nur77−/− mice develop more severe disease compared to WT mice. DSS colitis in Nur77−/− mice resulted in increased neutrophil
numbers in colon compared to WT and is attributed to increased levels of KC from colonic epithelial cells. This acute colitis can be extended into a chronic form when prolonging the animal experiment. The increased neutrophil influx that we observed may increase mucosal healing, which definitely makes it worth to perform a chronic DSS model and determine what the outcome is. Colons of TNBS-treated Nur77−/− mice show an increased macrophage influx and more T-cells in the mucosa. We also found diminished Foxp3 expression, corresponding to Nur77 regulating the Th1/Treg balance.22, 24 The different pathology in these two mouse models is likely due to the fact that TNBS colitis as we have utilized it, is a delayed-type hypersensitivity reaction to haptenized proteins with T-lymphocyte mediated mucosal damage32, whereas DSS-induced colitis involves disruption of the epithelial barrier function and direct mucosal damage.33 Again, depending on the type of disease model Nur77 may have a different role in acute inflammation.

**Nur77 reduces chronic inflammation**

In Chapter 2 we have investigated the role Nur77 in atherosclerosis, which may be considered as a chronic inflammatory process in the vessel wall. Ldlr−/− mice were transplanted with Nur77-deficient bone marrow and after a high-fat diet for 14 weeks this resulted in larger atherosclerotic lesions with more macrophages, T-cells, smooth muscle cells and larger necrotic cores compared to mice transplanted with WT bone marrow. The increased cellular influx into the atherosclerotic lesions was in part attributed to enhanced SDF-1α secretion by macrophages, since all these cell types migrate and/or proliferate in response to this chemokine.6 Most interestingly, Nur77 binds to the SDF-1a promoter thereby repressing the expression of this gene, which involves at least partly the interaction of Nur77 with an NBRE at −160 bp (Chapter 6). The research group of Hedrick reported a similar increase in atherosclerotic lesion size after feeding a western type diet for 20 weeks to Nur77−/− chimeric Ldlr−/− mice.9 In addition, they demonstrated enlarged vascular lesions in ApoE/Nur77 double knockout mice. Most recently, a study was published by Hu et al.34, demonstrating diminished atherosclerosis in ApoE−/− mice after lentiviral overexpression of Nur77 and an increased lesion size after silencing Nur77. Corresponding with our study6 and data from Hanna et al.9, they observed reduced macrophage content in the lesions of mice overexpressing Nur77. Nur77 has consistently shown to reduce macrophage foam cell formation and to downregulate inflammatory gene expression.3, 9, 34, 35 So far, only one study is not in consensus showing no difference in atherosclerotic lesion size in response to targeting Nur77 in mice,36 which could be due to different technical and methodological approaches. Multiple other studies have shown a protective function for Nur77 in atherosclerosis37, restenosis38, 39, and even vascular outward remodeling.40 In addition to atherosclerosis, our gene set enrichment analysis (GSEA) of the transcriptome of WT and Nur77−/− BMM (Chapter 6) revealed the association of Nur77 with several diabetes-related diseases, namely glucose intolerance, diabetic nephropathies, angiopathies, and retinopathy. Nur77 has indeed been shown to
diminish insulin resistance\textsuperscript{41}, involved in insulin signaling in white adipose tissue\textsuperscript{42} in mice. Regarding the recent publication stating that lipid storage by adipose tissue macrophages regulates systemic glucose tolerance\textsuperscript{43} and Nur77\textsuperscript{-/-} mice showing increased fasting serum glucose levels after high fat diet\textsuperscript{44}, it would be interesting to investigate glucose tolerance after transplantation of Nur77-deficient bone marrow to Ob/Ob mice. We detected reduced Nur77 mRNA expression in the stromal vascular fraction of white adipose tissue from Ob/Ob mice compared to WT (Figure 3), which may indicate a role for macrophage Nur77 in obesity. However, this may also be related to the function of Nur77 in endothelial cells, therefore we should assess Nur77 gene expression in sorted macrophages from the white adipose tissue in normal and Ob/Ob mice.

