1.4 Chemokine receptors CCR1 and CCR5 in allograft rejection

1.4.1 Role of Ccr1 and Ccr5 in rodent allograft models

Migration of leukocytes from the circulation into the graft as well as dendritic cell traffic from the graft to secondary lymphoid organs are crucial steps during the induction of allograft rejection and chemokines as well as their receptors have been implicated in both processes [101]. Moreover, several models of allograft rejection show that loss or blockade of the chemokine receptors Ccr1 or Ccr5 has a beneficial effect on allograft rejection and survival. In the year 2000, Gao *et al.* reported that loss of Ccr1 results in suppression of acute and chronic rejection and prolonged cardiac allograft survival in a murine transplantation model [102]. Another group showed that Ccr1-deficiency prolongs corneal allograft survival in a mouse model [103]. Bedke *et al.* used a Fischer to Lewis rat renal allograft model and observed a significant inhibition of chronic allograft damage by blocking CCR1 with the non-peptide antagonist BX 471 [104]. In addition, BX 471 was shown to be similar to cyclosporine in its ability to prevent extensive infarction of renal allografts and to prolong allograft survival in a rabbit model [105]. Furuichi *et al.* reported that CCR1 deficiency as well as application of BX 471 results in reduced infiltration by neutrophils and macrophages in a renal ischemia-reperfusion injury model [44]. Yun *et al.* utilized the potent chemokine receptor antagonist Met-RANTES (N-terminally methionylated RANTES (CCL5) [106]), for a combined blockade of the chemokine receptors CCR1, CCR3 and CCR5 in a murine model of chronic cardiac allograft rejection [107]. They found that application of Met-RANTES decreased chronic allograft vasculopathy and attributed this effect to reduced graft infiltration by macrophages and T cells as well as less intimal cell proliferation [107]. Met-RANTES blocked adhesion of monocytes to microvascular endothelium and decreased vascular and tubular damage during renal allograft rejection in rats [108]. Furthermore, TAK-779 – a small-molecule inhibitor of CCR5 and CXCR3 – was reported to enhance allograft survival and morphology in two allograft rejection models: TAK-779

prevented acute and chronic rejection of murine cardiac and islet allografts [109] and reduced numbers of infiltrating T cells in a rat model of small intestine transplantation [110].

Several groups observed that the rejection of cardiac and islet allografts is attenuated and accompanied by prolonged allograft survival in Ccr5-deficient mice. Gao and colleagues reported in 2001 that mice lacking Ccr5 or treated with a monoclonal antibody against CCR5 show prolonged cardiac allograft survival [111]. However, the mice used in this study were not backcrossed and displayed therefore a mixed genetic background. In 2004, our group investigated heart and carotid rejection in allografts transplanted to wildtype and CCR5$^{-/-}$ mice of fully MHC-mismatched genetic background [112]. Six days after transplantation, heart allografts of Ccr5$^{-/-}$ mice exhibited significantly diminished mRNA levels of four metalloproteinase genes (Mmp3, Mmp12, Mmp13 and Adam8), less tissue remodeling, better preservation of the myocardial architecture and prolonged cardiac allograft survival compared with wildtype allografts. At day 35 carotid allografts of Ccr5$^{-/-}$ recipients showed significant reduction of neointima formation and CD3^{+} T cell infiltration, suggesting that CCR5 plays an important role in transplant-associated arteriosclerosis and MMP-mediated vessel wall remodeling during the acute and chronic rejection [112]. Fairchild and colleagues suggested that acute cardiac and renal allograft rejection in Ccr5$^{-/-}$ recipients is mainly mediated by increased intragraft complement deposition and alloreactive antibody serum levels [113, 114]. Schnickel *et al.* demonstrated that combined blockade of CXCR3 and CCR5 in a mouse model of cardiac allograft rejection prolongs graft survival [115]. Furthermore, two groups showed that Ccr5-deficiency prolongs islet allograft survival in a murine rejection model [116, 117]. Interestingly, enhanced islet graft survival in Ccr5-deficient recipients was also found in a xenograft model using transfer of porcine pancreatic cell clusters under the capsules of murine kidneys [118]. Moreover, CCR5 plays a complex role in graft-versus-host disease (GvHD). While CCR5 blockade prevented GvHD [119], loss of donor CCR5 led to accelerated GvHD in a murine

model [120, 121]. Notably, a study from Wysocki *et al.* suggests that CCR5 might be important for immunosuppressive function of regulatory T cells during GvHD [122]. The finding that CCR5 expressing regulatory T cells are important suppressors of graft rejection is supported by results from Kallikourdis *et al.* showing accumulation of highly suppressive $CCR5^+$ regulatory T cells in the uterus of pregnant mice [123].

