Chapter 1

General introduction

Introduction

Summary

Macrophages (Mph) are major effector cells of the innate immune system. Mph are broadly divided into two types: pro-inflammatory Mph1 and anti-inflammatory Mph2. A distinctive feature described between human Mph1 and Mph2 is the high reactive oxygen species (ROS) production by Mph1, whereas Mph2 are being described as a non-ROS producing cell. ROS generated by Mph are usually associated with oxidative stress. However, ROS produced in the immunological synapse or produced in lower amounts can inhibit T cell activation. In addition, ROS serve as second messenger in various signaling pathways by changing specific proteins. These redox changes are in many cases associated with altered activity and effects on signaling pathways or cellular processes. ROS are thus being produced as a result of normal cell physiology or due to ageing or stress, but can also be produced in the context of immunological defense by a specific enzymatic system. A naturally occurring polymorphism in the NADPH oxidase complex member *Ncf1* was found to regulate the severity of arthritis in rats. This polymorphism caused reduced ROS production and promoted the activation of arthritogenic T cells. The main producers of phagocytic NADPH oxidase (NOX2)-derived ROS are neutrophils and Mph. It was shown using transgenic mice expressing a functional NOX2 restricted to Mph that Mph could inhibit T cell responses in a ROS-dependent fashion. Thus NOX2-derived ROS are involved in immune modulation and cellular signaling as well. The scope of this thesis was to obtain more insight in the role of ROS being produced by the human M-CSF-differentiated anti-inflammatory Mph2 in immune modulation.

Macrophage differentiation

In the bone marrow, hematopoietic progenitor cells give rise to neutrophils and cells from the mononuclear phagocyte system, including circulating blood monocytes (Mn), tissue macrophages (Mph) and dendritic cells (Figure 1) (1,2). Mn are released into the circulation upon maturation where they remain for a few days before migrating into all the tissue compartments in the body. There the Mn will differentiate into resident Mph and by adapting to their local environment, Mph such as Kupffer cells (liver), alveolar Mph (lung), and microglia (central nervous system) develop. These Mph types are morphologically and phenotypically very different, underscribing the plasticity of Mph. Mph are major effector cells of the innate immunity; they engulf and kill pathogens, but are also involved in tissue repair and resolution of inflammation. Under the influence of colony stimulating factors (CSF), including macrophage (M)-CSF and granulocyte-macrophage (GM)-SCF, Mn are differentiated from progenitor cells. This became evident when observing osteopetrotic mice having a genetic defect that makes them M-CSF deficient (3). These mice have severe reductions in circulating Mn, and almost complete absence of various myeloid cells including osteoclasts and resident peritoneal Mph. The majority of the tissue

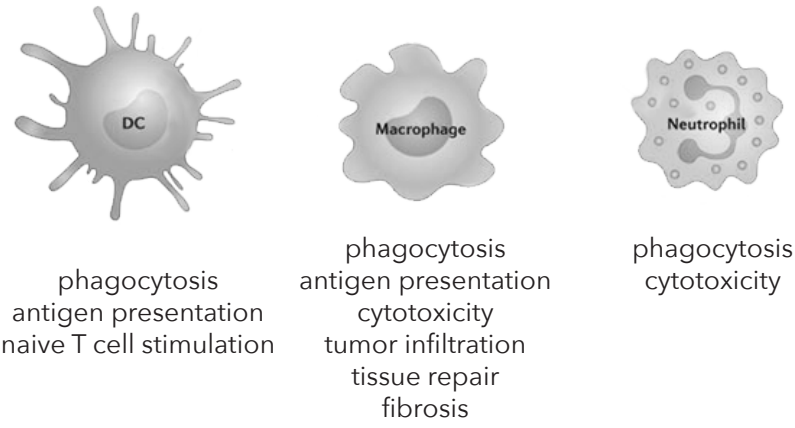

Figure 1: Functions of meyloid cells: DC, Mph, and neutrophil

Mph depend upon signaling through the CSF-1R for their migration, survival and (re)population (4).
Since M-CSF is constitutively present in blood, it is thought that an anti-inflammatory Mph phenotype is maintained under homeostatic conditions (5). However, under the influence of environmental factors, including signals like cytokines, chemokines, and TLR ligands, resident Mph can undergo phenotypic and functional differentiation, thereby resulting in different Mph populations (6-8). This was shown in an elegant study by Arnold et al. using *in vivo* tracing methods where inflammatory Mph switched to an anti-inflammatory Mph phenotype in response to a changed microenvironment (9).

Macrophage plasticity during inflammation

In response to various inflammatory and immune stimuli, tissue-resident Mph undergo local activation. In addition, Mn are recruited causing an accumulation of tissue Mph with a pro-inflammatory phenotype (Mph1) contributing to inflammation. Mph1 phagocytose debris, invading micro-organisms and opsonized particles, thereby triggering a pro-inflammatory response with production of pro-inflammatory mediators, including Interleukin (IL)-1β, IL-6, IL-12, and tumor necrosis factor-α (TNF)-α (10). During the phagocytosis of apoptotic cells, Mph dampen their pro-inflammatory activities, acquiring characteristics of anti-inflammatory Mph2; Mph that can initiate tissue healing and repair by producing the anti-inflammatory mediators TGF-β, IL-10, and prostaglandin E2 (11,12).
In a model of glomerulonephritis in the kidney, ablation of Mn and Mph diminished the amount of tissue injury (13,14). In addition, Kupffer cell depletion in a model of liver injury using clodronate liposomes attenuated liver damage (15). Mph depletion in a model of autoimmune diabetes blocked disease, and Mph inactivation led to increased graft survival in a model of islets of Langerhans

transplantation in the rat (16,17). This implicates that under several conditions Mph depletion could be of therapeutic interest. However, eliminating Mph can also have disadvantages. It has been demonstrated that hepatic Mph were necessary for matrix regression and regulating cell proliferation following liver injury (18). Similarly, in muscle Mph are needed to enhance myogenic growth by releasing trophic factors (19). These Mph depletion studies confirm *in vivo* that different Mph types exist: depletion does not only affect the pro-inflammatory function of Mph but also the wound healing function.

Macrophages in chronic inflammatory diseases

Lately there has been an increasing interest in the role of Mph in chronic inflammatory diseases. In atherosclerosis a persistent recruitment of Mn into atherosclerotic lesions is observed, where a variety of pro- and anti-inflammatory Mph can be found (20). A damaging role for Mph1 has been indicated in atherosclerosis, whereas Mph2 were shown to be protective (21,22). In addition, Mph play also a central role in the pathogenesis of rheumatoid arthritis (RA). In steady-state conditions a balance is established that allows normal joint function. This balance can shift towards an inflammatory pathway by increased production of growth factors that promote Mph1 development and increased production of pro-inflammatory cytokines such as TNF-α and IL-6 further favouring Mph1 progression (23). Furthermore, in obesity-induced inflammation Mph1 are considered to be the major inflammatory cell in adipose tissue, whereas normally resident adipose tissue Mph are non-inflammatory and regarded as Mph2 (24).

