Signal regulatory protein alpha in phagocyte function

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Chapter 1
General introduction

Our environment contains a great variety of infectious microorganisms. A complex and elaborate immune system has evolved to defend us against these invading pathogens. In evolutionary terms the innate immune system is the most ancient line of defense. The recognition of pathogens by the innate immune system relies on a number of germ line encoded pattern recognition receptors, which recognize components that are commonly conserved among broad groups of pathogens. In conjunction with the innate immune system a second defense system, called the adaptive or acquired immune system, can be activated. Recognition of pathogens by the adaptive immune system is mediated through highly specific antigen receptors on B and T lymphocytes, derived by gene rearrangement and clonal selection. In contrast to the innate immune system, which is present in essentially all groups of multicellular organisms, the adaptive system is only present in jawed vertebrates.

1. Phagocytes

Phagocytes, including macrophages and monocytes, play an indispensable role in the immune system with decisive function in both innate and adaptive immunity. However, they also contribute to an important extent to other (chronic) inflammatory conditions, such as autoimmune disease (e.g. rheumatoid arthritis, multiple sclerosis) and allergic reactions, and thus are considered to be a basic cellular ingredient of inflammation in general. Phagocytes also have important roles in normal homeostasis, including the clearance of apoptotic cells during development and adult life, in the elimination of cancer cells, and as specialized bone-resorbing cells, termed osteoclasts, in bone formation and maintenance. Phagocytes have a variety of specialized effector functions and these will be outlined in more detail below. However, it should be kept in mind that many of these effector functions may also inflict damage to the host and therefore a tight control over phagocyte activities is required. In fact a major aim of the research described in this thesis was to understand the role by which the activities of phagocytes are controlled by the host.

The word 'phagocyte' literally means 'eating cell'. Phagocytes are immune cells that have the capacity to engulf, internalize, and destroy pathogens and debris. Metchnikoff was the first one that observed phagocytes cells and their phagocytic activity in tissue[1]. The two major types of phagocytic cells are i) mononuclear phagocytes, such as monocytes and various subsets of macrophages and ii) granulocytes, including the neutrophilic and eosinophilic granulocytes.

1.1. Macrophages

Like all phagocytes, monocytes and macrophages derive from pluripotent hematopoietic stem cells in the bone marrow (Figure 1)[2]. Upon appropriate stimulation these stem cells differentiate into restricted myeloid progenitors, which undergo several precursor and differentiation stages, under the influence of the colony-stimulating factors (CSF), such as GM-CSF and M-CSF, to finally leave the bone marrow as monocytes[3]. The circulating monocytes may differentiate into resident tissue macrophages, of which a variety of subsets can be discriminated, and this occurs both during steady state as well as during inflammatory conditions, where also local

recruitment at inflammatory sites can occur. In addition, there is evidence to suggest that under normal conditions, certain subpopulations of resident macrophages in e.g. the spleen, respiratory tract and peritoneal cavity may renew from local stem cells instead of circulating monocytes[4]. However, inflammatory insults, such as trauma or infection, can lead to an increased dependence on the recruitment of circulating monocytes to aid repopulation of the tissue-resident populations in many of these inflamed tissues[5].