1.4.2 Chemokine and chemokine receptor expression in rejecting human renal allografts

In 1953 Jones wrote: "In the injured glomerulus increased capillary permeability is associated, as in other examples of inflammation, with a so-called increased stickiness of the endothelial cells. Circulating polymorphonuclear neutrophils adhere to these sticky walls and thus accumulate in the glomerulus" [124]. Today, infiltration of renal allografts by leukocytes is recognized as a hallmark of acute graft rejection [125]. The migration of inflammatory cells to sites of renal injury is mediated by chemokines and their receptors in concert with adhesion molecules such as integrins and selectins. Since different subsets of leukocytes express different chemokine receptors, chemokines are able to selectively control the migration of these subsets [101, 126, 127]. Yun *et al.* found an early/late pattern of chemokine expression in a murine model of chronic allograft vasculopathy [128]. In this regard, Shimizu and Mitchell summarized: "Chemokines likely affect all phases of transplantation injury by regulating intragraft leukocyte recruitment and inflammatory responses, as well as through modulation of APC homing to secondary lymphoid organs and clonal expansion or tolerance induction of alloantigen-specific T cells" [129]. They conclude that chemokines selectively and temporally control leukocyte immigration to the allograft.

In 1994, Pattison *et. al* were the first to show that CCL5 is abundantly expressed in human kidney allografts with ongoing acute cellular rejection [130]. CCL5 mRNA was detected in infiltrating mononuclear cells and tubular epithelial cells. CCL5 protein was found on

mononuclear cells and tubular epithelium. Additionally, CCL5 protein localized to endothelial cells at the surface of peritubular capillaries, although CCL5 mRNA was absent from these cells as shown by *in situ* hybridization. This finding suggested that CCL5 protein was deposited on the surface of endothelial cells to promote migration of monocytes and T cells into the graft. Moreover, Pattison *et al.* claim that CCR5 is expressed on graft infiltrating cells which are predominantly macrophages, T cells and to a lesser extent eosinophils [130].

Segerer *et al.* reported up-regulation of the chemokines CCL3, CCL4 and CCL5 as well as enhanced expression of their corresponding chemokine receptors CCR1, CCR2 and CCR5 during acute rejection of human renal allografts [125]. Furthermore, this group demonstrated absence of Th2-associated chemokine receptors (CCR3 and CCR8) and increased Th1 cytokine expression (Cxcl10 (IP-10)) during acute rejection, thereby confirming earlier results showing that acute renal allograft rejection in humans has characteristics of a Th1-type immune response [131-133].

In 2004, Rüster and colleagues analyzed chemokine receptor expression in human renal allografts undergoing acute or chronic rejection [134]. They observed that chemokine and chemokine receptor expression was localized to infiltrating mononuclear cells. In addition, they report that biopsies from patients with chronic allograft rejection display significantly lower expression of CCL2, CCL5, CCR1, CCR2 and CCR5 in addition to reduced numbers of infiltrating monocytes/macrophages as compared with biopsies from patients with acute allograft rejection. These findings are in accordance with observations by Oliveira *et al.* demonstrating that acute rejection is dominated by Th1 responses, while chronic rejection is associated with increased expression of Th2 cytokines [133].

Mayer *et al.* characterized CCR1$^+$ cells in rejecting human renal allografts and found that CCR1 protein localized to monocytes, B cells and dendritic cells [135]. Furthermore, CCR1, CCL3 and CCL5 mRNA were increased in biopsies with acute and chronic allograft rejection compared to

pre-transplant controls. Mayer *et al.* suggested that absence of CCR1 on macrophages might be the result of down-regulation after migration into the graft.

1.4.3 Renal allograft long-term survival correlates with CCR5 genotype in humans

In 2001, Fischereder and Luckow *et al.* conducted a retrospective study correlating the renal allograft survival with the CCR5 genotype of 1227 patients with a renal transplant [8]. These patients were recruited from six European transplantation centers (München, Berlin, Erlangen, Regensburg, Hamburg and Zürich) between January, 1998, and March, 2000. 958 patients (=78.0%) were homozygous for the wildtype allele of CCR5, whereas 248 (=20.2%) were heterozygous and 21 (=1.7%) of these patients were homozygous for the *CCR5Δ32* allele. The effect of *CCR5Δ32* homozygosity on allograft survival was analyzed in 576 patients with available demographic and clinical follow-up data (**Figure 7**). No obvious demographic or clinical