Macrophage 2-like phenotypes

Resident tissue Mph express the Mph2 marker CD163, indicating an Mph2 phenotype *in vivo* (25). Mph2 are also found in the lung where they function to protect against unwanted immune responses (26). They were also documented in a Th2-mediated inflammatory setting such as nematode infection (27). Embryonic Mph and Mph isolated from placentae and tumors also display characteristics of Mph2 (28-30). Additionally, coculturing monocytes with Tregs differentiated these cells toward Mph2 (31). Tumor-associated Mph (TAMs) also have Mph2 properties. TAMs originate from circulating monocytic precursors, which are recruited to the tumor in response to secreted chemokines and cytokines derived from cancer cells (32). They express high levels of the mannose receptor, are poor at presenting antigen and their cytokine profile consist of high IL-10 and low IL-12 production (33). The role of TAMs in tumors involves tumor progression and metastasis, immune suppression, and angiogenesis. Thus functionally different Mph2-like phenotypes have already been documented.

Although the results described above suggest fully polarized functions, Mph with overlapping phenotypes have been observed as well. In human Mn infected with cytomegalovirus and in adipose tissue Mph a mixed gene profile expressing both Mph1 and Mph2 genes was observed (34,35). In addition, oxidized phospholipid-treated murine Mph were identified as a new

Mph phenotype which is distinctly different from Mph1 and Mph2 (36). Thus ample Mph-like phenotypes have been observed, indicating the complexity of defining a specific Mph phenotype.

Macrophage activation

Interferon-γ (IFN-γ) was the first identified marker that could activate Mph (37). Later, LPS and GM-CSF were found to promote the generation of Mph1 (38). Factors that skew Mph towards Mph2 are M-CSF, IL-4, IL-13, immune complexes, Wnt5a, IL-10, or TGF-β, indicating great heterogeneity (39-43). These activators all induce a distinct, as well as partially overlapping, pattern of gene expression, thus presenting different Mph2 subsets. Mph2 are induced by M-CSF, Mph2a are induced by IL-4 or IL-13, Mph2b are induced by exposure to immune complexes, agonists of TLRs or IL-1R, and Mph2c are induced by IL-10 and glucocorticoid hormones (39). Functionally, Mph2a are responsible for wound healing and Mph2b together with Mph2c for immune regulation. Th2 driven inflammation is induced by Mph2b, whereas Mph2c are predominantly involved in deactivating immunoregulation (39). Overall, Mph2 participate in Th2 responses, dampen inflammation, promote tissue remodeling, angiogenesis, tumor progression and immune regulation, whereas Mph1 participates in Th1 responses and are protective against tumors and organisms (44).
Recently other Mph subsets have been described. Mph2d are the results of switching from Mph1 as a response to adenosine A2A receptor signaling induced by TLR agonists (45). These Mph have decreased expression of pro-inflammatory cytokines, including IL-12 and TNF-α, while concurrently produce high levels of IL-10 and vascular endothelial growth factor. Mox Mph are induced by ox-PL 1-palmitoyl- 2arachidonoyl-sn-glycero-3-phosphorylcholine, resulting in a population of Mph that express an unique set of genes like heme oxygenase-1 (HO-1) and thioredoxin-reductase in a Nrf2 dependent manner (36). Mha macrophages are differentiated from monocytes using hapto-hemoglobin complexes or oxidized red blood cells leading to the up-regulation of CD163, HO-1 and IL-10 in an Nrf2-dependent manner, suggesting that these two subsets might be related (46). In addition, Mph4 have combined features of Mph1 and Mph2 and were shown to be CSF/CXCL4-dependent (47). These Mph have a weak phagocytic capacity, but their function remains poorly understood. Mox, Mha and Mph4 have until now been poorly characterized. Considering the heterogeneous expression of cell surface markers in Mph, the number of Mph-subpopulations could be infinite (2).

Distinguishing between Mph1 and Mph2 subsets

Considering the great heterogeneity, it is a major challenge to distinguish different Mph subsets. This characterization is mainly based on the expression of cell surface markers as well as their cytokine and chemokine production profile (Table 1). Specific Mph2 markers in mice, arginase-1, FIZZ1, Ym1, Ym2 and MRC1 have been identified both *in vivo* and *in vitro* (1,48), whereas establishing specific markers for human Mph2 is still an ongoing challenge. Candidates like CD163, CD206 (mannose receptor), HO-1, folate receptor-β,

	Mph1 (IFN-g/LPS/GM-CSF)	**Mph2** (M-CSF)	**Mph2a** (IL-4)	**Mph2c** (IL-10)
markers	MHC class II CD80, CD86 CD16, CD32, CD64 CD206	CD16, CD32, CD64 CD163 MHC class II CD80, CD86	MHC class II CD80, CD86 CD163, CD206 CD16, CD32, CD64	CD16, CD32, CD64 CD163, CD206 MHC class II CD80, CD86
cytokines	TNF-a IL-1, IL6- IL-12 IL-18, IL-23 IL-10	IL-10 TNF-a IL-1, IL-6, IL-18	IL-10, IL-1ra TGF-b TNF-a IL-1, IL-6, IL-18, IL-12	IL-10, IL-1ra TGF-b TNF-a IL-1, IL-6, IL-18, IL-12
chemokines	CCL2,3,4,5,8,15,19,20 CXCL9,10,11,13,16 CX3CL1 CCR7 CCL17,18,22,24 CCR2	CCL2 CCL17,22	CCL2,13,14,17,18 CCL22,23,24,26 CXCL4,8 CXCR1,2 CCL5 CXCL9,10	CCL2,16, 18, 23 CXCL4,13 CCR2,5 CCL5 CXCL9,10
other	high ROS (NOX2) IRF5 STAT5 pathway Th1 response	 IRF4 Th2 response	low ROS (NOX2) STAT6 pathway Th2 response	low ROS (NOX2) STAT3 pathway Th2 response