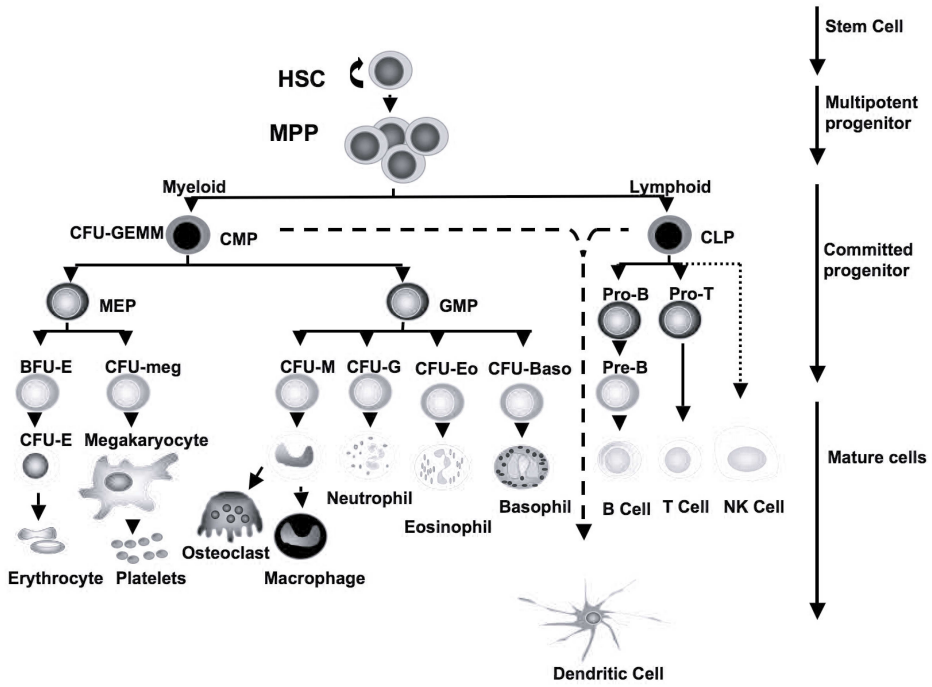

Figure 1. Pluripotent hematopoietic stem cells (HSC) and hematopoiesis. Throughout life, hematopoiesis continuously takes place to not only replenish the various lymphoid, myeloid and erythroid-megakaryocyte lineages, but also to maintain a small pool of HSC with the self-renewal capacity that is capable of carrying on hematopoiesis. From HSC to mature blood cells, extensive proliferation and expansion occurs that results in the production of millions of blood cells. Multi-potential progenitors (*e.g.* CFU-GEMM) and lineage-committed progenitors (*e.g.* CFU-E, CFU-GM), representing various stages along the differentiation pathway with various differentiation and proliferation potentials, can be identified by *in vitro* assays and by the expression of known surface antigens. Figure is from the website www.med.lu.se.

1.2 Neutrophils

Neutrophils comprise the majority of blood leukocytes. They belong to the polymorphonuclear granulocytes because of the characteristic multilobulated nucleus and rich content of granules. Other members of the polymorphonuclear granulocytes are the eosinophils and basophils. Neutrophils develop from the same early precursors as monocytes and macrophages. Their differentiation is under the control of two colony-stimulating factors (CSF), GM-CSF and G-CSF. Under normal conditions only 1-2% of the neutrophils are present in the blood. Like monocytes, they can migrate into tissues at sites of inflammation where they are very efficient in the recognition, phagocytosis and intracellular killing of pathogens, in particular bacteria and fungi. Neutrophils are

relatively short-lived cells, which under normal and inflammatory conditions rapidly die by apoptosis. The latter also helps to prevent excessive inflammation-associated damage to the host.

2. Functions of phagocytes

Phagocytes form the first line of defense against invading pathogens. They internalize and kill the invading pathogens at the site of the infection and support the formation of adaptive immune responses. Exuberant responses by phagocytes may result in inflammatory diseases, ranging from autoimmunity to atherosclerosis. Phagocytes, particularly macrophages, also have important homeostatic functions in the removal of apoptotic cells, the regulation of hematopoiesis, regulation of wound healing, and specialized macrophages such as osteoclasts play a critical role in bone formation and maintenance by means of bone resorption.