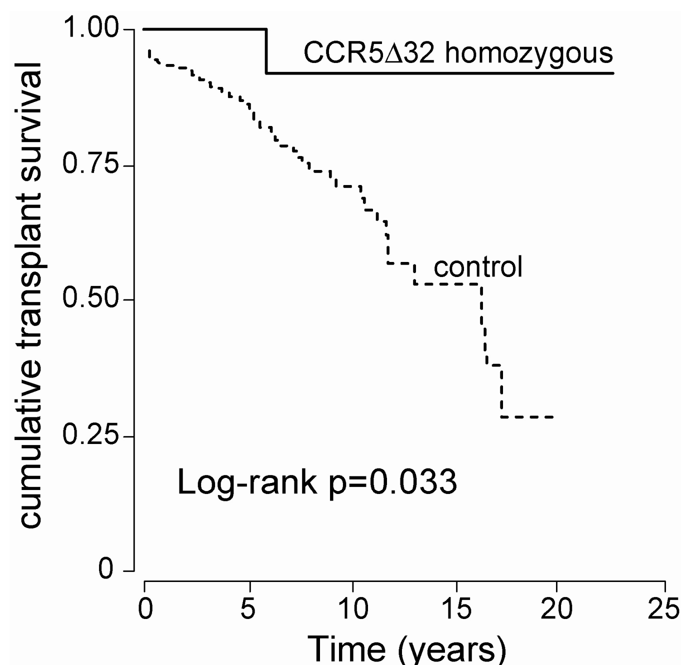

Figure 7. Kaplan-Meier plot showing renal allograft survival in patients with control (*CCR5* wildtype and *CCR5Δ32* heterozygous) or *CCR5Δ32* homozygous genotype (adapted from Fischereder and Luckow *et al.* 2001, [8]).

differences were found between the group of *CCR5Δ32* homozygous carriers and the control

group (wildtype plus *CCR5Δ32* heterozygous). Only one of the 21 patients with a homozygous *CCR5Δ32* genotype lost graft function and one patient died with a functioning graft. The other 19 patients were alive with functioning grafts in April, 2000. By contrast, in the control group graft failures occurred in 78 patients. Hence, 5 and 10 years after the transplantation significantly more patients of the control group lost graft function as compared with the group of *CCR5Δ32* homozygous transplant recipients (**Figure 7**. p=0.0108 after 5 years and p=0.0062 after 10 years, Fisher's exact test). Due to these results the authors suggested that homozygosity for the *CCR5Δ32* allele is associated with long-term graft survival representing an advantage in renal transplantation [8].

1.5 Macrophage biology

The investigation of renal allograft rejection in chemokine receptor deficient mice surprisingly showed that Ccr5 is involved in macrophage polarization. Therefore, the next two chapters are intended to give some background information on macrophage biology and activation phenotypes of macrophages.

The word "macrophage" stems from the Greek words for 'large' (*macros*) and 'to eat' (*phagein*) and means "big eater". This term was coined by the famous Russian zoologist Elie Metchnikoff (*1845-†1916) during his comparative studies of phagocytosis and the recruitment of phagocytes in different organisms in 1893 [136]. Metchnikoff was the first to fully recognize the significance of phagocytic cells for the host resistance against infectious agents and is therefore regarded today as the "Father of natural immunity" [137]. Two categories of phagocytic cells were already distinguished by Metchnikoff: small polymorphonuclear "microphages" (*i.e.* granulocytes, 10-15 µm in diameter) and larger sized "macrophages" (mononuclear phagocytes, 20-25 µm in diameter). Macrophages, monocytes and their precursors have been grouped into the mononuclear phagocyte system (MPS) due to common functional criteria:

they show avid phagocytosis and pinocytosis as well as the ability to attach firmly to glass surfaces [138]. Therefore, the cells of the MPS are separated from T and B lymphocytes, granulocytes and endothelial cells. The cells of the MPS derive from a common hematopoietic stem cell in the bone marrow (**Figure 8**). These precursor cells generate monoblasts which in turn give rise to promonocytes and these differentiate into monocytes [139]. Monocytes are incompletely differentiated non-dividing cells with irregular cell shape, bean-shaped nuclei, a high cytoplasm-to-nucleus ratio and a granular cytoplasm containing lysosomes and phagocytic vacuoles [1, 140]. Monocytes emigrate from the bone marrow into the circulation and the peripheral blood from where they transmigrate through the vascular endothelial cell layer into the tissues of the body – a process called diapedesis. Upon entry into the tissue the monocytes mature and become macrophages. In general, macrophages are large cells (20-25 µm in diameter) with an oval, bean-shaped or indented nucleus and distinct Golgi apparatus. They have a cytoplasm rich in lysosomes and endocytic vesicles as well as a plasma membrane covered by ruffles or microvilli [138]. However, macrophage morphology and expression of surface markers varies strongly depending on the anatomic site of tissue entry and different tissue macrophage populations have therefore been given different names. For example, in the CNS, tissue macrophages are called microglia cells, Kupffer cells line the vascular sinusoids of the liver, alveolar macrophages are found in pulmonary airways and multinucleated phagocytes in the bone are called osteoclasts. Macrophages in the spleen are even subdivided into at least 4 populations: red and white pulp macrophages, marginal zone and metallophilic macrophages [141]. Whether peripheral blood monocytes have the capacity to replenish each of these macrophage pools or if different monocyte subsets exist is currently a matter of debate. Besides replenishment by monocytes studies have also shown that local proliferation of tissue macrophages also plays a significant role for the maintenance of macrophage populations