Table 1: Characteristics of Mph1, Mph2, Mph2a, and Mph2c regarding markers, cytokines, chemokines and miscancellous

and CCL18 have been described (1,49-51). In general, the high expression of cell surface markers CD163 and CD206 on Mph2 is used to distinguish Mph1 and Mph2, although different levels have been reported depending on the Mph2 subtype (52-55). Mph1 and Mph2 produce distinct cytokines: Mph1 show high TNF-α, IL-6, IL-12, and IL-23 production and low IL-10 levels, whereas Mph2 typically produce high levels of IL-10 and TGF-β (40,41,53,55,56) (Table 1). In addition, the chemokine production of Mph1 and Mph2 is different. Mph1 produces inflammatory CC chemokines and IFN-γ-responsive chemokines CCL5 (RANTES), CXCL9, CXCL10 (IFN-γ-IP-10), and CXCL16 that will recruit Th1, Tc1, and NK cells and thus a type 1 immune response (39,56). IL-4 and IL-10 differentiated Mph2 will inhibit the expression of most of these chemokines, and thereby limiting inflammation. M-CSF, IL-4, and IL-10 all induce CCL2 (MCP-1). IL-4 induces CCL17, CCL24, and CCL22, which will be inhibited by IFN-γ differentiated Mph1 (39,57). CCL22 binds to CCR4 which is expressed on Th2 lymphocytes, thereby amplifying the Th2 response (58). Taken together, the cytokine and chemokine production of Mph1 will lead to a Th1 response, whereas that of Mph2 will lead a Th2 response.

The transcriptional regulation during differentiation is also different in Mph1 and Mph2 (Table 1). The transcription factor PU.1 is essential to Mph lineage

commitment and Mph-specific gene expression (59). Transcription factors are also involved in the Mph polarization. The transcription factors involved in Mph1 polarization are NF-κB, activator protein-1, CCAAT/enhancer-binding protein-α (C/EBP-α), and interferon regulatory factor (IRF)5, whereas STAT6, peroxisome proliferator-activated receptor γ (PPARγ), IRF4, C/EBP-β, and Krüppel-like factor 4 (KLF4) are associated with Mph2 polarization (60). STAT6 induces the transcription factor PPARγ, which acts together with STAT6 to regulate Mph2-specific genes and polarization (61). KLF4 was found to cooperate with STAT6 to induce an Mph2 profile, while inhibiting Mph1 targets (62). IRF4 has been implicated in Mph2 polarization, whereas IRF5 participates in the activation of genes encoding pro-inflammatory cytokines and represses the gene encoding IL-10, resulting into Mph1 activation (56,63).

ROS producing capacity of Mph1 and Mph2

A distinctive difference described between Mph1 and Mph2 is the high reactive oxygen species (ROS) production by Mph1, while IL-4 differentiated Mph2 are described as non-ROS producing cell (40,53,57,64). ROS generated by Mph are usually associated with oxidative stress (65). However, it has been shown that ROS produced in the immunological synapse or produced in lower amounts can inhibit T cell activation, or can serve as second messenger in various signaling pathways (66-69). These data indicate that the exact role of Mph-derived ROS in the immune system needs to be resolved.

Reactive oxygen species

Important insights into the potential role of ROS in immune regulation were obtained in experimental models of autoimmunity, where Dark Agouti (DA) rats were found to be more prone to develop RA compared with other rat strains. Genetic studies identified a naturally occurring polymorphism in *Ncf1*, component of the NADPH oxidase complex, linked to the severity of arthritis in rats (70). This polymorphism resulted in a reduced ROS production and promoted the activation of arthritogenic T cells.

Phagocytic NADPH oxidase complex

ROS are produced by the mitochondrial electron transport chain, peroxisomes, xanthine oxidase, endoplasmic reticulum and the NADPH oxidases. There are seven family members of the NADPH oxidases, namely NOX complexes 1-5, and DUOX 1 and 2. The main producers of phagocytic NOX2-derived ROS are PMN and Mph. The NOX2 complex is responsible for the oxidative burst, causing a sufficient concentration of ROS in the phagosome, required to kill pathogens. NOX2 consists of multiple components: the membrane-bound cytochrome-b558 consisting of gp91^{phox} and p22^{phox}, and the cytosolic components p47^{phox} (*Ncf1*), p67^{phox}, and p40^{phox}, and the small GTPase Rac. Upon phosphorylation p47^{phox}, together with p67^{phox} and p40^{phox}, form the NOX2 complex in the membrane by interacting with p22^{phox} and gp91^{phox}, resulting in the production of superoxide. This will dismutate to hydrogen peroxide, and

is then further processed to generate more reactive metabolites like hydroxyl radical, after interaction with transition metal ions, and hypochlorous acid, which is catalyzed by myeloperoxidase. NOX2-derived ROS are generated in the plasma membrane, but ROS production can also occur within intracellular organelles, although the exact compartment is yet to be determined (71).

Chronic granulomatous disease (CGD) is a disease that develops when mutations in one of the NOX2 components occur, resulting in defective ROS production. At this moment, mutations in the CYBB (gp91^{phox}), CYBA (p22^{phox}), NCF1 (p47^{phox}), NCF2 (p67^{phox}), and NCF4 (p40^{phox}) genes have been described (72,73). The predominant form of CGD, comprising about 70% of cases, results from mutations in gp91phox which are inherited in an X-linked recessive manner, whereas mutations in the other genes are inherited in an autosomal recessive manner (73). CGD patients have a ROS production of 0.1 – 27% compared to normal levels (74). The X-linked recessive cases are more severe than autosomal recessive cases, since these patients have a lower residual ROS production. In addition, with a modest residual ROS production there is a greater probability of long-term survival compared with those having little residual ROS levels (74). The consequences of the diminished or abrogated oxidative burst by the NOX2 complex in CGD patients are recurrent bacterial and fungal infections, as well as granuloma formation (75). In addition these patients suffer more often from autoimmune diseases such as RA or systemic lupus erythematosis compared to the normal population (76,77). Since a hyperinflammatory phenotype is presented under non-infectious conditions with suboptimal ROS production, this indicates that normal phagocytic ROS production suppress pro-inflammatory gene transcription and preserve homeostasis (71). Interestingly, CGD patients have defective apoptosis after phagocytosis, as a result of reduced induction of the pro-apoptotic protein BAX (78). This study indicates that ROS are involved in the resolution of inflammation as well.

Redox signaling

ROS are being produced as a result of normal cell physiology or due to ageing or stress. As defense against ROS, cells have anti-oxidants, like glutathione, Vitamins C and E, superoxide dismutases, catalase, peroxidases, thioredoxins, glutaredoxins, and peroxiredoxins (79). Oxidative stress generally involves non-specific oxidation of a wide variety of molecules and is associated with higher oxidation states like sulfinic and sulfonic acid, causing irreversibly damage. There are strong indications that oxidative stress is an important pathogenic mechanism in various disorders, including cancer, ageing, atherosclerosis, diabetes, and ischemia-reperfusion injury (80). Thus eliminating ROS by anti-oxidants would potentially be beneficial. However, it has been demonstrated that antioxidants could not improve the disease course of RA or atherosclerosis (81,82). Even more so, the use of pro-oxidant phytol, causing increased ROS production, actually decreased inflammation in animals with arthritis (83).