2.1. Migration

During inflammation phagocytes are recruited from the bloodstream to the inflammatory site. The transendothelial migration required for this is a well-orchestrated, multi-step process. The first step is rolling and tethering of phagocytes on the endothelium to reduce the velocity of circulating cells. This step is mediated by relatively weak interactions between selectins on the endothelium and their respective carbohydrate ligands on the phagocytes and vice versa[6;7]. Subsequently, chemokines, displayed or released by endothelial cells, activate the phagocytes via G-protein coupled chemokine receptors, which leads to integrin activation and enhances binding of integrins to their counter receptors on the endothelium. This results in firm adhesion of phagocytes to the endothelium. Important integrins involved in firm adhesion are $\alpha_4\beta_1$ (VLA-4), $\alpha_L\beta_2$ (LFA-1) and $\alpha_m\beta_2$ (Mac-1)[8;9] and genetic defects in for instance expression of the integrin β_2 chain lead to leukocyte adhesion deficiency (LAD) and recurrent infections[10]. The cellular adhesion molecules (CAM), VCAM-1 and ICAM-1, are the ligands for integrins on the endothelium[11]. Both VCAM-1 and ICAM-1 belong to the Immunoglobulin (Ig) superfamily. Signaling induced via cell adhesion molecules triggers the loss of endothelial cell-cell contact and allows the phagocyte to enter the next step of migration, i.e. crossing of the endothelial barrier, a process known as diapedesis. It is thought that diapedesis involves for instance PECAM-1 on the endothelial cells binding to PECAM-1 on the phagocytes[12]. In addition, it has been shown by blocking the interaction between transmembrane protein Signal Regulatory Protein alpha (SIRPα) on phagocytes and its endothelial counterligand CD47 that transendothelial migration is reduced, whereas the firm adhesion was not affected. This suggests that the SIRPα-CD47 interaction is required for the diapedesis process and not in the initial adhesion[13]. Phagocytes that have passed the endothelium accumulate in the extracellular matrix, where they encounter signals that direct it further towards the site of infection. These signals include chemotactic factors like chemokines (i.e. IL-8) and cleavage products of the complement system (C5a) or formylated bacterial peptides[14]. Release of the chemotactic factors leads to migration of the phagocytes to the actual site of infection, where they can finally perform their ultimate function: the killing of microbes.

2.2. Phagocytosis

Phagocytosis is the process by which macrophages and neutrophils engulf particles, including microorganisms. Internalization is initiated by the interaction of specific receptors on the surface of the phagocyte with ligands on the surface of the particle. These receptors include the Fc-receptors (FcR), complement receptors (CR) and pattern recognition receptors such as scavenger receptors and lectins. The two best characterized phagocytic receptors are the Fc gamma receptors (FcγRs) and complement receptor 3 (CR3). FcγRs bind to immunoglobulin G (IgG)-coated targets, whereas CR3 binds C3bi on complement-opsonized targets. Recognition by the phagocytic receptor triggers signaling events that induce reorganization in the actin cytoskeleton that is instrumental in particle uptake and lysosomal degradation. Depending on the phagocytic receptor, the signaling pathways and mechanisms of uptake appear to be different[15-17]. For instance, FcγR mediated uptake is accompanied by pseudopod extension and membrane ruffling and involves Rac1 and Cdc42, two Rho family GTPases, whereas complement-opsonized targets sink into the cell, producing little protrusive activity and requires only RhoA and not Rac1 and Cdc42[18;19]. Moreover, FcγR-mediated phagocytosis is accompanied by the activation of the respiratory burst, which leads to the production of reactive oxygen species, and by the production of arachidonic acid metabolites and cytokines[20;21], while CR3 dependent uptake occurs in the absence of these proinflammatory mediator production, although it should be noted that the production of reactive oxygen species does occur in neutrophils after CR3 ligation[22;23].

Phagocytosis is not only required for the clearance of microbes but also for homeostatic clearance of apoptotic cells during tissue remodeling and embryogenesis. Receptors that participate in phagocytosis of apoptotic cells include scavenger receptors of the class A and B[24] and CD14[25].

2.3. Antimicrobial activities

Phagocytosis results in engulfment of the microbe and its containment within a sealed phagosome. This is followed by fusion of the phagosome with lysosomal vesicles containing an array of microbicidal products. The microbicidal mechanisms of the phagocytes can be categorized as either oxygen-dependent or oxygen-independent. The oxygen-dependent microbicidal mechanism of phagocytes, which is also known as the respiratory burst, is mediated by a complex enzyme nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase. NADPH oxidase is composed of two membrane proteins, $gp91^{phox}$ and $p22^{phox}$ [26], which together form the catalytic component of the oxidase, the cytosolic proteins $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ [27;28] and the small GTPase Rac. There are two isoforms of Rac, Rac1 and Rac2. Rac1 is the predominantly expressed isoform in monocytes and macrophages and Rac2 is the isoform in neutrophils and eosinophils[29;30]. Upon stimulation, the cytosolic components and the small GTPase Rac translocate to the plasma membrane to form the active NADPH oxidase, which transfers two electrons from NADPH to two molecules of oxygen to form superoxide anion (O_2^-)[31;32]. The superoxide produced by the NADPH oxidase complex forms the basic compound from which other reactive oxygen species

(ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), are formed[33;34]. High concentrations of ROS are toxic and result in microbial killing, either directly or perhaps also indirectly by the activation of hydrolytic proteases[35]. The importance of the respiratory burst for host defense is exemplified by patients with chronic granulomatous disease (CGD) that have a dysfunctional NADPH oxidase. The phagocytes of these patients are unable to produce ROS and their efficiency in bacterial killing is significantly impaired, making these patients extremely susceptible to a range of bacterial and fungal infections, including in particular *Staphylococcus aureus* and various *Salmonella* and *Aspergillus* species[36].

In comparison with the oxygen-dependent microbicidal mechanisms, the oxygen-independent microbicidal mechanisms are less well defined. These mechanisms include acidification of the phagolysosome, nutrient depletion and the microbicidal actions of antimicrobial proteins or peptides. In neutrophils these antimicrobial components are present in granules, which upon phagocyte activation release their contents into the phagosome. Most of the antimicrobial proteins are stored within primary (azurophilic) granules. Lysozyme is contained in both the primary and the secondary (specific) granules of neutrophils[37]. It cleaves important linkages in peptidoglycan of bacterial cell walls and can act in concert with the complement membrane attack complex (MAC)[38]. Other antimicrobial proteins are bactericidal/ permeability-increasing protein[39], which is active only against gram-negative bacteria, the α-defensins[40], cathelicidin and a group of peptides called p15s[41]. α-Defensins are active against a range of bacteria, fungi, chlamydiae, and enveloped viruses[40].

2.4. Anti-tumor activities

In addition to a role in the elimination of cells infected with microorganisms, including viruses or intracellular bacteria, phagocytes also play a role in the killing of tumor cells. Their crucial role in the defense against tumor cells was confirmed by elimination of macrophages by intravenous injection of liposomes containing the toxic substance dichloro-methylene biphosphonate (Cl_2MDP-liposomes) or granulocyte depletion by intraperitoneal injection of Gr-1 antibody, which led to massive outgrowth of the tumors[42;43]. Phagocytes may kill the tumor cells by the release of cytokines, such as Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1β (IL-1β) and IL-6 and nitric oxide that recruit multiple cell types like T and NK cells[44-47]. Additionally, they can display antibody-dependent cellular cytotoxicity (ADCC), by binding antibody-coated tumor cells. After binding of the target through ligation of the Fc part of the antibodies with the Fc receptor (FcR) on monocytes and macrophages, lysis of the target is achieved by means of respiratory burst mechanisms. Monoclonal antibodies directed against the tumor cells are being employed to initiate antibody-dependent cell mediated cytotoxicity (ADCC) to tumor cells[48]. These therapeutic antibodies, which are directed against tumor cells, act by binding to tumor-associated antigens more or less specifically expressed on the tumors cell and turning the antibody-labeled tumor cells into targets for killing by immune cells. The monoclonal antibodies Rituximab directed against CD20 on both normal B cells and on most low-grade and some higher grade B-cell lymphomas, and Trastuzumab that recognizes the HER-2/neu growth factor receptor on

metastatic breast cancer cells have demonstrated that monoclonal antibodies are well tolerated and capable of initiating tumor regression in a significant percentage of patients[49]. The list of therapeutically available antibodies is likely to grow further in the next decade, since a considerable number of antibodies against different tumors is still being validated in clinical trials.

3. Phagocyte development and acute myeloid leukemia

The differentiation of stem cells into the two main myeloid lineages, the monocytic and the granulocytic lineages, is orchestrated by a hierarchical network of transcription factors. PU.1, an Ets family member, is one of the master transcription factors identified to regulate development of both granulocytes and monocytes/macrophages[50]. Further, C/EBPα, a basic-region leucine zipper DNA-binding protein responsible for the transactivation of G-CSF receptor gene, and retinoic acid receptors (RARs) have been suggested to be important in granulocyte differentiation. On the other hand, MafB, c-Maf, and Egr-1 have been suggested to promote monocytic differentiation at the cost of granulopoiesis[51]. In addition to the transcription factors, development of myeloid progenitors from stem cells also depends on the specific microenvironment of hematopoietic tissues. The close interaction of stem cells with stromal cells locally in the bone marrow has been suggested to play a crucial role in the maintenance of stem cells. Various signaling systems are involved in this process, such as stem cell factor/c-kit, Notch/Notch ligands, Wnt/Frissled, and Kiree. At later stages of myeloid cell maturation, soluble cytokines secreted by stromal cells such as G-CSF and M-CSF are important to maintain the homeostatic myelopoiesis[52]. Hematopoietic cytokines produced by T cells and macrophages, such as IL-3 and GM-CSF, are indispensable for steady-state myelopoiesis, but may also promote emergent myelopoiesis during infection[53].