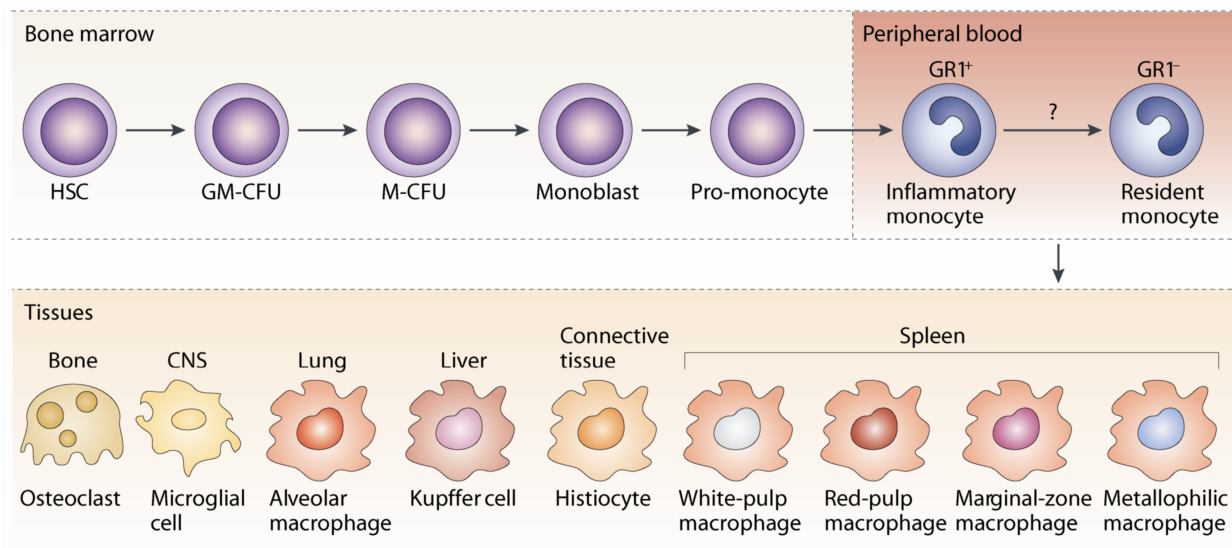

Figure 8. Heterogeneity of the mononuclear phagocyte system (adapted from Mosser *et al.* 2008, [2]). Monocytes originate in the bone marrow from a common haemopoietic stem cell (HSC). They undergo differentiation steps during which they commit to the myeloid and then to a monocyte lineage. In response to macrophage colony-stimulating factor, they divide and differentiate into monoblasts and then pro-monocytes before becoming monocytes, which exit the bone marrow and enter the bloodstream. Monocytes migrate to different tissues, where they replenish tissue-specific macrophages. CNS, central nervous system; GM-CFU, granulocyte/macrophage colony-forming unit; M-CFU, macrophage colony-forming unit.

under steady state conditions, whereas tissue injury and inflammation lead to recruitment of precursor cells from the blood [142, 143].

However, monocytes generate not only the various forms of tissue macrophages but give also rise to dendritic cells (DCs) raising the question if DCs can be placed in the system of mononuclear cells. In favor of this view, Hume summarized several lines of evidence and commented that “dendritic cells are a part of the mononuclear phagocyte system and are derived from a common precursor, responsive to the same growth factors (including CSF-1), express the same surface markers (including CD11c), and have no unique adaptation for antigen presentation that is not shared by other macrophages” [144]. From the point of this view, dendritic cells and macrophages are two sides of the same coin – the professional antigen presenting cell on the one side vs. the professional phagocyte on the other. So far, the best

feature to distinguish DCs from macrophages is by function: DCs are able to stimulate naïve T cells [143].