ROS serve as second messenger in various signaling pathways by changing

specific proteins. Hydrogen peroxide oxidizes thiol groups of protein cysteine residues forming disulfides either with other protein cysteines or with low-molecular weight thiols like glutathione, or forming sulfenic acids. Only a subset of cysteine residues in proteins is susceptible to oxidation, indicating the specificity of the reactions. These redox changes are in many cases associated with altered activity and effects on signaling pathways or cellular processes. In bacteria, the transcriptional activator OxyR, is directly oxidized in response to hydrogen peroxide (84). Even though both the oxidized and reduced OxyR are able to bind DNA, only the oxidized form can activate the transcription of antioxidant genes. In eukaryotes, proteins susceptible to thiol oxidation are protein tyrosine phosphatases (PTPs), kinases, and some transcription factors (85,86). PTPs are inactivated after oxidation, resulting in the activation of downstream signaling pathways. The transcription factor family NF-κB is also under redox control, although both oxidation and reduction by ROS are implicated in the activation process (87). In addition, the stress-responsive transcription factor Nrf2 is activated by the oxidation of its inhibitory partner Keap1, thereby disassociating Nrf2 from the complex, leading to the activation of target genes like HO-1 and superoxide dismutase (88). Since the above mentioned transcription factors also control the expression of genes involved in immunity and inflammation, understanding redox signaling is of great importance.

Effects of oxidation on signaling via the T cell receptor (TCR) has also been reported. In RA patients T cell hyporesponsiveness was dependent on the translocation of linker for activation (LAT) of T cells induced by oxidative stress, which impaired the TCR signaling (89). Also TCR zeta and $p56^{lck}$ signaling molecules have been indicated as redox targets after structural changes were observed after oxidation (90). The integrin VLA-4 was identified as redox target, which is a co-stimulatory molecule in the immunologic synapse (91). These data indicate an important role for ROS in modifying the T cell response, directly associating redox-dependent TCR signaling regulation and T cell function. This was also shown in a rat model where decreased ability to produce ROS in DA rats was associated with increased numbers of thiol groups present on the cell surface of their T cells (92). In addition, T cells from rats with normal ROS production could only transfer arthritis after increasing the number of thiol groups, whereas T cells from DA rats became less arthritogenic after decreasing the thiol groups (92).

ROS producing capacity of myeloid cells

During phagocytosis of pathogens, neutrophils produce NOX2-derived ROS to kill microorganisms (3). Interestingly, when comparing neutrophils from CGD patients with healthy controls increased expression of genes encoding for inflammation and host defense were observed, whereas anti-inflammatory mediators were down-regulated (78). A more recently discovered process stimulated by ROS is the formation of neutrophil extracellular traps (NETs) (93). Stimulated neutrophils generate these NETs in order to catch and kill pathogens. Interestingly, CGD patients cannot form NETs, further implicating

NOX2-derived ROS in the antimicrobial response (93).
Myeloid-derived suppressor cells (MDSC), a recently identified population of myeloid cells, continuously produce inflammatory mediators, like IL-1, IL-6, and nitric oxide, but also ROS (94,95). MDSC are predominantly found in tumour bearing patients where they proliferate and stay activated due to chronic inflammation in the tumour environment. ROS production of MDSC is mediated through increased activity of NOX2. The role of ROS in T cell suppression was confirmed when MDSC from gp91$^{-/-}$ mice were not able to induce T cell tolerance (96). MDSC, via generation of ROS and peroxynitrite ($O2\cdot-$ and NO interaction), induced the modification of the peptide-MHC binding of T cells rendering them unresponsive to antigen-specific stimulation (96).

ROS producing capacity of Mph

The *Ncf1* gene, encoding the NOX2 protein p47^{phox},was responsible for DA rats being more prone to develop RA or multiple sclerosis compared with other rat strains (70). This indicates that a lack of ROS production in these rats caused a higher susceptibility to disease. A mutation in the *Ncf1* gene in the mouse had similar results in models of RA and multiple sclerosis showing increased susceptibility and severity due to reduced oxidative burst (97). Interestingly, in rats with a reduced ROS producing capacity the increased susceptibility to pristane-induced arthritis was mediated in a T cell-dependent fashion (98). However, T cells have a very low NOX2 expression production, if any, suggesting that other cells were involved. Among murine APCs, Mph were the highest ROS producing cells compared with B cells and DCs (99). In addition, mice expressing functional p47phox only in their Mph were similarly protected against collagen induced arthritis compared to fully functional wild type mice (99). These data indicate that ROS derived from Mph inhibit T cell responses. Mn/Mph NADPH oxidase was also identified playing a central role in controlling fungal infecting and limiting lung inflammation in a mouse model (100). Furthermore, mice with Mn/Mph expressing functional *Ncf1* were protected against bacterial infections (101). These data indicate an anti-inflammatory role for Mph-derived ROS. However, IL-4 differentiated Mph2 have reduced respiratory burst, whereas Mph1 are known for their ROS-producing capacity (45,59,68,83). Mph-derived ROS are associated with oxidative stress (84), and therefore linked to Mph1, but apparently there is a dual role for Mph-derived ROS. The function of ROS in the immune system is thus not simply antimicrobial, but NOX2-derived ROS are also involved in immune modulation, cellular signaling, chemotaxis, antigen cross-presentation and in inducing autophagy (102).

Scope of thesis

ROS are implicated in cell signaling and also play a role in immune-modulating processes. Anti-inflammatory Mph could inhibit T cell responses in a ROS-dependent fashion, which was shown using transgenic mice expressing functional *Ncf1* restricted to Mph. However, there is limited information on the T cell inhibitory role of Mph-derived ROS in the human setting. For that reason the scope of this thesis was to obtain better insight in the role of

ROS being produced by anti-inflammatory Mph2 in immune regulation. First we investigated the human anti-inflammatory Mph2 for their functional capacity in a ROS-dependent fashion in **Chapter 2**. The anti-inflammatory potential of the ROS-producing capacity of Mph2 led us to investigate the possibility of using Mph2 as cellular therapy, therefore **Chapter 3** focuses on the effect of several commonly used immunosuppressive drugs on Mph1 and Mph2 regarding ROS production and the subsequent effect on T cell responses. Since inhibition of T cell activation in a ROS-dependent fashion was observed, we investigated a potential mechanism by which Mph2-derived ROS could affect T cell function in **Chapter 4**. Since an observed protective role of macrophages-derived ROS in autoimmune diseases was observed, in **Chapter 5** we investigated the impact of ROS production on the inflammatory response in a renal allograft transplantation model. **Chapter 6** summarizes the studies presented in this thesis and discusses the implications of these findings as well as some unpublished data, and further discusses the role of Mph2-derived ROS in immune modulation.

