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ROLE OF SOCS PROTEINS DURING MYCOBACTERIAL INFECTIONS

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Think outside the (SOCS) box.

ABSTRACT

Mycobacterium tuberculosis is the world's most successful bacterial killer. During infection, mycobacteria reside inside host cells encapsulated within a granuloma structure in the latent, asymptomatic phase of infection. Only 10% of latently infected develop active, infectious tuberculosis months or years after the initial infection. The mechanisms that determine latency and bacterial control as well as protection during active tuberculosis are still not fully understood. Members of the SOCS protein family are regulators of cytokine signaling via inhibition of JAK-STAT activation and their expression is increased during different kinds of infections. Therefore, the purpose of this thesis was to study the role of SOCS1, SOCS2 and SOCS3 during mycobacterial infections.

We demonstrated that infection with *M. tuberculosis in vitro* and in mice strongly upregulated SOCS1 expression. Interestingly, SOCS1 reduced IFN-γ secretion by macrophages in response to IL-12 rather than responses to IFN-γ itself. In line, SOCS1-deficient macrophages showed improved growth control of mycobacteria *in vitro*. Furthermore, in a mouse model of infection, we demonstrated that SOCS1 expression by macrophages impaired bacterial clearance before the onset of protective adaptive immune responses. However, SOCS1 did not hamper adaptive immune-controlled bactericidal mechanisms at later time points. At this stage of infection, SOCS1 expression by non-macrophage cells protected mice from severe immunopathology.

Additionally, we showed that SOCS2 expression was induced in an IRF3-dependent manner after infection with *M. bovis* BCG or incubation with LPS *in vitro*. However, SOCS2-deficient and control mice infected with *M. tuberculosis* displayed similar bacterial burdens in the lungs.

In studying the role of SOCS3 in different mouse models, we found that the lack of SOCS3 in either myeloid or T cells dramatically increased susceptibility to *M. tuberculosis* infection. During infection, SOCS3 expression in macrophages and dendritic cells was required to prevent an inhibitory effect of IL-6 on TNF and IL-12 secretion and elevated IFN-γ expression by CD4+ T cells. More detailed studies revealed that the lack of SOCS3 in myeloid cells could be mimicked by mutating the SOCS3 binding site of the gp130 receptor. This indicates that among the receptors, which can be regulated by SOCS3, the control over the IL-6 family gp130 receptor is fundamental for proper immune responses. Surprisingly, mice bearing SOCS3-deficient T cells were not susceptible to BCG infection. Moreover, a proper defense against challenge with *M. tuberculosis* infection was restored if mice deficient for SOCS3 in T cells had been BCG-vaccinated.

In conclusion, we demonstrated a pivotal role of SOCS1 and SOCS3 on the outcome of infection with *M. tuberculosis*. SOCS1 expression allows fast bacterial growth during the early phase of infection and protects from severe inflammation during later stages. SOCS3 expression in myeloid and T cells independently mediates resistance to *M. tuberculosis* infection by modulating T cell functions. Based on the obtained data, we suggest that SOCS3-regulated pathways are promising targets for future therapies as well as vaccination strategies.

POPULAR SCIENCE SUMMARY

Tuberculosis is a long known infectious disease that continues to cause 1.5 million deaths every year. Although treatment is available, it is long lasting and the numbers of antibiotic-resistant cases are increasing. Moreover, the available vaccine is not efficient in adults. To combat tuberculosis, more knowledge about how the immune system can protect against *M. tuberculosis* is required. Members of the SOCS protein family have important functions in the inhibition of immune signaling pathways. Since proper regulation and balance of immune responses during mycobacterial infections is crucial for infection control, we investigated the role of SOCS1, SOCS2 and SOCS3 during *M. tuberculosis* infection.

We chose mice as the animal model of infection for our studies. In order to investigate the role of the different SOCS molecules during mycobacterial infections, we took advantage of mice lacking these specific proteins and compared their outcome of infection with infected control mice.

Regarding the function of SOCS1 in *M. tuberculosis*-infected mice, we found that SOCS1 impaired early immune activation leading to increased bacterial numbers in the lungs. However, presence of SOCS1 was beneficial for the infected host restricting infection-induced inflammation at later time points.

For SOCS2, we demonstrated that SOCS2 expression was upregulated in mycobacteria-infected macrophages, but SOCS2-deficient mice did not show increased susceptibility to *M. tuberculosis* infection.

Furthermore, we studied the role of SOCS3 in different immune cells, which are known to be important during *M. tuberculosis* infection. We found that the lack of SOCS3 in myeloid cells, which belong to the first line of defense, as well as in T cells, which belong to the adaptive immunity as second line of defense, dramatically increased susceptibility to *M. tuberculosis* infections. Interestingly, if mice deficient for SOCS3 in T cells were vaccinated with the attenuated *M. bovis* strain BCG before *M. tuberculosis* infection, they could control *M. tuberculosis* infection equally well as mice expressing SOCS3.

Summarizing, we illustrated a role for SOCS1 and SOCS3 during mycobacterial infections, whereas the function of SOCS2 was shown to be minor or redundant. SOCS1 improves bacterial growth during the early phase of infection and protects from severe inflammation during later stages. SOCS3 expression in myeloid and T cells is required to mediate resistance to *M. tuberculosis* infection. This makes SOCS3-regulated pathways promising targets for future therapies and suggests that their modulation may improve vaccination as well as the control of mycobacterial infections.

LIST OF PUBLICATIONS

I. ***Carow B**, *Ye X, Gavier-Widén D, Bhuju S, Oehlmann W, Singh M, Sköld M, Ignatowicz L, Yoshimura A, Wigzell H, Rottenberg ME.
Silencing suppressor of cytokine signaling-1 (SOCS1) in macrophages improves Mycobacterium tuberculosis control in an interferon-gamma-dependent manner.
The Journal of Biological Chemistry 2011 Jul 29;286(30):26873-87.

II. Hu J, Lou DH, **Carow B**, Winerdal ME, Rottenberg ME, Wikström AC, Norstedt G, and Winqvist O.
LPS regulates SOCS2 transcription in a Type I interferon dependent autocrine-paracrine loop.
PLoS One. 2012;7(1):e30166. Epub 2012 Jan 23.

III. **Carow B**, Reuschl AK, Gavier-Widén D, Yoshimura A, Chambers B, Rottenberg ME.
Critical and independent role for SOCS3 in either myeloid or T cells in resistance to Mycobacterium tuberculosis.
Manuscript

* authors contributed equally

Publication not included in the thesis

Heuts F, **Carow B**, Wigzell H, Rottenberg ME.
Use of non-invasive bioluminescent imaging to assess mycobacterial dissemination in mice, treatment with bactericidal drugs and protective immunity.
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LIST OF ABBREVIATIONS

BMDC	Bone marrow-derived dendritic cells
BMM	Bone marrow-derived macrophages
CpG	Cytidine-phosphatate-guanosin
C. pneumoniae	*Chlamydia pneumoniae*
DC	Dendritic cell
EAE	Experimental autoimmune encephalitis
GH	Growth hormone
G-CSF	Granulocyte colony-stimulating factor
HIV	Human immunodeficiency virus
IFN	Interferon
IFNar1	Type I IFN receptor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRAK	Interleukin-1-associated kinase
IRF	Interferon regulatory factor
JAK	Janus kinase
JNK	c-Jun N-terminal kinase
KIR	Kinase inhibitory region
LCMV	Lymphocytic choriomeningitis virus
LIF	Leukemia inhibitory factor
L. major	*Leishmania major*
LPS	Lipopolysaccharide
MAL	MyD88-adaptor-like protein
M. bovis BCG	*Mycobacterium bovis* Bacillus Calmette-Guérin
MSMD	Mendelian susceptibility to mycobacterial diseases
M. tuberculosis	*Mycobacterium tuberculosis*
MyD88	Myeloid differentiation factor 88
NF-κB	Nuclear factor-κB
NK	Natural killer
NO	Nitric oxide
PIAS	Protein inhibitors of activated STAT
R	Receptor

RAG	Recombination activating gene
SH2	Src homology 2
SHP2	Src homology tyrosine phosphatase 2
siRNA	Small interfering RNA
SOCS	Suppressor of cytokine signaling
STAT	Signal transducer and activator of transcription
TGF-β	Transforming growth factor-β
TLR	Toll-like receptor
TNF	Tumor necrosis factor
T. gondii	*Toxoplasma gondii*
Treg	Regulatory T cell
TRIF	Toll-IL-1 receptor domain-containing adaptor-inducing IFN-β
WHO	World Health Organization
WT	Wild type

1 INTRODUCTION

1.1 *MYCOBACTERIUM TUBERCULOSIS*

Mycobacterium tuberculosis is the causative agent of the infectious disease tuberculosis[1]. It is estimated that around one third of the global population is currently infected with *M. tuberculosis*, but the probability of those infected to develop active tuberculosis during their lifetime is only around 10%[2]. Prediction for onset of clinical disease is difficult as critical biomarkers in infected but healthy individuals are not available[3]. However, the risk of developing active tuberculosis increases to 10% per year if a co-infection with the human immunodeficiency virus (HIV) and *M. tuberculosis* occurs[4], suggesting that immune responses are responsible for the asymptomatic infection. In 2010, the World Health Organization (WHO) reported for tuberculosis an incidence of over 8 million people and 1.2-1.5 million deaths (including deaths from tuberculosis among HIV-positive patients) making tuberculosis the second leading cause of death from an infectious disease worldwide, after HIV[5]. Other immune suppressive conditions such as diabetes, alcoholism, smoking, advanced age and malnutrition are known to promote disease[6,7]. However, a detailed understanding of why reactivation of *M. tuberculosis* occurs in some individuals has yet to be determined.