Macrophages, monocytes and polymorphonuclear cells have been called professional phagocytes due to the efficiency and rate with which they internalize particles [145]. Phagocytosis is a process whereby certain cells engulf relatively large particles (>0.5 µm) into intracellular vacuoles called phagosomes. This process is usually independent of clathrin but requires the polymerization of actin filaments thereby distinguishing classical phagocytosis from other endocytic mechanisms like pinocytosis (uptake of fluid and solutes) and receptor-mediated endocytosis (uptake of macromolecules, viruses and small particles) [146]. However, besides professional phagocytes some epithelial cell types are also capable of phagocytosis but to a lesser extent. Interestingly, transfection of fibroblasts with Fcγ receptors was found to confer phagocytic abilities to these cells. Thus, it was proposed that the phagocytic capacity depends on the range of phagocytic receptors expressed by a given cell type [146, 147]. Janeway named these receptors “pattern-recognition receptors” (PRRs). The targets of these receptors are conserved molecular patterns of foreign organisms like viruses, bacteria, fungi and parasites which are not normally found on host cells. Hence, these microbial structures were termed as “pathogen-associated molecular patterns” (PAMPs) [148]. Macrophages are able to express a variety of different PRRs. These can be categorized into opsonin receptors (*i.e.* receptors recognizing the Fc region of antibodies and receptors binding to complement proteins) and non-opsonic receptors (*i.e.* scavenger and lectin-like receptors as well as Toll-like receptors). PRRs are not only localized at the plasma membrane, they are also found in endosomal compartments (*e.g.* TLR3, TLR9) and in the cytosol (*e.g.* NOD-like receptors) thereby allowing the sensing of danger signals in phagocytosed material as well as in the cytoplasm in addition to sensing pathogens in the surrounding environment of the cell [149]. Upon ligand binding to these receptors signaling cascades are initiated leading to cytoskeletal

reorganization, activation of transcriptional programs, antimicrobial and secretory responses as well as phagocytosis [150].

1.6 Plasticity of macrophage activation and polarization

The cells of the mononuclear phagocyte system display a wide array of diverse functions. This is reflected by their heterogeneity and flexibility of transcriptional programs [151]. Resting tissue macrophages can be activated to provoke pro- or anti-inflammatory responses by appropriate stimuli. These stimuli trigger changes in macrophage phenotype and physiology resulting in different macrophage effector functions: host defense, wound healing or regulatory function (**Figure 9**) [2]. Initially, macrophage classification followed the scheme developed by Mosmann *et al.* for the division of Th1 and Th2 cells on the basis of mutually exclusive cytokine production leading to different functional states [152, 153]. Type 1 T helper cells (Th1) develop mainly in response to intracellular bacteria and viruses. Th1 cells produce pro-inflammatory cytokines like IFNγ, IL-2 and TNF, which trigger antimicrobial activity of macrophages and induce cell-mediated immunity. By contrast, type 2 T helper cells (Th2) are generated upon infection by extracellular parasites and induce the production of cytokines (*e.g.* IL-4, IL-5, IL-10 and IL-13) that initiate antibody production (mainly IgE), activation of eosinophils and mast cells as well as down-regulation of Th1 responses [1, 154]. Macrophages have been shown to produce either IL-12 or IL-10 in response to Th1 or Th2 stimuli, respectively. This finding set the basis for the M1/M2 paradigm of macrophage activation [155].

<u>Classically activated macrophages (CAMs)</u>

During a type 1 immune response, M1 macrophages are activated in reaction to bacterial stimuli (*e.g.* LPS) and pro-inflammatory cytokines such as IFN-γ, IL-12 and TNF that are generated by NK cells, APCs, activated $CD8^+$ cytotoxic T cells and $CD4^+$ Th1 cells (**Figure 9**).

These cytokines have several important effects on macrophages. They induce a program of enhanced phagocytotic activity and lysosomal enzyme synthesis, increased respiratory burst (by enhancement ROS and NO production) and increased expression of MHCII and co-stimulatory molecules. Furthermore, these macrophages show increased production of pro-inflammatory cytokines like IL-1, IL-6, IL-12 and TNF [156]. Moreover, M1 macrophages typically produce and secrete chemokine ligands for CCR5 and CXCR3 [157]. A hallmark of M1 macrophages is increased expression and activity of the enzyme 'nitric oxide synthase' (NOS2) which converts L-arginine to nitric oxide and citrulline, resulting in pronounced killing of pathogens by NO intermediates (**Figure 10**). Therefore, macrophages activated by IFN-γ and TLR ligands are potent killers of bacteria and intracellular pathogens and have tumoricidal capacity [158]. However, the M1 response is potentially dangerous for the host, due to production of radicals and substances not only toxic to bacteria but also to surrounding tissue. M1 macrophages have also been termed as classically activated macrophages or "*killer macrophages*".