References

1. Gordon, S., and F. O. Martinez. 2010. Alternative activation of macrophages: mechanism and functions. Immunity. 32: 593-604.
2. Hume, D. A. 2008. Differentiation and heterogeneity in the mononuclear phagocyte system. Mucosal. Immunol 1: 432-441.
3. Witmer-Pack, M. D., D. A. Hughes, G. Schuler, L. Lawson, A. McWilliam, K. Inaba, R. M. Steinman, and S. Gordon. 1993. Identification of macrophages and dendritic cells in the osteopetrotic (op/op) mouse. J Cell Sci 104 (Pt 4): 1021-1029.
4. Chitu, V., and E. R. Stanley. 2006. Colony-stimulating factor-1 in immunity and inflammation. Curr. Opin. Immunol 18: 39-48.
5. Martinez, F. O., S. Gordon, M. Locati, and A. Mantovani. 2006. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: New molecules and patterns of gene expression. Journal of Immunology 177: 7303-7311.
6. Lin, S. L., A. P. Castano, B. T. Nowlin, M. L. Lupher, and J. S. Duffield. 2009. Bone Marrow Ly6C(high) Monocytes Are Selectively Recruited to Injured Kidney and Differentiate into Functionally Distinct Populations. Journal of Immunology 183: 6733-6743.
7. Geissmann, F., C. Auffray, R. Palframan, C. Wirrig, A. Ciocca, L. Campisi, E. Narni-Mancinelli, and G. Lauvau. 2008. Blood monocytes: distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses. Immunology and Cell Biology 86: 398-408.
8. Cassetta, L., E. Cassol, and G. Poli. 2011. Macrophage polarization in health and disease. ScientificWorldJournal. 11: 2391-2402.
9. Arnold, L., A. Henry, F. Poron, Y. Baba-Amer, R. N. van, A. Plonquet, R. K. Gherardi, and B. Chazaud. 2007. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J. Exp. Med. 204: 1057-1069.
10. Duffield, J. S. 2003. The inflammatory macrophage: a story of Jekyll and Hyde. Clin. Sci. (Lond) 104: 27-38.
11. Chan, A., R. Seguin, T. Magnus, C. Papadimitriou, K. V. Toyka, J. P. Antel, and R. Gold. 2003. Phagocytosis of apoptotic inflammatory cells by microglia and its

therapeutic implications: termination of CNS autoimmune inflammation and modulation by interferon-beta. Glia 43: 231-242.
12. Huynh, M. L., V. A. Fadok, and P. M. Henson. 2002. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. J Clin Invest 109: 41-50.
13. Duffield, J. S., P. G. Tipping, T. Kipari, J. F. Cailhier, S. Clay, R. Lang, J. V. Bonventre, and J. Hughes. 2005. Conditional ablation of macrophages halts progression of crescentic glomerulonephritis. American Journal of Pathology 167: 1207-1219.
14. Guo, S. H., T. A. Wietecha, K. L. Hudkins, Y. Kida, M. W. Spencer, W. Pichaiwong, I. Kojima, J. S. Duffield, and C. E. Alpers. 2011. Macrophages are essential contributors to kidney injury in murine cryoglobulinemic membranoproliferative glomerulonephritis. Kidney International 80: 946-958.
15. Schumann, J., D. Wolf, A. Pahl, K. Brune, T. Papadopoulos, R. N. van, and G. Tiegs. 2000. Importance of Kupffer cells for T-cell-dependent liver injury in mice. Am J Pathol 157: 1671-1683.
16. Bottino, R., L. A. Fernandez, C. Ricordi, R. Lehmann, M. F. Tsan, R. Oliver, and L. Inverardi. 1998. Transplantation of allogeneic islets of Langerhans in the rat liver: effects of macrophage depletion on graft survival and microenvironment activation. Diabetes 47: 316-323.
17. Jun, H. S., C. S. Yoon, L. Zbytnuik, R. N. van, and J. W. Yoon. 1999. The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Exp Med 189: 347-358.
18. Duffield, J. S., S. J. Forbes, C. M. Constandinou, S. Clay, M. Partolina, S. Vuthoori, S. Wu, R. Lang, and J. P. Iredale. 2005. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J. Clin. Invest 115: 56-65.
19. Chazaud, B., C. Sonnet, P. Lafuste, G. Bassez, A. C. Rimaniol, F. Poron, F. J. Authier, P. A. Dreyfus, and R. K. Gherardi. 2003. Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. J. Cell Biol. 163: 1133-1143.
20. Martinet, W., D. M. Schrijvers, and G. R. De Meyer. 2012. Molecular and cellular mechanisms of macrophage survival in atherosclerosis. Basic Res Cardiol. 107: 297.
21. Babaev, V. R., P. G. Yancey, S. V. Ryzhov, V. Kon, M. D. Breyer, M. A. Magnuson, S. Fazio, and M. F. Linton. 2005. Conditional knockout of macrophage PPARgamma increases atherosclerosis in C57BL/6 and low-density lipoprotein receptor-deficient mice. Arterioscler. Thromb. Vasc. Biol. 25: 1647-1653.
22. Park, S. H., Y. Sui, F. Gizard, J. Xu, J. Rios-Pilier, R. N. Helsley, S. S. Han, and C. Zhou. 2012. Myeloid-specific IkappaB kinase beta deficiency decreases atherosclerosis in low-density lipoprotein receptor-deficient mice. Arterioscler. Thromb. Vasc. Biol. 32: 2869-2876.
23. Li, J., H. C. Hsu, and J. D. Mountz. 2012. Managing macrophages in rheumatoid arthritis by reform or removal. Curr. Rheumatol. Rep. 14: 445-454.
24. Osborn, O., and J. M. Olefsky. 2012. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 18: 363-374.
25. Van den Heuvel, M. M., C. P. Tensen, J. H. van As, T. K. van den Berg, D. M. Fluitsma, C. D. Dijkstra, E. A. Dopp, A. Droste, F. A. Van Gaalen, C. Sorg, P. Hogger, and R. H. J. Beelen. 1999. Regulation of CD163 on human macrophages: cross-linking of CD163 induces signaling and activation. Journal of Leukocyte Biology 66: 858-866.
26. Holt, P. G. 1986. Down-regulation of immune responses in the lower respiratory tract: the role of alveolar macrophages. Clin. Exp. Immunol. 63: 261-270.