The treatment regiment for tuberculosis with combined antibiotics is long, but efficient when followed properly. Due to insufficient drug treatment, drug-resistant *M. tuberculosis* strains have developed over the last decades, including strains resistant to isoniazid and rifampicin, the standard treatment antibiotics, as well as extensively drug-resistant strains that additionally resist treatment with one fluoroquinolone or injectable second-line drug[5,8].

The only available vaccine against tuberculosis is the attenuated *Mycobacterium bovis* Bacille Calmette Guérin (BCG) that was first introduced in 1921. BCG is one of the most used vaccines worldwide, but protection to tuberculosis, especially in adults, varies from 0% to 80%[9-11]. Moreover, BCG vaccination does not provide sterilizing immunity that is needed to prevent the establishment of a latent persistent *M. tuberculosis* infection or active pulmonary tuberculosis. However, over the last years several new tuberculosis vaccines have reached phase I clinical trials[12].

Mycobacteria are non-motile rods of 2-4 micrometers length. Due to the high lipid content of its cell wall, *M. tuberculosis* cannot be classified as Gram-positive or

Gram-negative, but it can be identified via acid fast staining (Ziehl-Neelsen stain). The cell wall consists of peptidogycans and complex lipids, where the major lipid components are mycolic acid, cord factor and wax-D. Different compositions of the cell wall can contribute to the virulence of different mycobacteria strains[13]. *M. tuberculosis* is an obligate aerobe and a facultative intracellular pathogen, and it usually infects myeloid cells. *M. tuberculosis* has a slow growing rate with a doubling time of 18 to 24 hours, so that cultures of clinical specimens must be held for 6-8 weeks, which makes diagnostics difficult.

1.1.1 Experimental models of *M. tuberculosis*

As *M. tuberculosis* has a very limited host range with no known natural hosts beyond humans[14], alternative models to study the disease have been tested. Based on the infection of phagocytes by *M. tuberculosis*, cell cultures of primary murine macrophages, human monocyte-derived macrophages and dendritic cells, have been applied to investigate pathogen-host interactions on intracellular and cellular levels[15].

The first animal model, the guinea pig, was used by Robert Koch when he identified *M. tuberculosis* as the causative agent of tuberculosis in 1882[1]. Guinea pigs show clinical symptoms around 30 days after infection and survive for 100-140 days[16,17]. Importantly, lung responses to mycobacterial stimuli in guinea pigs are similar to human responses, even leading to the development of primary pulmonary granulomas, which closely resemble human granulomas[17-19]. The hallmark of human granulomas, necrotic centers, are found in infected guinea pigs making them a valuable model to test efficacy of tuberculosis vaccines and anti-mycobacterial drugs[20,21].

Mice are the most widely used experimental animal model today due to their lower overhead costs as compared to monkeys or guinea pigs, as well as their high reproductive rate, the availability of reagents to characterize mouse responses and established methods for manipulation of gene expression with knockout, conditional knockdown and transgenic mice. The infection of mice is performed either intravenously, intranasally or via aerosol, the latter mimics the natural infection route in humans. A major drawback of the mouse model is the relatively small protective effect of BCG vaccination that reduces bacteria loads after *M. tuberculosis* challenge around 1 log, in comparison to the 2-3 logs reduction seen in guinea pigs[21]. Furthermore, the formed granulomas in infected mice are not highly structured;

consisting of cellular infiltrations with irregular borders, whereas a central necrosis and caseation, as found in human tuberculosis, is absent[22].

Mycobacteria in aerosol-infected mice replicate progressively in the lungs during the first 3 weeks after infection (Fig. 1)[23]. The onset of adaptive immune responses slows down the bacterial growth, reduces bacteria levels approximately 1 log, and after 4-5 weeks of infection, bacterial levels reach a level that will remain constant throughout the infection (Fig. 1)[24].

Tuberculosis is typically manifested in the lungs but can also affect other sites, described as extra-pulmonary tuberculosis. In mice, bacterial dissemination to other organs such as the draining lymph node, spleen and liver can be detected after 10 days of aerosol infection[25]. Depending on the initial dose and genetic background of infected mice, they may survive for longer than 200 days, but since all mice develop active disease, they do not provide a model to study latency[26].

One experimental option to mimic latency in mice is the treatment of *M. tuberculosis*-infected mice with anti-mycobacterial drugs to reduce the bacterial load to undetectable levels followed by either spontaneous or induced reactivation of infection[27,28]. However, it is difficult to evaluate how well this model reflects *M. tuberculosis* latency in humans.

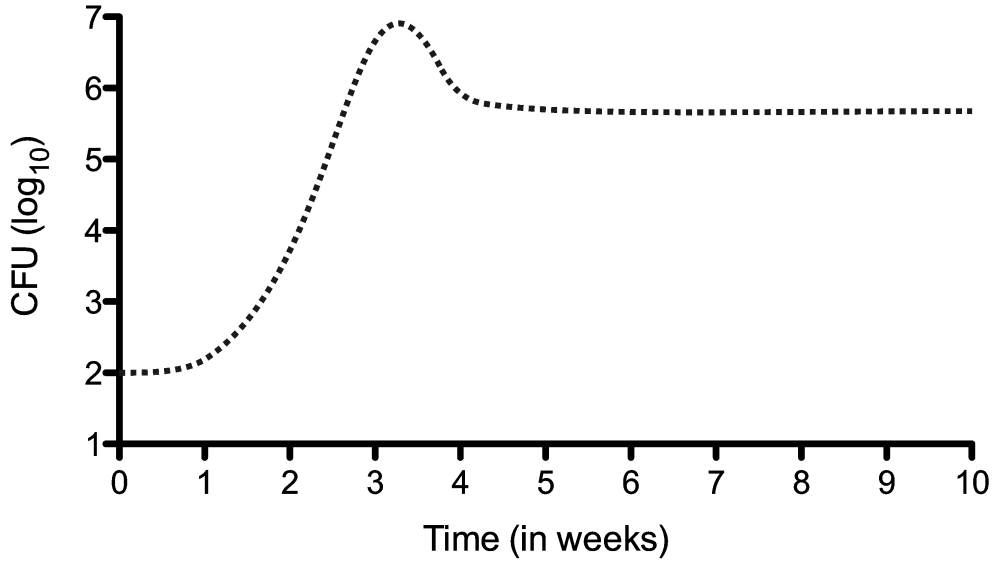

Figure 1: Kinetic of colony forming units (CFU) of *M. tuberculosis* in lungs of aerosol-infected mice (adapted from [29]).
Mycobacteria replicate progressively in the lungs during the first 3 weeks of infection, bacterial numbers peak after around 4 weeks and stay at a plateau for more than 10 weeks.

The outcome of infection in cynomolgus macaques displays strong similarities with human disease progression. After a low dose infection some primates generate latent infections, an important stage of *M. tuberculosis* infection that cannot be investigated in the commonly used mouse model[30].

1.1.2 Transmission of *M. tuberculosis*

When infected humans with active pulmonary tuberculosis cough or sneeze, they can spread infectious droplets that are required for transmission of *M. tuberculosis*. Infection occurs if inhaled droplets of bacilli reach the alveoli of the lungs. Exhaled droplets or nuclei are known to remain in the atmosphere for several hours and the infectious dose is low, probably under 10 bacteria[31].

1.1.3 Innate immune responses

The first contact between inhaled mycobacteria and the host is the uptake of *M. tuberculosis* by resident alveolar macrophages within the airways. The ingested mycobacteria are often destroyed directly depending on the mycobactericidal capacities of the host phagocyte and the virulence of the mycobacterium. Pathogenic mycobacteria replicate in the phagosome of the primary host cell and successfully prevent fusion of the phagosome with late endosome/lysosome organelles[32,33]. Moreover, virulent mycobacteria were shown to escape from the lysosomes into the cytosol[34]. Phagocytosis of mycobacteria can be mediated by several receptors such as complement, mannose and scavenger receptors[35-37]. The binding of *M. tuberculosis* molecules to various pattern recognition receptors stimulates the activation of innate immune responses in the host. For *M. tuberculosis*, recognition mainly by toll-like receptor (TLR) 2, 4 and 9 and subsequent activation of MyD88 and TRIF-dependent pathways has been described[38-42]. This innate immune activation leads to up-regulation of co-stimulatory molecules and production of cytokines by infected phagocytes. In addition to macrophages, dendritic cells and neutrophils can also be infected with *M. tuberculosis*[43]. However, innate immune mechanisms are not sufficient to control mycobacterial growth, so that adaptive immune responses need to be activated as a support. Infected dendritic cells migrate to the draining lymph nodes to activate naïve T cells, whereas macrophages recruit immune cells to the site of infection by secretion of attractant chemokines[24]. The following steps are required for a successful protective immune response to *M. tuberculosis*: (1) activation of

antigen-specific effector T cells that migrate back to the lung to activate infected macrophages and (2) the formation of granulomas.

1.1.4 Adaptive immune responses

Antigen-specific CD4+ and CD8+ T cells were shown to be required for the control of *M. tuberculosis* infection[44-46]. This is especially apparent in HIV patients, in which a decline of CD4+ T cells coincides with tuberculosis disease progression[47]. Moreover, deficiency of these cell populations increased susceptibility in murine models of the acute and chronic infection[48,49]. Dissecting the role of T cell subsets in mice demonstrated that the control of *M. tuberculosis* infection was to a greater extent dependent on CD4+ than on CD8+ T cells[46].