Alternatively activated macrophages (AAMs)

In 1992 Gordon and colleagues introduced the concept of alternatively macrophage activation (AMA) by IL-4 to distinguish this process from the mechanism of classical activation by IFN-γ [159]. IL-4-activated macrophages acquire several functions distinct from classically activated macrophages (CAMs). IL-4 inhibits the respiratory burst by reduction of NOS2 activity and concomitant stimulation of arginase activity, thereby further reducing NO production since arginase as well as nitric oxide synthase use L-arginine as a substrate (**Figure 10**). The increase in arginase activity leads to enhanced production of proline and polyamines – both are important precursors for collagen synthesis. Furthermore, IL-4 induces up-regulation of mannose-receptor (MRC1) expression, MRC1-mediated endocytosis and stimulates MHCII expression and antigen presentation. IL-4 induces expression of mediators of tissue remodeling,

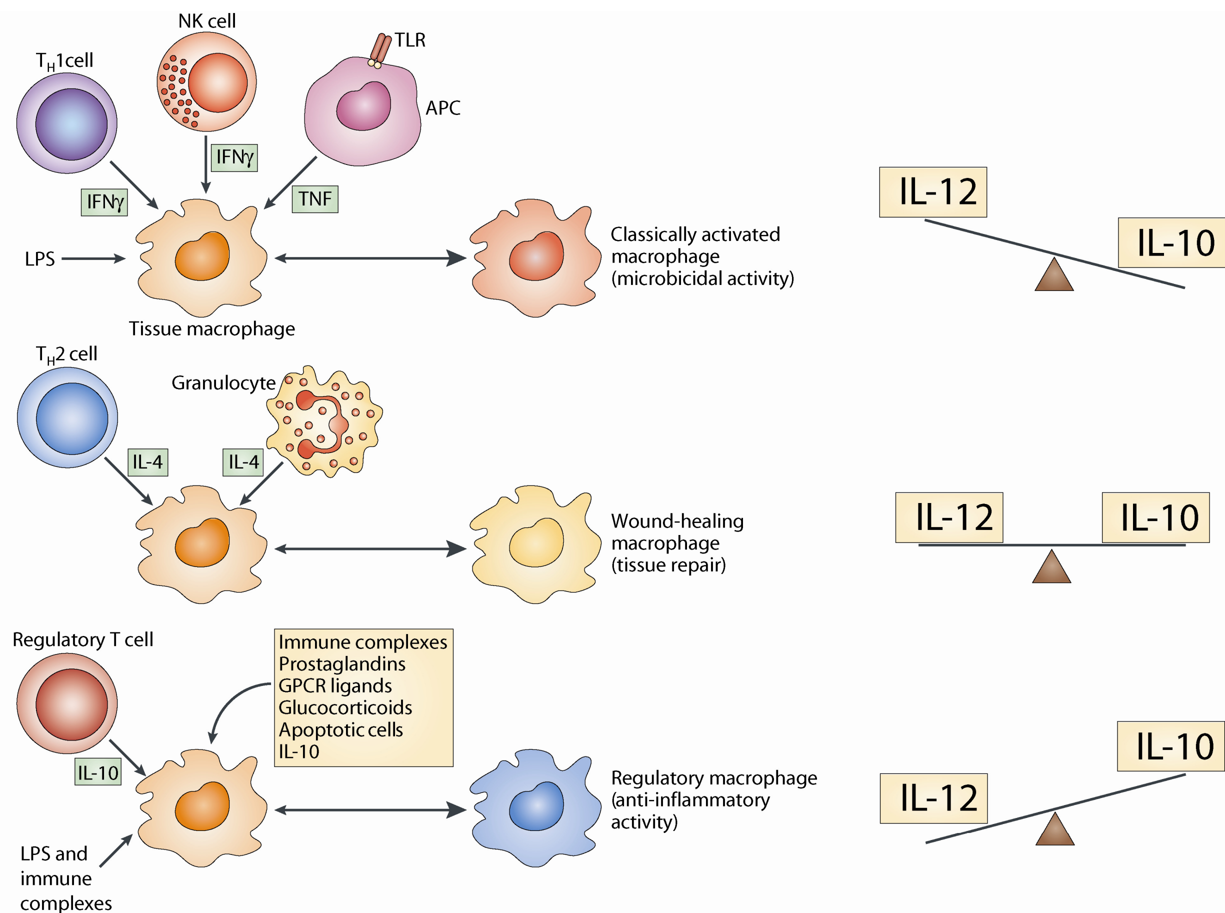

Figure 9. Plasticity of activated macrophages (adapted from Mosser *et al.* 2008, [2]). Classically activated macrophages (CAMs) arise in response to IFN-γ, which can be produced by T_h1 cells or $CD8^+$ T cells (not shown) or by natural killer (NK) cells and TNF, which is produced by antigen-presenting cells (APCs). CAMs produce high levels of IL-12 and modest levels of IL-10. Wound-healing macrophages which are similar in phenotype to alternatively activated macrophages (AAM) arise in response to IL-4, which can be produced by T_h2 cells or by granulocytes. AAMs produce only low levels of IL-10 and IL-12, but express resistin-like molecule α (Retnla) intracellularly, a marker that is not expressed by the other macrophage populations. Regulatory macrophages are generated in response to various stimuli, including immune complexes, prostaglandins, G-protein coupled receptor (GPCR) ligands, glucocorticoids, apoptotic cells or IL-10 and these macrophages produce high levels of IL-10 and low levels of IL-12. Each of these three populations has a distinct physiology. CAMs have microbicidal activity, whereas regulatory macrophages produce high levels of IL-10 to suppress immune responses. Wound-healing macrophages have a role in tissue repair.