27. Loke, P., M. G. Nair, J. Parkinson, D. Guiliano, M. Blaxter, and J. E. Allen. 2002. IL-4 dependent alternatively-activated macrophages have a distinctive *in vivo* gene expression phenotype. BMC. Immunol. 3: 7.
28. Gustafsson, C., J. Mjosberg, A. Matussek, R. Geffers, L. Matthiesen, G. Berg, S. Sharma, J. Buer, and J. Ernerudh. 2008. Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype. PLoS. One. 3: e2078.
29. Mantovani, A., A. Sica, P. Allavena, C. Garlanda, and M. Locati. 2009. Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. Human Immunology 70: 325-330.
30. Mues, B., D. Langer, G. Zwadlo, and C. Sorg. 1989. Phenotypic Characterization of Macrophages in Human Term Placenta. Immunology 67: 303-307.
31. Tiemessen, M. M., A. L. Jagger, H. G. Evans, M. J. C. van Herwijnen, S. John, and L. S. Taams. 2007. CD4(+)CD25(+)Foxp3(+) regulatory T cells induce alternative activation of human monocytes/macrophages. Proceedings of the National Academy of Sciences of the United States of America 104: 19446-19451.
32. Traves, P. G., A. Luque, and S. Hortelano. 2012. Macrophages, inflammation, and tumor suppressors: ARF, a new player in the game. Mediators. Inflamm. 2012: 568783.
33. Mantovani, A., B. Bottazzi, F. Colotta, S. Sozzani, and L. Ruco. 1992. The origin and function of tumor-associated macrophages. Immunol. Today 13: 265-270.
34. Chan, G., E. R. Bivins-Smith, M. S. Smith, P. M. Smith, and A. D. Yurochko. 2008. Transcriptome analysis reveals human cytomegalovirus reprograms monocyte differentiation toward an M1 macrophage. Journal of Immunology 181: 698-711.
35. Shaul, M. E., G. Bennett, K. J. Strissel, A. S. Greenberg, and M. S. Obin. 2010. Dynamic, M2-Like Remodeling Phenotypes of CD11c+Adipose Tissue Macrophages During High-Fat Diet-Induced Obesity in Mice. Diabetes 59: 1171-1181.
36. Kadl, A., A. K. Meher, P. R. Sharma, M. Y. Lee, A. C. Doran, S. R. Johnstone, M. R. Elliott, F. Gruber, J. Han, W. S. Chen, T. Kensler, K. S. Ravichandran, B. E. Isakson, B. R. Wamhoff, and N. Leitinger. 2010. Identification of a Novel Macrophage Phenotype That Develops in Response to Atherogenic Phospholipids via Nrf2. Circulation Research 107: 737-U155.
37. Nathan, C. F., H. W. Murray, M. E. Wiebe, and B. Y. Rubin. 1983. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med 158: 670-689.
38. Gordon, S. 2003. Alternative activation of macrophages. Nature Reviews Immunology 3: 23-35.
39. Mantovani, A., A. Sica, S. Sozzani, P. Allavena, A. Vecchi, and M. Locati. 2004. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 25: 677-686.
40. Ricardo, S. D., H. van Goor, and A. A. Eddy. 2008. Macrophage diversity in renal injury and repair. J. Clin. Invest 118: 3522-3530.
41. Verreck, F. A., T. de Boer, D. M. Langenberg, M. A. Hoeve, M. Kramer, E. Vaisberg, R. Kastelein, A. Kolk, R. de Waal-Malefyt, and T. H. Ottenhoff. 2004. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. Proc. Natl. Acad. Sci. U. S. A 101: 4560-4565.
42. Xu, W., A. Roos, N. Schlagwein, A. M. Woltman, M. R. Daha, and K. C. van. 2006. IL-10-producing macrophages preferentially clear early apoptotic cells. Blood 107: 4930-4937.
43. Bergenfelz, C., C. Medrek, E. Ekstrom, K. Jirstrom, H. Janols, M. Wullt, A.

Bredberg, and K. Leandersson. 2012. Wnt5a induces a tolerogenic phenotype of macrophages in sepsis and breast cancer patients. J Immunol 188: 5448-5458.
44. Mantovani, A., S. K. Biswas, M. R. Galdiero, A. Sica, and M. Locati. 2013. Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229: 176-185.
45. Grinberg, S., G. Hasko, D. Wu, and S. J. Leibovich. 2009. Suppression of PLCbeta2 by endotoxin plays a role in the adenosine A(2A) receptor-mediated switch of macrophages from an inflammatory to an angiogenic phenotype. Am J Pathol 175: 2439-2453.
46. Boyle, J. J., M. Johns, J. Lo, A. Chiodini, N. Ambrose, P. C. Evans, J. C. Mason, and D. O. Haskard. 2011. Heme induces heme oxygenase 1 via Nrf2: role in the homeostatic macrophage response to intraplaque hemorrhage. Arterioscler. Thromb. Vasc. Biol. 31: 2685-2691.
47. Gleissner, C. A., I. Shaked, K. M. Little, and K. Ley. 2010. CXC chemokine ligand 4 induces a unique transcriptome in monocyte-derived macrophages. J. Immunol. 184: 4810-4818.
48. Raes, G., P. De Baetselier, W. Noel, A. Beschin, F. Brombacher, and G. Hassanzadeh. 2002. Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. Journal of Leukocyte Biology 71: 597-602.
49. Puig-Kroger, A., E. Sierra-Filardi, A. Dominguez-Soto, R. Samaniego, M. T. Corcuera, F. Gomez-Aguado, M. Ratnam, P. Sanchez-Mateos, and A. L. Corbi. 2009. Folate Receptor beta Is Expressed by Tumor-Associated Macrophages and Constitutes a Marker for M2 Anti-inflammatory/Regulatory Macrophages. Cancer Research 69: 9395-9403.
50. Schraufstatter, I. U., M. Zhao, S. K. Khaldoyanidi, and R. G. DiScipio. 2012. The chemokine CCL18 causes maturation of cultured monocytes to macrophages in the M2 spectrum. Immunology 135: 287-298.
51. Sierra-Filardi, E., M. A. Vega, P. Sanchez-Mateos, A. L. Corbi, and A. Puig-Kroger. 2010. Heme Oxygenase-1 expression in M-CSF-polarized M2 macrophages contributes to LPS-induced IL-10 release. Immunobiology 215: 788-795.
52. Ambarus, C. A., S. Krausz, M. van Eijk, J. Hamann, T. R. Radstake, K. A. Reedquist, P. P. Tak, and D. L. Baeten. 2011. Systematic validation of specific phenotypic markers for *in vitro* polarized human macrophages. J. Immunol. Methods 375: 196-206.
53. Bogdan, C., and C. Nathan. 1993. Modulation of Macrophage Function by Transforming Growth-Factor-Beta, Interleukin-4, and Interleukin-10. Annals of the New York Academy of Sciences 685: 713-739.
54. Verreck, F. A. W., T. de Boer, D. M. L. Langenberg, L. van der Zanden, and T. H. M. Ottenhoff. 2006. Phenotypic and functional profiling of human proinflammatory type-1 and anti-inflammatory type-2 macrophages in response to microbial antigens and IFN-gamma- and CD40L-mediated costimulation. Journal of Leukocyte Biology 79: 285-293.
55. Tugal, D., X. Liao, and M. K. Jain. 2013. Transcriptional control of macrophage polarization. Arterioscler. Thromb. Vasc. Biol. 33: 1135-1144.
56. Tomioka, H., Y. Tatano, W. W. Maw, C. Sano, Y. Kanehiro, and T. Shimizu. 2012. Characteristics of Suppressor Macrophages Induced by Mycobacterial and Protozoal Infections in relation to Alternatively Activated M2 Macrophages. Clinical & Developmental Immunology.
57. Biswas, S. K., and A. Mantovani. 2010. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nature Immunology 11: 889-896.
58. Bonecchi, R., S. Sozzani, J. T. Stine, W. Luini, G. D'Amico, P. Allavena, D. Chantry, and A. Mantovani. 1998. Divergent effects of interleukin-4 and interferon-gamma on macrophage-derived chemokine production: an amplification circuit of polarized