IL-12 produced by dendritic cells is crucial for the initiation of the adaptive immune response and stimulates the development of the CD4+ T cell subpopulation Th1 in response to *M. tuberculosis* infection. The importance of IFN-γ-secreting Th1 cells during *M. tuberculosis* infection is well established in mice and humans[50-53]. Moreover, lack of IFN-γ as well as the Th1 specific transcription factor Tbet, increases susceptibility to *M. tuberculosis* infection in mice[50,54].

IFN-γ produced by Th1 cells during *M. tuberculosis* infection counteracts the generation of Th17 cells and thereby regulates the balance between Th1 and Th17 cells[55]. Th17 cells are characterized by the secretion of IL-17 and the expression of RORγ transcription factor,[56] and have been shown to be induced during *M. tuberculosis* infection[57].

Interestingly, the onset of adaptive immune responses after *M. tuberculosis* infection via aerosol is delayed in comparison to other pathogens. In the mouse model, the arrival of antigen-specific T cells into the lung associated with mycobacterial growth restriction, typically occurs 3 to 4 weeks after infection. Studies using transgenic mice that express T cell receptors specific for mycobacterial antigens suggested two time limiting steps: (1) the arrival of infected dendritic cells in the draining lymph node and (2) a threshold of around 1500 mycobacteria in the lymph node that are required for naïve antigen-specific T cells activation[24]. Studies comparing relatively resistant to more susceptible mouse strains showed that bacteria in resistant mouse strains appeared earlier in draining lymph node and could therefore sooner mount an antigen-specific T cell response [25].

The induction of pathogen-specific regulatory T cells (Tregs) may contribute to the delay of T cell responses during *M. tuberculosis* infection. In humans and mice, regulatory T cells were found to expand and accumulate at the site of infection[58-61]. Regulatory T cells, characterized by expression of transcription factor foxp3 and surface markers CD4 and CD25, are important to down-regulate immune activation to prevent autoimmune diseases[62], but their role during tuberculosis infection is under debate. On the one hand, transfer of *M. tuberculosis*-specific T regs in mice resulted in increased bacterial burdens in the lungs, whereas others reported an only transient reduction of bacterial loads after depletion of CD25+ T cells[63,64].

1.1.5 Granuloma formation

The hallmark of tuberculosis and other diseases, the granuloma, typically consists of a core of infected macrophages or epithelioid cells, and giant cells surrounded by foamy macrophages and other mononuclear phagocytes encircled by a mantle of lymphocytes. Mature granulomas are further encapsulated by collagen fibers. The initiation of a stable granuloma during a subclinical stage of infection leads to containment of the mycobacteria and thus is considered to be a host-protective structure[65,66]. This exclusive perspective is questioned by recent findings suggesting granulomas as a niche, in which the mycobacterium can grow and persist[67]. The environment of the granuloma is believed to respond to mycobacteria, and mycobacterial antigens were shown to be required for the granuloma formation[68]. Mycobacteria-infected macrophages undergo necrosis, resulting in a caseous center of the lesion that is characterized by low oxygen, pH and nutrient levels[69]. The replication of mycobacteria is reduced, and bacteria differentiate into a latent stage, but are not eradicated[69,70]. Yet, in less immune-competent or immune-compromised individuals, the fibrous walls lose their integrity, allowing the bacteria to reach the bronchi (transmission of infection) or the blood vessels (dissemination of infection). Tuberculosis can be manifested in almost any organ, but it occurs most frequently in the lungs and lymph nodes, or as generalized lesions resulting from haematogenous dissemination. Cases of genital, skin, meningeal, bone and hepatic tuberculosis are frequent in highly endemic countries[71].

1.2 CYTOKINES

Cytokines are usually soluble protein messengers that coordinate the development and function of immune cells, and therefore are indispensable for the initiation, maintenance and termination of immune responses[72]. The group of cytokines includes interleukins and other cell signal molecules, such as tumor necrosis factor and interferons, which are secreted by activated cells to trigger inflammation and respond to infections[73]. Cytokines have a short half-life; acting in either an autocrine or paracrine fashion. Their mode of action is regarded as pro- or anti-inflammatory, depending on the change of activation status of the target cell. The balance of pro- and anti-inflammatory cytokines is an important mechanism to modulate the effect of immune responses: excess of anti-inflammatory cytokines can suppress immune activation, whereas excess of pro-inflammatory cytokines can create harmful immunopathology.

Cytokines exert their function by binding to specific cytokine receptors that can be either membrane bound or soluble, which need to bind to co-receptors on the target cell[73].

Cytokine receptors can be divided in different groups regarding structural homologies: type I and II cytokine receptors, TNF receptors, IL-1 family receptors, seven transmembrane receptors and G-protein-coupled receptors[73]. Type I and type II cytokine receptors do not possess an intrinsic cytoplasmic kinase activity and therefore are dependent on associated Janus kinases (JAKs) to mediate intracellular signaling after cytokine binding[74]. Binding of cytokines to their type I or II receptor chain leads to the formation of active receptor heterodimers (or cluster of heterodimers) and activation of associated JAKs, which cross-phosphorylate themselves and phosphorylate the cytokine receptor creating binding sites for the SH2 domain of the transcription factors "signal transducers and activators of transcription" (STATs)[74]. Recruited STATs are in turn phosphorylated by the adjacent JAKs and act as binding sites for the SH2 domain of another STAT, which also will be phosphorylated. The phosphorylated STAT dimer is released from the cytokine receptor and translocates into the nucleus where it acts as a transcription activator of specific target genes (Fig. 2). The specificity of the intracellular pathways is due to the presence of 7 different STAT molecules, activated by different receptors[75]. Moreover, STATs can form homo- or heterodimers depending on the activated

receptor, which increases the diversity of target promoters and thereby of gene patterns that can be activated.

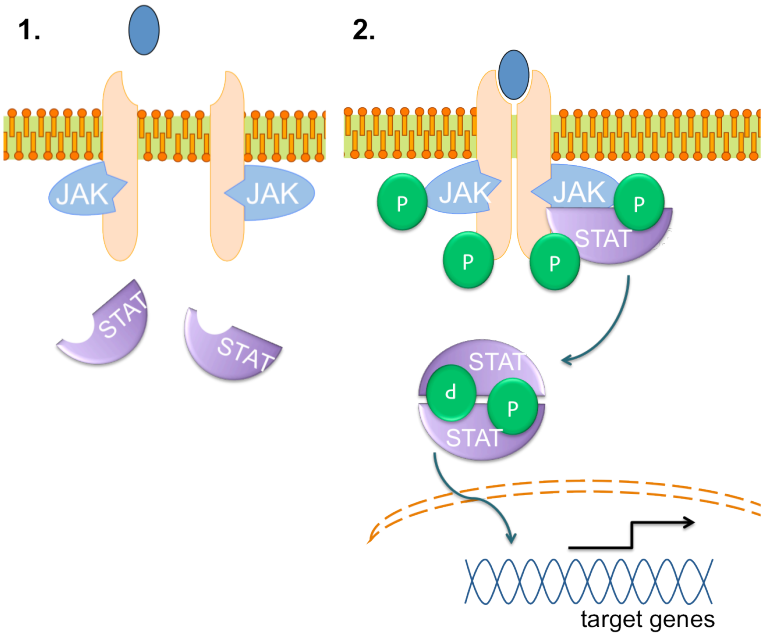

Figure 2: JAK-STAT pathway mediates responses to cytokines.
Binding of cytokines to their receptors leads to activation of the associated JAKs. Thereafter, STAT molecules get recruited to the receptor and are phosphorylated resulting in activated STAT dimers that act as transcription factors in the nucleus.

A tight regulation of cytokine functions is crucial for both the control of infections and the prevention of infection-associated immunopathology. Different intracellular mechanisms of cytokine signal inhibition are involved in the regulation of innate and adaptive immune responses. The JAK-STAT pathway can be negatively regulated at different stages: protein tyrosine phosphatases remove phosphates from cytokine receptors and activated STATs, SOCS proteins inhibit STAT activation and label cytokine receptors for degradation, whereas PIAS (protein inhibitors of activated STATs) act in the nucleus[76-79]. The role of several of these molecules in the control of infection and disease is not completely understood.

1.2.1 Tumor necrosis factor

Tumor necrosis factor (TNF, formerly TNF-α) is a pro-inflammatory cytokine, mainly produced by activated mononuclear phagocytes, but also by antigen-stimulated T cells and mast cells[80]. TNF is a typical mediator of acute inflammatory responses, sepsis, cachexia and can cause fever but also systemic complications leading to septic shock[81]. During infections, TNF is an important early cytokine that

recruits neutrophils and granulocytes to the site of infection and evokes microbicidal activities in macrophages and neutrophils[80].

TNF plays a diversified role during *M. tuberculosis* infection contributing to the control of mycobacterial growth as well as to the immunopathology as it participates in granuloma formation. Genetic deletion and neutralization of TNF in mice led to increased bacterial burden and lethality after *M. tuberculosis* infection[82,83]. Furthermore, patients receiving anti-TNF therapy (used for treatment of immune disorders as rheumatoid arthritis) showed an increased rate of reactivation of latent tuberculosis[84]. Similarly in mice, TNF was found to be essential to prevent reactivation in a model of latent *M. tuberculosis* infection[85-87].

As TNF-deficient mice displayed decreased or disorganized granulomas after mycobacteria infection, part of its protective role could be assigned to its influence on granuloma formation and maintenance[82,86,88-91]. Accordingly, TNF modulates the expression of the chemokines by *M. tuberculosis*-infected macrophages that recruit effector T cells, monocytes and macrophages to the site of infection[90,92,93]. Neutralization of TNF in a chronic infection, when granulomas were already formed, disorganized existing granulomas and aberrant pathology was observed[87]. However, the exact mechanisms for how TNF contributes to the control of mycobacterial growth are poorly understood and recent data in the zebrafish model showed that granuloma formation in *M. marinum* infection may be independent of TNF[94].