Acute myeloid leukemia (AML) is the result of malignant transformation of myeloid progenitor cells. AML is characterized by a block in normal myeloid differentiation from stem cells to monocytes or granulocytes[54]. According to standard French-American-British (FAB) classification, AML is divided into subgroups (M0-M7), which are defined by their degree of differentiation along one of the myeloid lineages. A common genetic aberration responsible for the block in differentiation of AML blasts is chromosomal translocation. Genes encoding transcription factors that are important for normal hematopoietic development are frequently targeted by balanced chromosomal translocations in AML. These include core binding factor (CBF), retinoic acid receptor alpha (RARα), and members of the HOX family of transcription factors[54-56]. Further, transcriptional coactivators, such as Creb-binding protein (CBP), p300, MOZ, TIF2, and MLL, are also targets of chromosomal translocations in AML[55;56]. The consequence of chromosomal translocations found in AML is a loss-of-function and therefore impaired hematopoietic differentiation. For example, CBF is a heterodimeric transcription factor composed of a DNA-binding component, AML1, and the CBFβ subunit that functions as a transcriptional activator of AML1. CBF controls the expression of genes that are critical for normal hematopoietic development. The three most common translocations involving CBF are the t(8;21), inv(16) and t(12;21), which result in expression of the

AML1/ETO, CBFβ/SMMHC and TEL/AML1 fusion proteins, respectively. Each of these fusion proteins is a dominant negative inhibitor of CBF-mediated transcription. Transcriptional repression of CBF target genes by CBF-related fusion proteins is thought to be mediated through aberrant recruitment of the nuclear corepressor/histone deacetylase complex. Another example is formed by fusion genes involving RARα, such as PML/RARα associated with the t(15;17) translocation that is specifically found in acute promyelocytic leukemia (APL). Expression of the PML/RARα fusion protein results in a block of differentiation at the promyelocyte stage caused in part by the recruitment of the nuclear corepressor complex. This block is relieved by all-*trans*-retinoic acid (ATRA), which forces the release of the nuclear corepressor complex by binding to PML/RARα. In addition to these direct inhibitory effects on transcriptional activation, the fusion proteins may also have additional roles in impairment of hematopoietic differentiation[55]. Although the contribution of many leukemic fusion proteins in AML is evident, it has become clear from studies with transgenic animals that express AML fusion proteins and from the analysis of patient samples that secondary mutations are generally also required for the establishment of AML[55]. In a significant fraction of AML, activating mutations are found in Ras proteins (N-ras and K-Ras) and in receptor tyrosine kinases (RTK) that promote the growth of normal hematopoietic cells (e.g. c-kit and FLT-3).

3.1. Phagocyte heterogeneity

Not only granulocytes but also macrophages constitute a highly heterogeneous population of cells. The heterogeneity reflects the specialization of function that is adopted by macrophages in different anatomical locations, such as the Kupffer cells in the liver, alveolar macrophages in the lungs, mesangial macrophages in the kidneys and the osteoclasts in the bone. Macrophage heterogeneity is not only reflected by the location and function but also the receptors they express. Both the alveolar macrophages, which are involved in clearing microorganisms, viruses and environmental particles in the lung, and marginal-zone macrophages, located adjacent to the marginal sinus in the marginal zone of the spleen and aiding in the clearance of blood-borne pathogens, have a high expression of pattern-recognition receptors and scavenger receptors[57-59]. Even within a single organ considerable macrophage heterogeneity can be observed. For example in the spleen, which harbours red pulp macrophages, tingible-body macrophages, marginal-zone macrophages and metallophilic macrophages. The red pulp macrophages play an important role in the clearance of aged erythrocytes and also have the capacity to support extramedullary erythropoiesis. In contrast, the tingible-body macrophages located in the germinal centers are primarily involved in the clearance of apoptotic lymphocytes that are generated during the development of an adaptive immune response[60]. The marginal zone macrophages are probably involved in the killing of at least certain blood-borne pathogens, and the function(s) of the metallophilic macrophages is still not known.