T helper 2 responses. Blood 92: 2668-2671.
59. DeKoter, R. P., and H. Singh. 2000. Regulation of B lymphocyte and macrophage development by graded expression of PU.1. Science 288: 1439-1441.
60. Lawrence, T., and G. Natoli. 2011. Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 11: 750-761.
61. Odegaard, J. I., R. R. Ricardo-Gonzalez, M. H. Goforth, C. R. Morel, V. Subramanian, L. Mukundan, A. R. Eagle, D. Vats, F. Brombacher, A. W. Ferrante, and A. Chawla. 2007. Macrophage-specific PPAR gamma controls alternative activation and improves insulin resistance. Nature 447: 1116-1U12.
62. Liao, X. D., N. Sharma, F. Kapadia, G. J. Zhou, Y. Lu, H. Hong, K. Paruchuri, G. H. Mahabeleshwar, E. Dalmas, N. Venteclef, C. A. Flask, J. Kim, B. W. Doreian, K. Q. Lu, K. H. Kaestner, A. Hamik, K. Clement, and M. K. Jain. 2011. Kruppel-like factor 4 regulates macrophage polarization. Journal of Clinical Investigation 121: 2736-2749.
63. Satoh, T., O. Takeuchi, A. Vandenbon, K. Yasuda, Y. Tanaka, Y. Kumagai, T. Miyake, K. Matsushita, T. Okazaki, T. Saitoh, K. Honma, T. Matsuyama, K. Yui, T. Tsujimura, D. M. Standley, K. Nakanishi, K. Nakai, and S. Akira. 2010. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nature Immunology 11: 936-U89.
64. Abramson, S. L., and J. I. Gallin. 1990. Il-4 Inhibits Superoxide Production by Human Mononuclear Phagocytes. Journal of Immunology 144: 625-630.
65. Duffield, J. S. 2010. Macrophages and immunologic inflammation of the kidney. Semin. Nephrol. 30: 234-254.
66. Matsue, H., D. Edelbaum, D. Shalhevet, N. Mizumoto, C. Yang, M. E. Mummert, J. Oeda, H. Masayasu, and A. Takashima. 2003. Generation and function of reactive oxygen species in dendritic cells during antigen presentation. J. Immunol. 171: 3010-3018.
67. Pillay, J., V. M. Kamp, H. E. van, T. Visser, T. Tak, J. W. Lammers, L. H. Ulfman, L. P. Leenen, P. Pickkers, and L. Koenderman. 2012. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. J Clin Invest 122: 327-336.
68. Schmielau, J., and O. J. Finn. 2001. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. Cancer Res. 61: 4756-4760.
69. Stone, J. R., and S. Yang. 2006. Hydrogen peroxide: a signaling messenger. Antioxid. Redox. Signal. 8: 243-270.
70. Olofsson, P., J. Holmberg, J. Tordsson, S. M. Lu, B. Akerstrom, and R. Holmdahl. 2003. Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. Nature Genetics 33: 25-32.
71. Bylund, J., K. L. Brown, C. Movitz, C. Dahlgren, and A. Karlsson. 2010. Intracellular generation of superoxide by the phagocyte NADPH oxidase: how, where, and what for? Free Radic. Biol. Med 49: 1834-1845.
72. Matute, J. D., A. A. Arias, N. A. M. Wright, I. Wrobel, C. C. M. Waterhouse, X. J. Li, C. C. Marchal, N. D. Stull, D. B. Lewis, M. Steele, J. D. Kellner, W. M. Yu, S. O. Meroueh, W. M. Nauseef, and M. C. Dinauer. 2009. A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40(phox) and selective defects in neutrophil NADPH oxidase activity. Blood 114: 3309-3315.
73. van den Berg, J. M., E. van Koppen, A. Ahlin, B. H. Belohradsky, E. Bernatowska, L. Corbeel, T. Espanol, A. Fischer, Kurenko-Dept, R. Mouy, T. Petropoulou, J. Roesler, R. Seger, M. J. Stasia, N. H. Valerius, R. S. Weening, B. Wolach, D. Roos, and T. W. Kuijpers. 2009. Chronic Granulomatous Disease: The European Experience. Plos