1.2.2 Interleukin-12

Interleukin-12 (IL-12) is an important initiator of adaptive immune responses promoting differentiation of naïve CD4+ T cells to Th1 cells, which produce IFN-γ and augment cell-mediated immune responses. Bioactive IL-12 (IL-12p70) is composed of two covalently linked subunits, p35 and p40, that bind to the receptor subunits IL-12Rβ2 and β1[95]. Binding of IL-12 to the receptor leads to activation of the Janus kinases JAK2 and TYK2, mediating phosphorylation and activation of the transcription factor STAT4 (Fig. 3)[95].

The role for IL-12 during *M. tuberculosis* infection was highlighted by the discovery that deficiencies for IL-12p40 and IL-12Rβ1 can lead to "mendelian susceptibility to mycobacterial disease" (MSMD) due to non virulent mycobacteria[96]. Moreover, mice lacking IL-12p40 or IL-12p35 showed highly increased susceptibility to *M. tuberculosis* infection[97]. In turn, exogenous administration of IL-12p70 increased resistance of BALB/c mice to *M. tuberculosis* infection[98].

IL-12p40 can be induced in response to *M. tuberculosis* in mononuclear cells by ligation of TLR2 and TLR9 *in vitro* while TLR9 is required for IL-12p40 induction *in vivo*[38]. Early induction of IL-12p40 during *M. tuberculosis* infection is crucial to enable the migration of infected dendritic cells to the draining lymph nodes followed by the activation of naïve T cells[99]. Although IL-12p40 homodimers IL-12$(p40)_2$ can restore the delayed hypersensitivity response in IL-12p35/IL-12p40-deficient mice, optimal expression of IFN-γ by antigen-specific T cells can only be achieved in the presence of IL-12p70[97,100]. Moreover, IL-12p70 is needed to maintain Th1 effector functions during *M. tuberculosis* infection, since exogenous IL-12p70 can reconstitute protection in *p40*$^{-/-}$ mice that is again lost after IL-12p70 removal[101,102]. This observation could be confirmed in humans with IL-12Rβ1-deficiency that fail to maintain a Th1 effector memory population showing that IL-12 plays indispensable roles not only in initiation but also maintenance of protection against mycobacteria[102].

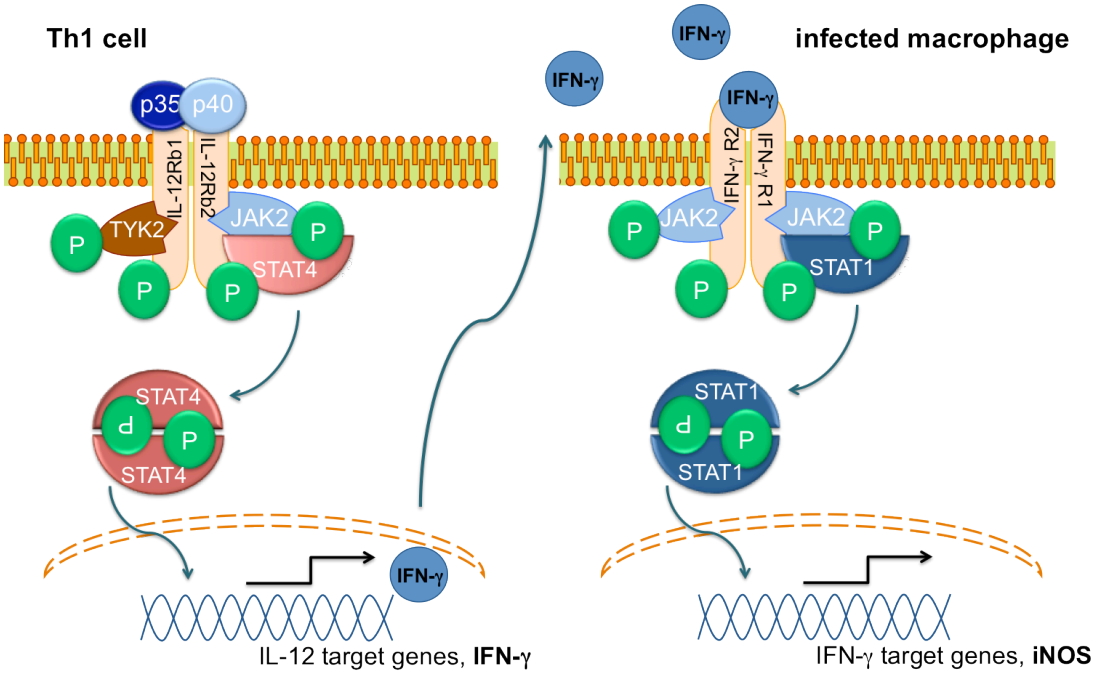

Figure 3: Th1 cells activate infected macrophages.
IL-12 induces IFN-γ production in Th1 cells via STAT4 activation that in turn activates infected macrophages. These macrophages induce the transcription of IFN-γ-responsive genes as *iNos*.

1.2.3 Interferon-γ

Interferon-γ (IFN-γ) is critical during intracellular bacterial and protozoal infections as well as tumor growth control, but aberrant IFN-γ production can induce autoimmune diseases[103-107]. Responses to IFN-γ are mediated through its interaction with the IFN-γ receptor that initiates the activation of STAT1 (Fig. 3)[108].

The importance of IFN-γ for the defense against *M. tuberculosis* has been demonstrated in experimental studies using knockout mice and was confirmed in patients with defects in signaling or production of IFN-γ as both show dramatically increased susceptibility to mycobacterial infections[50,109-112]. As previously discussed, CD4+ T cells play an important role during *M. tuberculosis* infection and are considered to be the main source of IFN-γ, whereas IFN-γ secreted by natural killer (NK) cells appears to be redundant[113,114].

Early appearance of antigen-specific IFN-γ-producing CD4+ T cells in the lungs coincided with earlier restriction of growth after aerosol *M. tuberculosis* infection[115]. Furthermore, a delay in priming of effector T cells led to increased bacterial burden in the lungs[63].

T cells elicit the bactericidal effects of infected macrophages. IFN-γ-activated macrophages were found to overcome bacteria-induced blockage of phagosome maturation and increased presentation of mycobacterial peptides to T cells and upregulated nitric oxide synthase 2 (iNOS2), an enzyme that produces nitric oxide (NO) and is involved in bacterial killing[116,117]. The role of NO in the control of mycobacterial intracellular growth is well established in the mouse model where *Nos2*$^{-/-}$ mice displayed increased susceptibility to *M. tuberculosis* infection. In humans, iNOS and NO production has been reported in alveolar macrophages in response to infection[106,118-122]. However, the role of NO in protection during human infection is controversial.

In the addition to the effect of IFN-γ on macrophages, it also was found in mouse radiation chimeras that responsiveness to IFN-γ by non-hematopoietic cells is involved in the protective immunity to *M. tuberculosis*[55].

Although the relevance of IFN-γ-producing T cells in protection is not up for debate, the numbers of IFN-γ-secreting T cells after vaccination do not always correlate with increased protection against *M. tuberculosis* infection[123,124]. This

suggests that more factors than IFN-γ production by CD4+ T cells should be assessed to determine protective immune responses to *M. tuberculosis* infection[49].

1.2.4 Interleukin-6

IL-6 is involved in innate and adaptive immunity and is produced by mononuclear phagocytes, endothelial cells, fibroblasts, adipocytes and muscle cells[73]. It is induced in response to pathogens, TNF and IL-1. IL-6 signals are transmitted via a receptor complex consisting of IL-6Rα and gp130, the common IL-6 family co-receptor. Binding of IL-6 to the receptor complex initiates activation of JAK1 followed by recruitment of several signaling molecule such as STAT3 and SHP2. STAT3, once phosphorylated, dimerizes and acts as a transcription factor in the nucleus, and SHP2 links IL-6 to the Ras-MAP kinase pathway. Depending on the target cell, IL-6 stimulates secretion of acute phase proteins by hepatocytes, neutrophil production in the bone marrow, growth of B lymphocytes and generation of Th17 cells[73].

Historically, IL-6 has been regarded as a pro-inflammatory cytokine, but recently anti-inflammatory functions of IL-6 have also been described, which will be discussed in detail later[125,126].

M. tuberculosis-infected human and murine macrophages secrete IL-6 and elevated IL-6 serum levels are found in tuberculosis patients[127-129]. However, the role of IL-6 during infection with *M. tuberculosis* is not well understood.

IL-6-deficient mice displayed increased susceptibility to *M. tuberculosis* infection compared to control mice, but only after a systemic high dose infection[130]. In the low-dose aerosol model, which mimics the natural route of infection, *Il-6*$^{-/-}$ mice only initially showed elevated bacterial levels[131]. In contrast, *in vitro* studies have shown that high levels of IL-6 impair T cell proliferation of antigen-specific T cells, reduce the responsiveness to IFN-γ of infected macrophages and counteract TNF-mediated mycobactericidal activities in macrophages[132-134].

1.2.5 Interleukin-17

IL-17 can be produced by γδ T cells and Th17 cells and has been shown to be involved in the attraction of neutrophils to the site of infection[135-138]. The

development of Th17 cells depends on STAT3 activation (by IL-6, IL-21, IL-23) and TGF-β and is counter-regulated by Th1 cells[139,140].