3.1.1. Osteoclasts

Osteoclasts are the principal, if not the only cells that resorb bone. They are specialized

macrophages with a clear ontogenetic relationship to macrophages. Early nonspecific differentiation along the osteoclast pathway is dependent on PU.1 and the MITF family of transcription factors, as well as the macrophage proliferation and survival cytokine M-CSF[61-64]. TNF-related factor, known as receptor activator of NF-κB ligand (RANKL), commits the cell to the osteoclast fate, and activates tumour necrosis factor receptor associated factor 6 (TRAF6), c-Fos and calcium signaling pathways, all of which are indispensable for the induction and activation of nuclear factor of activated T cells c1 (NFATc1), the master transcription factor for osteclastogenesis[65-68].

The bone resorption activity of mature osteoclasts is determined by a balance between positive and negative signals from the environment. Reorganization of the actin cytoskeleton plays a pivotal role in this context, as it allows for the formation of an acidified extracellular milieu that supports bone resorption[69;70]. For instance, binding of the mineralized bone matrix by integrins, such as by the αvβ3-integrin, promotes actin reorganization and bone resorption. This involves an intracellular signaling pathway composed of the αvβ3 integrin-associated c-Src tyrosine kinase, the immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor proteins, DAP12 and FcRγ, the tyrosine kinase Syk, the GEF Vav3 and the small GTPase Rac[71]. Consistently, mice deficient in c-Src or Syk develop a severe osteopetrosis, which is marked by increased bone density, and apparently due to a diminished osteoclast activity and not to a defect in osteoclast development[72-74]. Osteopetrosis, albeit less severe, is also observed in mice that lack DAP12 and/or FcRγ, and this is due to defects in both osteoclast differentiation and activation[75;76]. On the other hand, *motheaten* me(v)/me(v) mice with a defect in protein tyrosine phosphatase (PTPase) SHP-1, develop severe osteoporosis (lose of bone), which is associated with an increase in the extent of differentiation and activation of osteoclasts[77;78]. SHP-1 is a major cytosolic PTPase present in hematopoietic cells, including osteoclasts, that is activated upon recruitment to a phosphotyrosine immunoreceptor tyrosine-based inhibitory motifs (ITIMs) present in the cytoplasmic regions of a variety of inhibitory receptors. Clearly, dysregulation of differentiation and function may lead to pathological conditions such as osteoporosis or osteopetrosis.

4. Regulation of phagocyte function

The specialized functions of phagocytes, such as migration, phagocytosis, microbial killing or bone resorption, have to be under tight control in order to prevent unwanted or prolonged activation, which could result in severe damage to the host. Activation of these phagocyte functions relay on cytokine receptors, pattern recognition receptors such as Toll-like receptors, or immunoreceptor tyrosine-based activating motifs (ITAMs). ITAMs can be found in the intracellular tail of the activating immune receptors or in chains associating to the transmembrane domain of receptors, and are characterized by the amino acid sequence YxxL/Ix$_{(6-12)}$YxxL/I, where x denotes any amino acid[79]. Receptors signaling via ITAMs include for instance the Fc receptors. Upon crosslinking of the receptor, tyrosines in the ITAM are phosphorylated by Src family kinases, and activating signal transduction molecules such as protein tyrosine kinases are recruited[80].