promotes fibroblast proliferation as well as collagen synthesis (**Figure 10**). AAM preferentially produce chemokine ligands that bind to CCR3, CCR4 and CCR8 [157]. Interestingly, IL-13 – the nearest chromosomal neighbor of IL-4 – shares many overlapping effects with IL-4; for instance

inhibition of respiratory burst, tissue remodeling and autophagy [160]. Due to their promotion of tissue repair, production of extracellular matrix and return to homeostasis IL-4/Il-13 activated macrophages have been called "*wound healing*" macrophages [2]. IL-4 is mainly produced by activated $CD4^+$ Th2 cells, but also $CD8^+$ cytotoxic T cells, NK T cells, mast cells, basophils, eosinophils as well as human neutrophils have been reported to synthesize IL-4 suggesting that alternative macrophage activation is a feature of innate as well as adaptive immunity [160-162]. In accordance with the role of IL-4 in Th2-driven immune responses, alternative activation of macrophages occurs during host defense against extracellular parasites and enhanced expression of marker genes associated with AAMs were found in mouse models of asthma. Referring to the Th1/Th2 paradigm, AAMs have also been termed M2 macrophages [160].

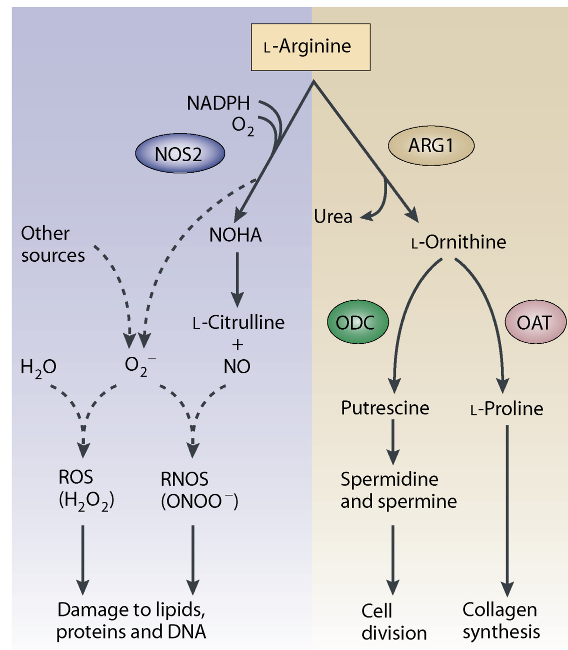

Figure 10. Schematic representation of arginase 1- and nitric-oxide synthase 2-dependent metabolic pathways (adapted from Bronte *et al.* 2005, [7]). The activities of the enzymes arginase 1 (ARG1) and nitric oxide synthase 2 (NOS2) are illustrated, together with the downstream pathways that are activated by L-arginine metabolites. Solid lines indicate the main enzymatic activity, whereas dashed lines indicate alternative metabolic pathways. NOHA, *N*G-hydroxy-L-arginine; OAT, ornithine amino-transferase; ODC, ornithine decarboxylase; RNOS, reactive nitrogen-oxide species.