In mice, IL-17 is mainly secreted by γδ T cells during *M. tuberculosis* and early BCG infection[141,142]. Additionally, numbers of IL-17 producing γδ T cells were elevated in tuberculosis patients compared to healthy donors[142,143]. Although the lack of IL-17 or IL-17R does not impair the ability of mice to control a low dose aerosol *M. tuberculosis* infection, deficient mice were unable to control *M. tuberculosis* after a high dose intratracheal infection[144,145]. Thus, the relevance of IL-17 for the control of *M. tuberculosis* infection needs to be further investigated.

However, the impact of IL-17 on granuloma formation and pathology have been suggested since BCG-infection of *Il-17a*$^{-/-}$ mice showed impaired granuloma maturation[146,147]. On the other hand, repeated BCG-immunizations of *M. tuberculosis*-infected mice led to increased infiltration of neutrophils and tissue damage in the lungs associated to elevated IL-17 levels[146].

Interestingly, high numbers of neutrophils have been associated with susceptibility to *M. tuberculosis* infection in mice [55,146-148]. In *Ifn-γ*$^{-/-}$ mice infiltrating neutrophils could be related to high levels of IL-17[55]. Another hint for a detrimental effect of high IL-17 levels in *M. tuberculosis* infection was found in IL-27R deficient mice. These mice showed lower bacterial burdens compared to control mice but developed enlarged granulomas and succumbed earlier to infection most likely due to enhanced IL-17 responses[148].

1.3 SOCS PROTEINS

The family of suppressor of cytokine signaling (SOCS) proteins consists of 8 members (cytokine inducible SH2 protein, CIS, SOCS1-7), and all are main regulators of intracellular signal transduction mediated through the JAK-STAT pathway. All SOCS proteins share a modular organization with a C-terminal SOCS box, a central SH2 domain and an amino-terminal domain of variable length (Fig. 4). The SOCS-box was shown to interact with additional proteins to form an E3 ubiquitin ligase complex, initiating ubiquitination of target proteins, followed by proteosomal degradation of bound signaling complexes as JAKs and cytokine receptors[149-151]. The SH2 domain of SOCS proteins determines the specificity for their receptors[152,153].

Both deletion and overexpression of SOCS proteins in animal models provided insights into their importance in regulating the responsiveness to cytokines, growth

factors and hormones but also showed the specificity of SOCS proteins for certain receptors[154]. In particular, SOCS1 and SOCS3 were found to balance immune functions by influencing sensitivity to cytokines, modulating the differentiation of immune cell populations as well as their activation.

To date, SOCS1 and SOCS3 are the most studied members of the SOCS family. They both contain an additional N-terminal kinase inhibitory region (KIR) that is absent in other SOCS proteins. The KIR of SOCS1 and SOCS3 can directly inhibit JAK tyrosine kinase activity by acting as a pseudo-substrate, and thereby blocks the interaction of JAK with their substrate STAT molecules. Although SOCS1 preferably binds directly to the JAK activation loop, the SH2 domain of SOCS3 binds to the cytokine receptor. Using this mechanism, JAK inhibition by SOCS1 and SOCS3 takes place even in the absence of the SOCS box[155,156].

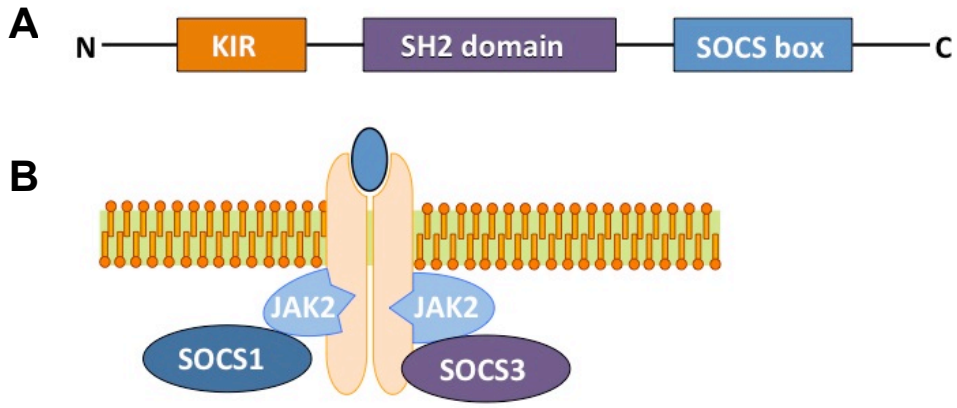

Figure 4: SOCS proteins. A: Schematic structure of SOCS1 and SOCS3 proteins is shown. B: SOCS1 and SOCS3 binding to JAK or cytokine receptor.

1.4 SOCS1

SOCS1 expression can rapidly be induced by many cytokines, especially IFNs, and serves as a classical feedback loop inhibiting its inducing pathways[157,158]. Importantly, *Socs1* mRNA expression increases even in response to microbial molecules such as LPS, Pam_3Cys and CpG oligonucleotides that signal via TLR[159-162]. Furthermore, hormones like insulin[163], cardiotrophin[164] or glucocorticoids[165] have been shown to stimulate SOCS1 expression.

SOCS1 can bind to the catalytic domain of JAK2 and to TYK2, another molecule of the JAK family, which mediates IFN-α/β signaling. SOCS1 has also been shown to bind directly to type I IFN receptors (IFNar1)[166] and to the IFN-γ receptor, efficiently inhibiting STAT1-mediated signaling[154,166,167].

1.4.1 SOCS1$^{-/-}$ mice

Mice deficient for SOCS1 die within 3 weeks after birth due to fatty degeneration and necrosis of the liver[168,169]. Additionally, these mice show retarded growth, lymphopenia and multi-organ haematopoetic infiltrates[168,169]. This inflammatory disease could be related to hyper-responsiveness of *Socs1*$^{-/-}$ mice to IFN-γ, because mice with an additional knockout of IFN-γ or STAT1 survive until adulthood[170,171]. Moreover, *Socs1*$^{-/-}$ mice that were treated with neutralizing IFN-γ antibodies from birth reached adulthood but died of myocarditis[172]. Indications for an involvement of T cells and NK T cells as a probable source of enhanced levels of IFN-γ secretion were found in T and NK T cell-deficient *Rag2*$^{-/-}$/*Socs1*$^{-/-}$ mice that are healthy to at least 3 month of age, and in the finding that *Socs1*$^{-/-}$ mice transgenic for SOCS1 in T cells survive[171]. However, conditional deletion of SOCS1 in T and NKT cells or in macrophages is not sufficient for the hyper-inflammation[173]. Requirement of non-hematopoietic cells for detrimental inflammation could be excluded, as chimeric mice, which received *Socs1*$^{-/-}$ bone marrow after radiation, showed decreased survival[174]. Altogether, lethality caused by SOCS1-deficiency is mainly due to an exacerbated secretion of IFN-γ by T and NKT cells during the neonatal period and to an increased sensitivity of myeloid cells to IFN-γ.

In contrast, *Socs1*$^{-/-}$ mice crossed to *IFNar1*$^{-/-}$ mice survived beyond weaning, indicating that even type I IFN contributes to the lethal inflammation[166]. Besides the rescue of SOCS1-deficient mice by IFN depletion, *Socs1*$^{-/-}$ mouse strains crossed to *Stat1*$^{-/-}$, *Stat4*$^{-/-}$ or *Stat6*$^{-/-}$ mice are partially protected from neonatal lethality, but nevertheless displayed chronic inflammation with aberrant T cell activation[175-177]. STAT6 and STAT4 are two STAT family members that specifically mediate signals that emanate from the IL-4/ IL-13 and IL-12 receptors, showing that SOCS1 also affects IL-4 and IL-12 signaling. Uncontrolled responses to IL-12, which is an important activator of IFN-γ secretion by T cells, could contribute to increased IFN-γ levels and therefore to the development of inflammation in *Socs1*$^{-/-}$ mice[175].

1.4.2 SOCS1 in immunity

Socs1$^{-/-}$ mice are hypersensitive to LPS-induced endotoxic shock, associated with increased levels of IL-12 and TNF[178,179]. Surprisingly, even though *Ifn-γ*$^{-/-}$ mice are highly resistant to endotoxic shock[180], additional deletion of IFN-γ or STAT1 does not rescue *Socs1*$^{-/-}$ mice from lethal LPS injection, demonstrating that SOCS1 even attenuates IFN-γ-independent mechanisms[178-180]. Furthermore, elevated sensitivity to endotoxin shock was observed in *Socs1*$^{fl/fl}$ *LysM-cre* mice (see description of mouse strain in material and methods) lacking SOCS1 specifically in macrophages and neutrophils[181].

Increased sensitivity to LPS suggests an interaction of SOCS1 with components of the TLR signaling. In fact, binding of SOCS1 to IRAK1 and the p65 subunit of NF-κB has been shown to destabilize and limit NF-kB activation[178,179,182,183]. SOCS1 can also bind to the apoptosis-regulating kinase 1 and regulates mitogen-activated protein kinases JNK and p38[184]. Moreover, SOCS1 was shown to mediate the degradation of the adaptor protein MAL that is involved in TLR2 and TLR4 signaling[185]. However, results from other investigators could not confirm a direct effect of SOCS1 on TLR signaling[186,187]. SOCS1 overexpression did not affect TLR signaling; instead the inhibition of IFN-α/β-mediated STAT1 activation by SOCS1 may account for the observed sensitivity to LPS in *Ifn-γ*$^{-/-}$/*Socs1*$^{-/-}$ mice[186,187]. Thus, further work is required to clarify whether SOCS1 is directly involved in the regulation of TLR signaling.