As counterbalance to the activating immunoreceptor signaling immune cells, including phagocytes, express a variety of inhibitory receptors. Most inhibitory immune receptors contain one or more immunoreceptor tyrosine based inhibitory motifs (ITIMs), defined as the amino acid sequence I/V/L/SxYxxV/L. The inhibitory receptors signal via phosphorylation of the tyrosine in the ITIM, which leads to recruitment of phosphatases such as SHP-1, SHP-2 or SHIP. These phosphatases can then dephosphorylate activating molecules, leading to suppression of the activation signal. SHP-1 is restricted to hematopoietic cells and epithelial cells, whereas SHP-2 is broadly expressed. The SHP-1 motheaten mice, which have a mutation in SHP-1, have an increased number of macrophages and an increased level of macrophage activation[81]. As a result of this and/or defects in other leukocytes, these mice die from pneumonia a few weeks after birth[82]. In contrast, SHP-2-deficient mice die at the blastocyst stage, indicating a requirement of SHP-2 for normal development[83]. SHIP accumulates not only in immune cells, but also in non-hematopoietic cell types. SHIP-deficient mice have a number of hematopoietic perturbations, including enhanced cytokine responsiveness. This suggests that SHIP has a major role in modulating pathways important in homeostasis and regeneration of hematopoietic stem cells, and emphasize the importance of negative cytokine regulation at the earliest stages of hematopoiesis[84].

In recent years it has become clear that a large family of such ITIM-containing inhibitory receptors is present on phagocytes and other immune cells. Perhaps the best characterized are the killer inhibitory receptor (KIR/CD94) families on NK cells, which act as MHC class I receptors and negatively regulate the killing of target cells. Inhibitory receptors on phagocytes include ILT (Ig-like Transcripts), which is also known as LIR (Leukocyte Ig-like Receptor) or MIR (Monocyte/ macrophage Ig-like Receptor)[85;86], LAIR-1 (Leukocyte associated Inhibitory receptor 1)[87;88] and SIRPα[89]. SIRPα is a transmembrane glycoprotein abundantly expressed by phagocytes but also by neuronal cells. SIRPα is involved in the regulation of a variety of cellular functions of phagocytes, including growth, differentiation, adhesion and transendothelial migration[13]. In addition SIRPα was shown to negatively regulate the clearance and phagocytosis of antibody- or complement-opsonized erythrocytes by binding its ligand CD47[90]. Collectively, SIRPα acts as a versatile regulator of phagocyte cell function.

5. Outline of the thesis

Inhibitory receptors are hypothesized to regulate phagocyte effector functions and thereby to limit innate responses in both time and/or magnitude. SIRPα is one of the inhibitory receptors that may modulate these effector functions of the phagocytes. The aim of this thesis is to investigate the putative regulatory role(s) of SIRPα in phagocyte functions. In chapter 2 we review in detail the different functions that have been described until now of SIRPα and other SIRP family members in the immune system.

Phagocytes play a crucial role in host defense through their ability to recognize, ingest, and destroy invading microorganisms. One of the most important anti-microbial activities of phagocytes is the abrupt formation of reactive oxygen species (ROS), a process known as the respiratory burst. In chapter 3 we demonstrate that SIRPα is a

critical negative regulator of the phagocyte respiratory burst. Inhibition of the respiratory burst by SIRPα involves interactions with its ligand as well as direct signaling through the SIRPα ITIMs, which acts to selectively suppress the expression of the key catalytic component of the respiratory burst complex.

Phagocytes also have important roles in maintenance of the homeostasis, such as bone resorption. Chapter 4 shows that SIRPα signaling has no effect on osteoclast formation but inhibits the bone resorption capacity of mature osteoclast both *in vivo* and *in vitro*.

Previous reports have demonstrated that SIRPα is involved in the regulation of the clearance of erythrocytes and platelets by inhibition of the antibody (Ab)-dependent phagocytosis. In Chapter 5 we investigated whether SIRPα also inhibits the Ab-dependent phagocytosis and/or killing of tumor cells. We report significant reduction of tumor load in the presence of antibodies in SIRPα mutant mice, which lack the SIRPα cytoplasmic tail and therefore its capacity to signal. This suggests that CD47-SIRPα may be an important limiting factor for Ab therapy against tumor cells.

Chapter 6 and 7 provide evidence for a growth regulatory role of SIRPα in AML, including APL, and suggest that, at least in some AML subtypes, epigenetic SIRPα gene silencing contributes to the uncontrolled growth. Furthermore, the SIRPα-derived growth suppressive signal synergized with chemotherapeutic agents routinely used for treatment of AML.

Finally, in chapter 8 the results presented in this thesis are summarized and discussed.

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