Regulatory macrophages

A third group of activated macrophages has been emerging recently showing an additional flavor: regulatory macrophages [2]. This population of macrophages has an immunosuppressive

phenotype that is characterized by high expression of IL-10 and down-regulation of IL-12 synthesis. Their phenotype resembles that of myeloid-derived suppressor cells found in solid tumors. Among the various stimuli that have been identified to induce regulatory macrophages are glucocorticoids, TLR agonists in presence of IgG complexes, apoptotic cells, adenosine and histamine [2].

Originally sub-grouped as M2c macrophages [157] regulatory macrophages have recently been proposed to represent an own division [2]. Although, the M1/M2 annotation reflects that the primary Th1 cytokine IFN-γ induces M1 activation (and IL-12 synthesis in macrophages) whereas IL-4 – the prototypic Th2 cytokine – induces M2 activation (and IL-10 synthesis in macrophages), this scheme underscores that M1 and M2 phenotypes are endpoints in a wide spectrum of activation states found in macrophages [163]. Moreover, the finding that other stimuli like glucocorticoids, TGF-β and IL-10 or Fc receptor ligation and TLR ligands are also able to activate macrophages with similar activation phenotypes like IL-4/IL-13 additionally complicates such classification attempts [2]. Furthermore, several studies have demonstrated that macrophage activation states are fully reversible [164-166]. Therefore, Mosser *et al.* suggested a new classification system based on macrophage function: host defense, wound healing or regulatory (**Figure 9**) [2]. In summary, the cells of the mononuclear phagocyte system exhibit a unique plasticity that confers flexibility to the innate immune system and assists the immune system in adaptive immune responses.

1.7 Rationale and aim of this study

Kidney is the most frequently transplanted organ in human patients [167]. However, most immunosuppressive drugs available on the market to date, target the effects of acute allograft rejection and there are no real treatment options for chronic rejection [168]. This situation is reflected by excellent first-year survival rates and only moderate improvements in long-term

graft survival [169]. At best, prolonging allograft survival could supersede follow-up transplantations, help to alleviate the shortage of accessible organs and decrease waiting time for transplantation.

The murine model of renal allograft transplantation provides a useful method to study acute and chronic rejection. In this experimental setting antigen-independent factors such as donor age, graft size and time of cold ischemia can be controlled. Long-term survival of renal allografts in certain murine donor-recipient strain combinations allows studying chronic phase of rejection without the need for immunosuppression. Hence, chronic alloimmune responses can be studied without interfering effects of drug toxicity on chronic injury [170]. In contrast to heterotopic heart transplantation (*i.e.* transplantation into the abdominal cavity and vascularization of the graft via the aorta and vena cava below the renal vessels [171]), the orthotopic (*i.e.* in place of the recipient organ) renal allograft model used in this study is a more physiologic model, provides longer allograft survival times and graft failure ultimately leads to death of the animal defining an endpoint of rejection [172]. There is already a considerable amount of data available for the role of several chemokine receptors in cardiac allograft rejection. However, due to the underlying disease, different organs behave differently during transplant rejection [167] and it is still unclear if the beneficial effects of chemokine receptor deficiency are limited to certain organs or can be assigned to all transplanted organs [173-175]. The chemokine receptors CCR1 and CCR5 appear to play an important role in various processes during allograft rejection. Key findings from previous experiments (see section 1.4) are:

1. Mouse models of allograft rejection showed enhanced allograft function and survival upon blockade or deletion of Ccr1 or Ccr5.
2. Human renal allografts undergoing rejection show increased expression of the chemokines CCL3 and CCL5. Additionally, graft infiltrating cells express the corresponding chemokine receptors CCR1 and CCR5.

3. Long-time renal allograft survival in humans has been correlated with a lack of functional CCR5 protein.

To date, improved allograft outcome in mice lacking functional chemokine receptors due to blockade or genetic deletion has mostly been accounted to reduced graft infiltration by inflammatory leukocytes. However, the function of chemokines and their receptors is not limited to chemotaxis of leukocytes and other cells. Chemokines and chemokine receptors were shown to initiate distinct transcriptional programs leading to activation and/or differentiation of different target cell types [157, 176-178]. Therefore, it was tempting to speculate whether loss of Ccr1 or Ccr5 results in altered immune response phenotypes and whether those changes might contribute to reduced allograft rejection. On the other hand, the finding that loss of either Ccr1 or Ccr5 has a beneficial effect on allograft survival in several mouse models (see 1.4.1), prompted us to generate $Ccr1^{-/-}$/$Ccr5^{-/-}$ double-deficient mice and analyze whether loss of both chemokine receptors has additional or synergistic effects on allograft rejection leading to prolonged allograft survival times. Furthermore, these mice serve as an important tool to answer the question whether CCR1 and CCR5 have redundant function during allograft rejections.