1.4.3 SOCS1 in infectious diseases

A wide range of pathogens including parasites, bacteria and viruses are potent stimulators of SOCS1 expression. Despite obvious differences in the type of protective or deleterious immune response elicited by different pathogens, for most intracellular infections studied, SOCS1 expression is associated to an increase in susceptibility to the infection by facilitating pathogen replication. However, SOCS1 may on the other hand also reduce the pathological outcome of infections by hampering inflammatory reactions.

Several viral infections including herpes simplex virus, human respiratory virus, HIV and hepatitis C virus were found to induce SOCS1 expression[188-191]. Importantly, silencing of SOCS1 increased type I IFN signaling and resulted in inhibited viral replication in a human respiratory virus model[189]. Accordingly, *Socs1*-

deficient mice showed increased type I IFN-mediated resistance to Semliki Forest-virus[166,170]. However, unrestrained immune responses cause damaging immunopathology in other virus infections. In these cases, impairment of cytokine responses with SOCS1 may prevent inflammatory damage. Thus, in a vaccinia virus infection model, mice could be protected against lethal virus infection by the administration of a peptide mimicking the SOCS1 KIR region[192].

In parasite infections, SOCS1 has been shown to inhibit responsiveness to IFN-γ, as in an *in vitro* model *Socs1*$^{-/-}$ macrophages were capable of killing *Leishmania major* at significantly decreased IFN-γ concentrations compared to SOCS1-sufficient macrophages[170]. However, increased pro-inflammatory signaling was not beneficial *in vivo* as *Socs1*$^{+/-}$ mice infected with *L. major* showed worsened pathology without a reduction in numbers of parasites[193].

Studies on the role of SOCS1 in bacterial infections mainly focused on intracellular infections, in which IFN-γ plays a major protective role. Research from our laboratory demonstrated that *Chlamydia pneumoniae* infection of macrophages induced SOCS1 expression[194]. Infected *Socs1*$^{-/-}$ macrophages displayed lower bacterial titers and higher levels of IFN-regulated genes as *iNos* and indoleamine dioxygenase that participate in the control of intracellular bacteria. *Rag1*$^{-/-}$/*Socs1*$^{-/-}$ mice showed 10-fold lower bacteria numbers in lungs than controls 6 days after infection. However, *Rag1*$^{-/-}$/*Socs1*$^{-/-}$ mice died within seven days after infection with *C. pneumoniae* showing a severe pulmonary inflammation, whereas *Rag1*$^{-/-}$ mice survived for more than 60 days. Thus, SOCS1 has a crucial role in preventing acute lethal inflammation in *C. pneumoniae* infection.

Infections with different mycobacterial species such as *M. bovis*[195], *M. avium*[196] and *M. tuberculosis* were found to induce SOCS1 expression in macrophages[195-198]. This SOCS1 induction was shown to be mediated by different innate immune receptors as TLR2 and DC-SIGN in mycobacterial-infected myeloid cells[198.] Knockdown of SOCS1 using siRNA in mouse DCs resulted in increased killing of virulent *M. tuberculosis*[198]. However, whether deletion of SOCS1 is beneficial for the outcome of mycobacterial infections *in vivo* remains to be investigated and will be discussed in this thesis.

1.5 SOCS2

SOCS2 is induced in response to growth hormone (GH), prolactin, insulin, lipoxin, LPS and several cytokines[199,200]. Compared to SOCS1 and SOCS3, SOCS2 shows a complete different spectrum of functions, with GH and insulin-like growth factor signaling as main targets of SOCS2 inhibition. Additionally, several reports indicate that SOCS2 not only regulates GH receptor signaling but may also be involved in responses to infection[201,202]. In contrast to SOCS1 and SOCS3, SOCS2 lacks the N-terminal KIR domain so that the inhibitory function of SOCS2 is dependent on competitive binding via its SH2 domain and, even more importantly, on the ubiquitination and proteasomal degradation of the interacting receptor[200].

In contrast to *Socs1*$^{-/-}$ mice, *Socs2*$^{-/-}$ mice are viable. However, *Socs2*$^{-/-}$ mice show a 40% increase in body weight alike GH-transgenic mice, and overgrowth of *Socs2*$^{-/-}$ mice could be attenuated by an additional deletion of STAT5, the STAT molecule that mediates GH signal transduction [203,204].

1.5.1 SOCS2 in immunity and infection

Silencing of SOCS2 in human immature DCs demonstrated that the expression of SOCS2 was required for maturation of DCs after LPS stimulation[199]. Furthermore, *Socs2*$^{-/-}$ DCs were refractory to anti-inflammatory mediators like lipoxin (LXA4) and showed in turn increased expression of pro-inflammatory cytokines in response to microbial stimulation[202]. *Socs2*$^{-/-}$ mice infected with *Toxoplasma gondii* showed uncontrolled production of pro-inflammatory cytokines, aberrant leukocyte infiltration and elevated mortality that was most probably due to hyper-responsive DCs[202].

Regarding T cell responses, SOCS2-deficient CD4+ T cells displayed a Th2 predisposition, and *Socs2*$^{-/-}$ mice showed enhanced Th2 responses to helminthic infections, atrophic dermatitis and allergic lung inflammation[201]. This suggested SOCS2 as an inhibitor of Th2 development.

Even though SOCS2 is expressed in Th1 cells, *Leishmania major*-infected *Socs2*$^{-/-}$ mice showed the same level of disease progression than control mice, indicating that despite its potential involvement in Th1 immune responses, SOCS2 is not involved in the control of cutaneous leishmaniasis[193]. Yet, a role for SOCS2 in other Th1-controlled infections has not been studied and will be discussed later as part of this thesis.

1.6 SOCS3 AND GP130

SOCS3 can be induced by different cytokines like leukemia inhibitory factor (LIF), IL-11, IL-10, IL-2, IL-6,[158,205-208] hormones including ciliary neutrophilic factor, leptin, prolactin and growth hormones[209-212] but also by microbial molecular patterns as LPS or CpG[162,213].

SOCS3 is an important endogenous regulator of STAT3-mediated signaling. Interestingly, not all cytokine signaling pathways that activate STAT3 are SOCS3-regulated since SOCS3 binds to the gp130 and selected receptor subunits, whereas the signaling of IL-10 that also stimulates STAT3-activation is unaffected by SOCS3 (Fig. 5)[77,125,126,214,215]. This is explained by the ability of SOCS3 to bind to the IL-6 receptor subunit gp130 but not to the IL-10 receptor (Fig. 5)[126,153,216]. Furthermore, SOCS3 inhibits non-gp130-mediated cytokine and hormone receptor signaling pathways activated by G-CSF, leptin, IL-12 and IFN[206,215,217-220].

The glycoprotein gp130 is a promiscuous cytokine receptor subunit that mediates signaling by IL-6 and all other cytokines belonging to the IL-6 family such as IL-11, IL-27, oncostatin M, cardiotropin 1, cardiotropin-like cytokine and LIF[221]. IL-6 binds to the IL-6R alpha chain, recruits gp130 and forms a hexameric complex consisting of two IL-6 bound to two IL-6Rα and gp130 subunits[222,223]. It was found that gp130 could be present on hematopoetic cells depending on their activation status but also on non-hematopoetic cells[224-226].

Binding of the ligand to gp130 induces activation of JAK1 kinases, which results in tyrosine phosphorylation of gp130. This creates binding sites for signaling transmitting factors at two different sites: phosphorylation of the four membrane-distal tyrosines leads to binding of STAT1 and STAT3, whereas phosphorylation of the membrane-proximal tyrosine (Y757) enables binding of the Src-homology tyrosine phosphatase-2 (SHP2)[227-230]. These bound molecules are in turn activated and trigger distinct intracellular signaling cascades.