One 4.
74. Kuhns, D. B., W. G. Alvord, T. Heller, J. J. Feld, K. M. Pike, B. E. Marciano, G. Uzel, S. S. DeRavin, D. A. Priel, B. P. Soule, K. A. Zarember, H. L. Malech, S. M. Holland, and J. I. Gallin. 2010. Residual NADPH oxidase and survival in chronic granulomatous disease. N. Engl. J Med 363: 2600-2610.
75. Segal, B. H., P. Veys, H. Malech, and M. J. Cowan. 2011. Chronic Granulomatous Disease: Lessons from a Rare Disorder. Biology of Blood and Marrow Transplantation 17: S123-S131.
76. De Ravin, S. S., N. Naumann, E. W. Cowen, J. Friend, D. Hilligoss, M. Marquesen, J. E. Balow, K. S. Barron, M. L. Turner, J. I. Gallin, and H. L. Malech. 2008. Chronic granulomatous disease as a risk factor for autoimmune disease. Journal of Allergy and Clinical Immunology 122: 1097-1103.
77. Rosenzweig, S. D. 2008. Inflammatory manifestations in chronic granulomatous disease (CGD). Journal of Clinical Immunology 28: S67-S72.
78. Kobayashi, S. D., J. M. Voyich, K. R. Braughton, A. R. Whitney, W. M. Nauseef, H. L. Malech, and F. R. DeLeo. 2004. Gene expression profiling provides insight into the pathophysiology of chronic granulomatous disease. J. Immunol. 172: 636-643.
79. Biswas, S., A. S. Chida, and I. Rahman. 2006. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem. Pharmacol. 71: 551-564.
80. Droge, W. 2002. Free radicals in the physiological control of cell function. Physiological Reviews 82: 47-95.
81. Bae, S. C., W. J. Jung, E. J. Lee, R. Yu, and M. K. Sung. 2009. Effects of Antioxidant Supplements Intervention on the Level of Plasma Inflammatory Molecules and Disease Severity of Rheumatoid Arthritis Patients. Journal of the American College of Nutrition 28: 56-62.
82. Tardif, J. C., J. J. V. McMurray, E. Klug, R. Small, J. Schumi, J. Choi, J. Cooper, R. Scott, E. F. Lewis, P. L. L'Allier, and M. A. Pfeffer. 2008. Effects of succinobucol (AGI-1067) after an acute coronary syndrome: a randomised, double-blind, placebo-controlled trial. Lancet 371: 1761-1768.
83. Hultqvist, M., P. Olofsson, K. A. Gelderman, J. Holmberg, and R. Holmdahl. 2006. A new arthritis therapy with oxidative burst inducers. Plos Medicine 3: 1625-1636.
84. Storz, G., L. A. Tartaglia, and B. N. Ames. 1990. Transcriptional Regulator of Oxidative Stress-Inducible Genes - Direct Activation by Oxidation. Science 248: 189-194.
85. Sareila, O., T. Kelkka, A. Pizzolla, M. Hultqvist, and R. Holmdahl. 2011. NOX2 Complex-Derived ROS as Immune Regulators. Antioxidants & Redox Signaling 15: 2197-2208.
86. Salmeen, A., and D. Barford. 2005. Functions and mechanisms of redox regulation of cysteine-based phosphatases. Antioxidants & Redox Signaling 7: 560-577.
87. Bubici, C., S. Papa, K. Dean, and G. Franzoso. 2006. Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance. Oncogene 25: 6731-6748.
88. Kundu, J. K., and Y. J. Surh. 2010. Nrf2-Keap1 Signaling as a Potential Target for Chemoprevention of Inflammation-Associated Carcinogenesis. Pharmaceutical Research 27: 999-1013.
89. Gringhuis, S. I., E. A. M. Papendrecht-van der Voort, A. Leow, E. W. N. Levarht, F. C. Breedveld, and C. L. Verweij. 2002. Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways. Molecular and Cellular Biology 22: 400-411.
90. Cemerski, S., J. P. M. van Meerwijk, and P. Romagnoli. 2003. Oxidative-stress-induced T lymphocyte hyporesponsiveness is caused by structural modification

rather than proteasomal degradation of crucial TCR signaling molecules. European Journal of Immunology 33: 2178-2185.
91. Laragione, T., V. Bonetto, F. Casoni, T. Massignan, G. Bianchi, E. Gianazza, and P. Ghezzi. 2003. Redox regulation of surface protein thiols: Identification of integrin alpha-4 as a molecular target by using redox proteomics. Proceedings of the National Academy of Sciences of the United States of America 100: 14737-14741.
92. Gelderman, K. A., M. Hultqvist, J. Holmberg, P. Olofsson, and R. Holmdahl. 2006. T cell surface redox levels determine T cell reactivity and arthritis susceptibility. Proceedings of the National Academy of Sciences of the United States of America 103: 12831-12836.
93. Fuchs, T. A., U. Abed, C. Goosmann, R. Hurwitz, I. Schulze, V. Wahn, Y. Weinrauch, V. Brinkmann, and A. Zychlinsky. 2007. Novel cell death program leads to neutrophil extracellular traps. Journal of Cell Biology 176: 231-241.
94. Gabrilovich, D. I., and S. Nagaraj. 2009. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9: 162-174.
95. Gabrilovich, D. I., S. Ostrand-Rosenberg, and V. Bronte. 2012. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12: 253-268.
96. Nagaraj, S., K. Gupta, V. Pisarev, L. Kinarsky, S. Sherman, L. Kang, D. L. Herber, J. Schneck, and D. I. Gabrilovich. 2007. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. Nat Med 13: 828-835.
97. Hultqvist, M., P. Olofsson, J. Holmberg, B. T. Backstrom, J. Tordsson, and R. Holmdahl. 2004. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene. Proceedings of the National Academy of Sciences of the United States of America 101: 12646-12651.
98. Holmberg, J., J. Tuncel, H. Yamada, S. M. Lu, P. Olofsson, and R. Holmdahl. 2006. Pristane, a non-antigenic adjuvant, induces MHC class II-restricted, arthritogenic T cells in the rat. Journal of Immunology 176: 1172-1179.
99. Gelderman, K. A., M. Hultqvist, A. Pizzolla, M. Zhao, K. S. Nandakumar, R. Mattsson, and R. Holmdahl. 2007. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. Journal of Clinical Investigation 117: 3020-3028.
100. Grimm, M. J., R. R. Vethanayagam, N. G. Almyroudis, C. G. Dennis, A. N. Khan, A. C. D'Auria, K. L. Singel, B. A. Davidson, P. R. Knight, T. S. Blackwell, T. M. Hohl, M. K. Mansour, J. M. Vyas, M. Rohm, C. F. Urban, T. Kelkka, R. Holmdahl, and B. H. Segal. 2013. Monocyte- and macrophage-targeted NADPH oxidase mediates antifungal host defense and regulation of acute inflammation in mice. J Immunol 190: 4175-4184.
101. Pizzolla, A., M. Hultqvist, B. Nilson, M. J. Grimm, T. Eneljung, I. M. Jonsson, M. Verdrengh, T. Kelkka, I. Gjertsson, B. H. Segal, and R. Holmdahl. 2012. Reactive oxygen species produced by the NADPH oxidase 2 complex in monocytes protect mice from bacterial infections. J Immunol 188: 5003-5011.
102. Lam, G. Y., J. Huang, and J. H. Brumell. 2010. The many roles of NOX2 NADPH oxidase-derived ROS in immunity. Seminars in Immunopathology 32: 415-430.