Westbrook et al.\textsuperscript{45} have shown that Nur77-deficient rats on a kidney injury susceptible genetic background exhibited decreased renal function and attenuated kidney injury and that this is completely rescued by bone marrow transplanted from control animals; suggesting strong immune cell mediation. Transplanting LysMCre/Cre-Nur77fl/fl bone marrow in this study may be even more interesting, because this will reveal the role of macrophages in kidney injury more specifically.\textsuperscript{45-50} For now, during chronic inflammation Nur77 seems to have a dampening effect.

**Is there a role for Nur77 in autoimmune disease?**

In this Chapter I placed atherosclerosis under the heading “chronic inflammation”, even though there is a certain level of similarity between atherosclerosis and autoimmune diseases. Atherosclerosis has been associated with both Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) and cardiovascular disease.
has been implicated as a major contributor to the adverse prognosis of these patients, and vice versa.49, 51-54 For example, the inflammatory burden in RA increases the oxidation process of LDL, promoting the initiation of atherosclerosis.55 Interestingly, around 10% of all T-cells in human atherosclerotic lesions specifically recognize LDL or oxLDL, pointing even more towards an auto-immune classification.56 In addition, IgG autoantibodies against apolipoprotein A-1 (constituent of high density lipoprotein) have been found to be associated with a worsened cardiovascular prognosis.57, 58 In Chapter 6, the detailed analyses of the BMM transcriptome (applying GSEA and IPA) uncovered a potential involvement of macrophage Nur77 in osteoarthritis (OA), SLE signaling, psoriasis and several signaling pathways related to RA. Nur77 expression is elevated in synovial tissue, cartilage and prostaglandinE2 (PGE2) stimulated chondrocytes from patients with RA, psoriatic arthritis or osteoarthritis, making Nur77 an attractive protein to study in macrophages in rheumatic diseases.59-62 The microarray analysis described in Chapter 6 showed that S100A9 is dramatically upregulated in Nur77-Ko BMM; 7 fold. Increased protein levels of S100A9 in synovial fluid have been strongly correlated to RA and OA severity.63 So far, only the effect of T-cell specific Nur77 overexpression was studied in the mouse collagen-induced arthritis model, showing a reduction in incidence and severity of arthritis by promoting activation-induced T-cell apoptosis and inhibition of Collagen II-specific antibody production.63, 64 Interestingly, closely related Nurr1 has been implicated in both psoriasis and RA59, 60, 62, 65 making it rather intriguing to also study the role of Nur77 in these auto-immune diseases.

**Nur77 as future target for intervention in inflammatory diseases**

Many Nuclear Receptors are active modulators of inflammatory processes and at the same time they regulate crucial processes in metabolism. Well-known examples are the glucocorticoid receptor (GR), liver X receptors (LXRs) and Peroxisome proliferation activated receptors (PPARs). For each of these Nuclear Receptors synthetic ligands have been developed, with often unique properties to activate only specific activities of the receptors. Especially orphan nuclear receptors such as Nur77 are of interest for the discovery of novel agonists to design innovative treatments for human diseases. Since Nur77, Nurr1 and NOR-1 have no ligand-binding pocket, it is unlikely that traditional ligands/agonists will be discovered. The first NR4A agonist identified was 6-mercaptopurine (6-MP), the active metabolite of the immunosuppressive drug Azathioprine. 6-MP enhances the transcriptional activity of all three NR4A receptors.66, 67 This activation is mediated through the N-terminal AF-1 domain, most likely involving recruitment of co-activators such as TRAP220, but without direct binding of the NR4A proteins.68 6-MP inhibits smooth muscle cell proliferation crucially involving Nur77 however, 6-MP does not show specificity for Nur77 in other cell types/functions70, 71. We for example demonstrated that 6-MP enhances macrophage apoptosis and induces an anti-inflammatory macrophage phenotype, independent of Nur77.72 To further substantiate those data, we stimulated WT and Nur77/−/ BMM with LPS and showed that the anti-
inflammatory effect of 6-MP does not require the presence of Nur77 in these cells (Figure 4). In **Chapter 5** we demonstrate that the underlying mechanism of 6-MP function in RAW macrophages and Caco-2 gut epithelium cells involves inhibition of the small GTPase Rac1. A number of compounds have been identified, which enhance Nur77-mediated apoptosis in different cancer cells, such as 9-cis-retinoic acid, 1-di(3-indolyl)-1-(4-X-phenyl)methanes, etoposide and 5,8-diacetoxyl-6-(1′-acetoxyl-4′-methyl-3′-pentenyl)-1,4-naphthaquinones. At present, it is unknown whether these compounds also enhance the activity of Nur77 in monocytes/macrophages, smooth muscle cells, endothelial cells or T-cells.