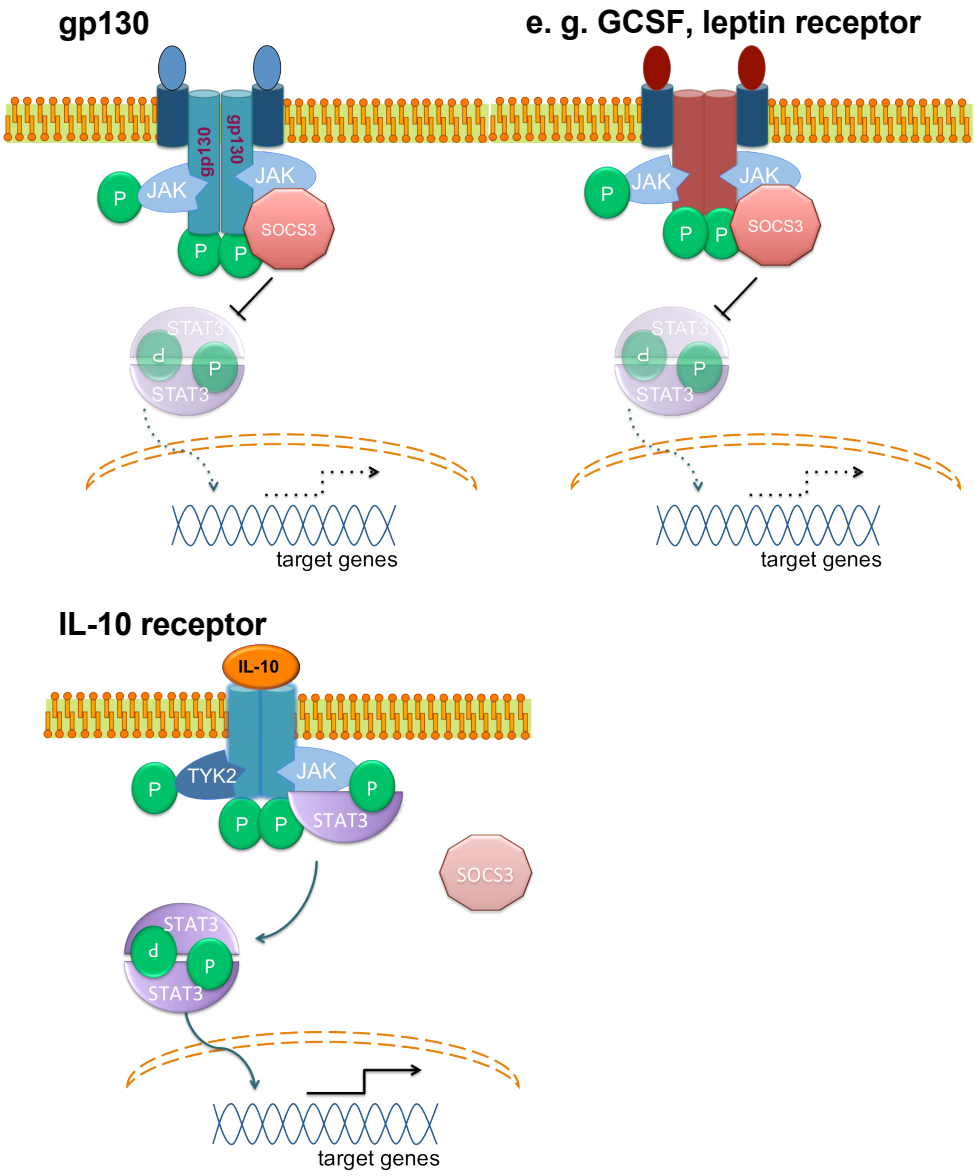

Figure 5: SOCS3-mediated inhibition of gp130 and non-gp130 signals but not of IL-10-signaling.
Since SOCS3 cannot bind to the IL-10 receptor, STAT3 activation in response to IL-10 is unimpaired in the presence of SOCS3, whereas gp130, GSCF and leptin receptor signals are inhibited by SOCS3.

Interestingly, Y757 (SHP2-binding site) is also the binding site for SOCS3 and required for SOCS3-mediated inhibition of STAT3 activation[231]. Therefore, a mutation at Y757 has two effects: abrogation of SHP2 signaling and deletion of SOCS3-mediated STAT3 inhibition.

1.6.1 SOCS3 and gp130 mutations in mice

SOCS3 knockout mice die during embryogenesis due to placental defects[232,233]. Mortality is caused by enhanced LIF signaling in the absence of SOCS3 causing an altered trophoblast differentiation[234]. However, mice with a conditional knockdown for SOCS3 in specific tissues or cell linages survive and have been used for targeted SOCS3 studies (see in materials and methods).

Since the knockdown of gp130 leads to embryonic or perinatal lethality, mice with conditional knockdowns for gp130 or mutations ablating either the STAT1/3 binding or SHP2/SOCS3 binding are used to dissect the role of gp130 in mouse models[235]. To study the role of SOCS3 within gp130 signaling, two different models can be used: One model, *gp130*^{F759} mice, the mouse *gp130* is replaced with human *gp130* mutant cDNA, in which the crucial tyrosine for SOCS3 binding in position 759 (corresponds to 757 in the mouse) is replaced by phenylalanine (*gp130*^{F759} mice), and the other model, in which tyrosine 757 is directly substituted by phenylalanine (*gp130*$^{F/F}$ mice)[236,237]. These models show similarities, a prolonged activation of STAT3 in response to gp130-activating factors was found in both models confirming the impairment of SOCS3 function by these mutations[236,238]. Phenotypically these mice developed splenomegaly that could be accounted to increased STAT3 activation by IL-6 as it is also found in IL-6/IL-6Rα transgenic mice[236,237,239]. Furthermore, *gp130*^{F759} mice showed autoimmune arthritis and lymphadenopathy, whereas in *gp130*$^{F/F}$ mice gastric adenoma formation and a reduced life span was found[225,226,240]. Combination of *gp130*$^{F/F}$ mice with deletion of IL-6 (*gp130*$^{F/F}$/*Il-6*$^{-/-}$) or reduction of STAT3 levels (*gp130*$^{F/F}$/*Stat3*$^{+/-}$), allows to study the underlying mechanisms caused by the changed gp130 signaling[238].

1.6.2 SOCS3 in myeloid cells

Mice with a conditional knockdown of SOCS3 in myeloid cells highlighted the crucial role of SOCS3 in suppression and termination of IL-6/gp130-mediated signaling. SOCS3-deficient macrophages stimulated with IL-6 displayed an increased magnitude and duration of STAT1 and STAT3 activation in comparison to controls[125,126,241]. This prolonged STAT3 activation is a hallmark of anti-inflammatory IL-10 signaling and LPS-induced pro-inflammatory responses like secretion of TNF and IL-12 were reduced in the presence of IL-6 in SOCS3-deficient macrophages (Fig. 6). The importance of SOCS3-regulated STAT3 activation was confirmed in *gp130*$^{F/F}$ macrophages that responded to IL-6 similar as to IL-10 under LPS stimulation[242]. Additionally, Croker (2003), Lang (2003) and co-workers found an increased STAT1 activation and elevated levels of IFN-γ responsive genes in response to IL-6 using SOCS3-deficient macrophages (Fig. 6)[125,241].

The anti-inflammatory properties of IL-6 in the absence of SOCS3 or SOCS3-binding may explain why mice deficient for SOCS3 in myeloid cells are resistant to LPS-induced endotoxic shock whereas *Stat3*$^{-/-}$ mice are highly susceptible[126,243]. Moreover, SOCS3-deficient DCs were found to be capable of suppressing the development of experimental autoimmune encephalitis (EAE)[244,245].

Furthermore, deletion of SOCS3 in the myeloid or hematopoetic cells increased the numbers of neutrophils, which showed increased survival and proliferative capacity[214,247]. Following GCSF injection *in vivo*, SOCS3-deficient mice developed neutrophilia, and a spectrum of inflammatory pathologies characterized by neutrophil infiltration in multiples tissues[248].

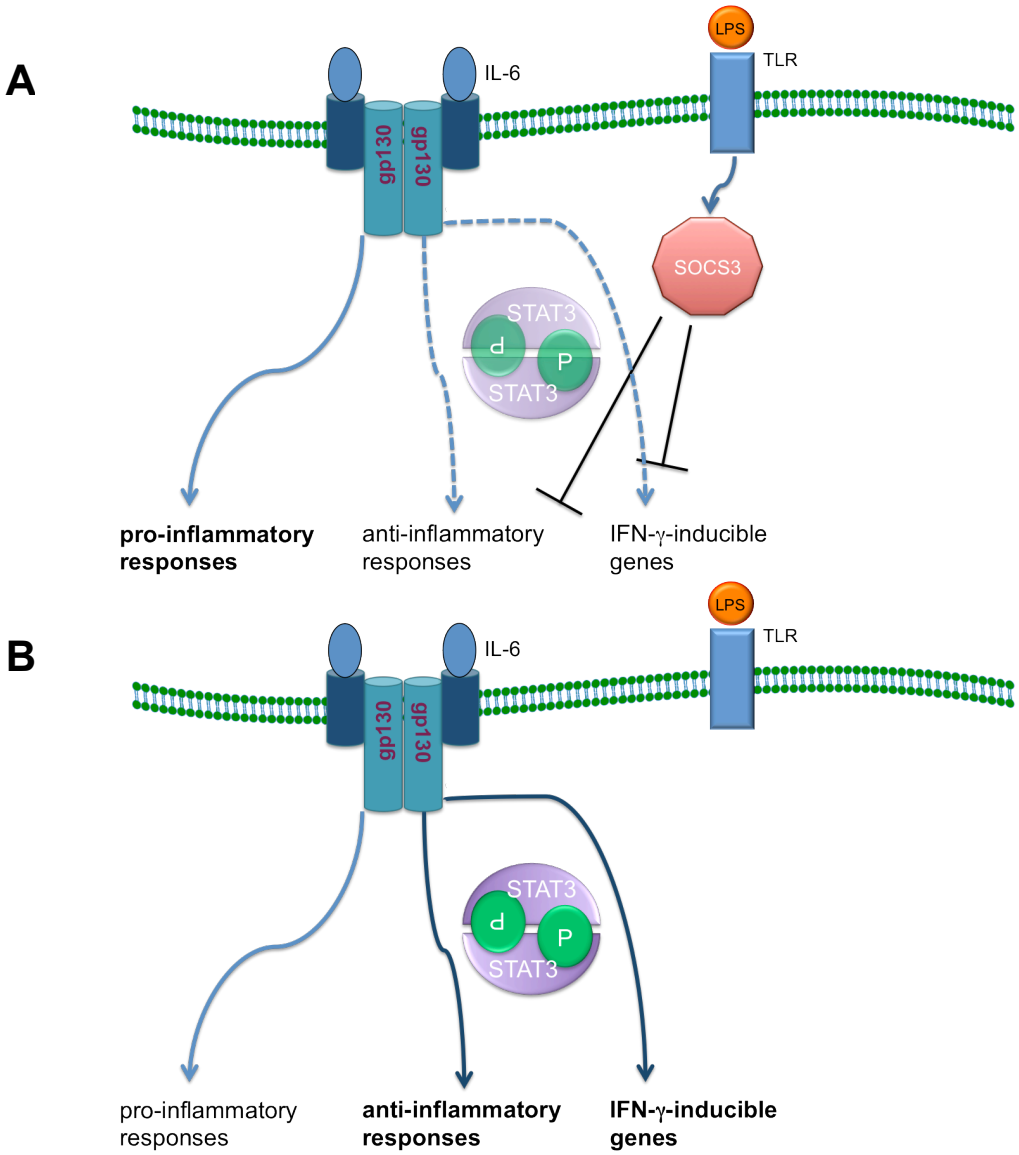

Figure 6: SOCS3 inhibits anti-inflammatory and IFN-inducible genes in response to LPS in presence of IL-6 (adapted from [246])**.**
LPS-induced SOCS3 modulates IL-6 signaling leading to pro-inflammatory responses (A). In contrast in the absence of SOCS3, anti-inflammatory responses and expression of IFN-γ-inducible genes are observed (B).