Cytosporone B (Csn-B) was identified as a naturally occurring agonist of Nur77 by the group of Wu, who demonstrated that it induces apoptosis through translocation of Nur77 to the mitochondria. Furthermore they have shown that Csn-B elevates blood glucose levels in fasting mice and that this effect was absent in the Nur77−/− mice. Furthermore, it has been shown that Csn-B represses intestinal cancer through Wnt signalling as well as bladder cancer growth via Nur77. Csn-B also decreases atherosclerosis in mice, although a direct link with Nur77 has not been proven in vivo. Interestingly, Hu et al did show siNur77 to inhibit Csn-B mediated effects in THP-1 macrophage-derived foam cells, HepG2 and Caco-2 cells.

None of the compounds discussed above are highly specific for Nur77, which indicates the necessity to identify compounds that target Nur77 with high specificity and sensitivity. Given that Nur77 has many functions in multiple organs and cell types, it is extremely relevant to target potential Nur77 agonists to the site of inflammation/disease avoiding side-effects. This may for example be achieved with a drug-eluting stent in the case of atherosclerosis or via encapsulation into nanoparticles for the treatment of inflammatory diseases. For instance, liposomes are delivered to sites of inflammation based on endothelium leakiness and can directly fuse with macrophages. These carriers have been tested in clinical trials and some formulations are already on the market (DOXIL®). Another carrier is rHDL, which inherently interacts with plaque macrophages. It is possible to label these particles with for example antibodies or proteins to target a specific cell type at the site of inflammation, such as phosphatidylserine to target macrophages. If it will be proven to difficult to find a ligand for Nur77, maybe even siRNA or genes could be delivered like this to respectively knockdown or overexpress Nur77 locally. Even more attractive will be to encapsulate microRNAs into nanocarriers to locally control/treat epigenetic changes of Nur77 and other genes.

Altogether, an extensive amount of research has been performed to gain crucial novel insight on the role of Nur77 in inflammation, yet multiple questions were raised in this thesis and important issues remain to be resolved.

**References**

(1) Pei L, Castrillo A, Chen M, Hoffmann A, Tontonoz P. Induction of NR4A orphan nuclear


(22) Fassett MS, Jiang W, D’Alise AM, Mathis D, Benoist C. Nuclear receptor Nr4a1 modulates both regulatory T-cell (Treg) differentiation and clonal deletion. Proc Natl Acad Sci U S A 2012 March 6;109(10):3891-6.


(35) Shao Q, Shen LH, Hu LH, Pu J, Qi MY, Li WQ, Tian FJ, Jing Q, He B. Nuclear receptor Nur77 suppresses inflammatory response dependent on COX-2 in macrophages induced by oxLDL. J Mol Cell Cardiol 2010 August;49(2):304-11.


Zhang XL, Guo YF, Song ZX, Zhou M. Vitamin D Prevents Podocyte Injury Via Regulation Of Macrophage M1/M2 Phenotype In Diabetic Nephropathy Rats. Endocrinology 2014 September 4;en20141020.


Ralph JA, Ahmed AU, Santos LL, Clark AR, McMorrow J, Murphy EP, Morand EF.
Identification of NURR1 as a mediator of MIF signaling during chronic arthritis: effects on glucocorticoid-induced MKP1. Am J Pathol 2010 November;177(5):2366-78.


(74) Marinkovic G, Hamers AA, de Vries CJ, de W, V. 6-Mercaptopurine reduces macrophase activation and gut epithelium proliferation through inhibition of GTPase Rac1. Inflamm Bowel Dis 2014 September;20(9):1487-95.


CD105(+) hMSCs. Stem Cells Int 2014;2014:197154.