1.6.3 SOCS3 in T cells

Even though T cell development in the thymus is unaffected by SOCS3[249], several models suggest a role for SOCS3 in T cell proliferation. One indication for its regulatory role is the fact that SOCS3 levels are high in resting T cells, whereas they are low in activated T cells[250]. Furthermore, T cells overexpressing SOCS3 showed a reduced proliferation upon T cell receptor stimulation[251,252], whereas SOCS3-deficient T cells were hyper-proliferative towards T cell receptor stimulation[253,254]. Interestingly, T cells from *gp130*$^{F/F}$ mice behaved alike SOCS3-deficient T cells,

suggesting that hypersensitivity to gp130 cytokines may drive this enhanced proliferation[253].

SOCS3 protein levels may influence the Th1/Th2 balance, as expression was found to be low in Th1 cells and high in Th2 cells[255,256]. Accordingly, overexpression of SOCS3 in mice resulted in elevated Th2 responses and hypersensitivity to allergic diseases[256]. A possible explanation for the increased Th2 development under high SOCS3 levels might be that binding of SOCS3 to IL-12Rβ2 inhibits IL-12-mediated STAT4 activation that in turn impairs Th1 differentiation[256,257].

Interestingly, a conditional knockdown for SOCS3 in T cells mice showed reduced Th2 responses together with increased numbers of Th3-like T cells secreting IL-10 and TGF-β[258]. Unexpectedly, Th1 responses were not elevated in the absence of SOCS3, which may have been caused by the presence of immunosuppressive cytokines[258].

Other reports indicated only minimal effects of SOCS3 deletion in T cells on the Th1/Th2 balance but found an increased Th17 generation and enhanced STAT3 activation in response to IL-23[249]. This data was confirmed in a study of an atherosclerosis model using mice with a T cell specific SOCS3 knockdown as well as in a rheumatoid arthritis model, in which mice lacking SOCS3 in hematopoietic and endothelial cells showed increased Th17 development[259,260]. These findings fit well with the known mechanism for Th17 differentiation, as STAT3 activation either by IL-23 or IL-6 is crucial for Th17 development (Fig. 7). Furthermore, TGF-β, another cytokine necessary for Th17 generation, was shown to inhibit SOCS3 expression with the resulting prolonged STAT3 activation and thereby promoting Th17 development[261].

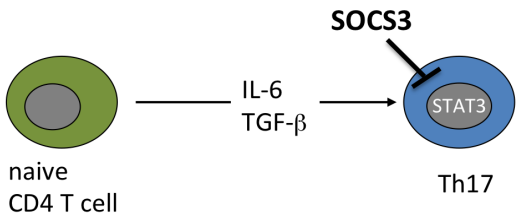

Figure 7: Role of SOCS3 in Th17 cell development.
STAT3 activation is essential for the generation of Th17 cells and can be inhibited by SOCS3.

No SOCS3 protein expression was found in Tregs[262]. Accordingly, in SOCS3-deficient T cells no defects in Treg differentiation and function were reported[254]. Recently, SOCS3 has been assigned a role in development of memory CD8+ T cells since numbers of memory CD8+ T cells were dramatically reduced in the absence of SOCS3 in T cells[263,264].

1.6.4 SOCS3 in infectious diseases

Similar to SOCS1 and SOCS2, SOCS3 expression can be stimulated by both cytokines and TLR agonists. Additionally, several pathogens including viruses, bacteria and parasites have been shown to induce SOCS3 expression. Due to the diversity of described SOCS3 actions, it is hard to predict in which cases SOCS3 expression will be beneficial for the host.

SOCS3 was found to inhibit STAT1 activation in response to type I IFNs but was less effective than SOCS1 in such function[215,219]. Infections with several viruses stimulated SOCS3 expression that correlated with reduced STAT1 activation in response to type I IFNs. Silencing of SOCS3 significantly increased IFN signaling and hampered viral replication. Similar findings were obtained during different viral infections e.g. HSV, Influenza A, hepatitis C virus and Epstein Barr virus infection indicating that increased SOCS3 expression improves viral replication[265-269].

Surprisingly, mice with a T cell-specific SOCS3 deletion showed increased T cell activation and viral clearance without development of immunopathology during the infection with lymphocytic chriomeningitis virus (LCMV)[270]. Treatment of LCMV-infected mice with IL-7 repressed SOCS3 expression and promoted IL-6 production, which resulted in enhanced T cell effector functions and viral clearance. On the contrary, *Leishmania major*-infected mice were more susceptible in the absence of SOCS3 in T cells. In this case, SOCS3 deficiency in T cells led to increased anti-inflammatory TGF-β secretion promoting the infection[258].

Infection of macrophages with *M. avium* and *M. bovis* raised SOCS1 and SOCS3 levels in a TLR2-NOTCH1 dependent pathway[195,196,271]. In clinical studies, patients with active tuberculosis were found to have higher SOCS3 expression levels in whole blood and T cells in comparison to latently infected controls[272,273]. Accordingly, SOCS3 expression decreased under chemotherapy treatment indicating that SOCS3 expression could be interpreted as an on-going infection response[272,273].

However, to prove that SOCS3 plays a crucial role for tuberculosis control, more work needs to be done. This was one of the objectives of my thesis.

Regarding SOCS3-ablations due to gp130 mutations, *gp130*^{F759} mice showed an attenuated early phase of defense against *Listeria monocytogenes* infection resulting in higher bacterial loads and mortality most probably due to insufficient elevation of IFN-γ levels[274]. Furthermore, low IFN-γ titers led to increased susceptibility to *Toxoplasma gondii* in mice deficient for SOCS3 in myeloid cells as well as in *gp130*$^{F/F}$ mice.[275,276] In both mouse strains, lower IL-12p40 and therefore lower IFN-γ levels after infection compared to control mice were found. Interestingly, addition of IL-12 as well as neutralization of IL-6 could restore the wild type (WT) phenotype in both models demonstrating that an altered IL-6 signaling was responsible for the observed increased susceptibility[275,276].

2 AIMS

M. tuberculosis is a highly successful pathogen. Even though the literature is extensive, detailed reasons why a latent tuberculosis patient will convert to active disease and the mechanisms that regulate the control of *M. tuberculosis* are not completely understood. More knowledge about protective immune responses during *M. tuberculosis* infection is required for the design of prophylaxis and therapy.

SOCS proteins are non-redundant regulators of immune responses through inhibition of cytokine signaling. Therefore, these molecules are interesting target candidates that may modulate the outcome of infections.

In my thesis work, I have studied the role of SOCS1, SOCS2 and SOCS3, which have different specific cytokine signaling pathways as their targets, in the outcome of mycobacterial infections.

Specific questions:

SOCS1

- How is SOCS1 expression regulated in mycobacteria-infected macrophages?
- What is the role of SOCS1 in the regulation of intracellular bacterial levels and cytokine expression in mycobacteria-infected macrophages *in vitro*?
- What is the role of SOCS1 *in vivo* during *M. tuberculosis* or BCG infection regarding
    - Bacterial loads
    - Cytokine responses
    - Immunopathology?

SOCS2

- How is SOCS2 expression regulated in response to TLR stimulation?
- How is SOCS2 expression regulated in mycobacteria-infected macrophages?
- Is there a role for SOCS2 during *M. tuberculosis* infection *in vivo*?

SOCS3

- How is SOCS3 expression regulated in mycobacteria-infected macrophages?
- Which function has SOCS3 in macrophages/DCs during mycobacterial infections regarding the regulation of
    - Bacterial loads
    - Cytokine responses
    - Immunopathology?
- What is the role of SOCS3 in T cells during *M. tuberculosis* infection *in vivo* regarding the regulation of
    - Bacterial loads
    - Cytokine responses
    - Immunopathology?
- Which function has gp130 signaling in SOCS3-deficient models *in vivo* and *in vitro* during mycobacterial infections?

3 MATERIAL AND METHODS

The methods applied in our studies are described in detail in the published articles and in the manuscript. Here, I want to give an overview about the genetically modified mice that have been used.

SOCS-unrelated

mouse strain	type of modification	effect
Rag1$^{-/-}$	knockout	disruption of the *Rag1* gene in all cells no mature B and T cells
MyD88$^{-/-}$	knockout	disruption of the *MyD88* gene in all cells
Irf3$^{-/-}$	knockout	disruption of the *Irf3* gene in all cells
Ifn-γ$^{-/-}$	knockout	disruption of the *Ifn-γ* gene in all cells

SOCS proteins and SOCS-related

mouse strain	type of modification	effect
Rag1$^{-/-}$/*Socs1*$^{-/-}$	double knockout	no mature B and T cells disruption of the *Rag1* and *Socs1* gene in all cells
Ifn-γ$^{-/-}$/ *Socs1*$^{-/-}$	double knockout	disruption of the *Ifn-γ* and *Socs1* gene in all cells
Socs1$^{fl/fl}$ *LysM cre**	conditional knockdown	excision of the *Socs1* gene in myeloid cells
Socs2$^{-/-}$	knockout	disruption of the *Socs2* gene in all cells
Socs3$^{fl/fl}$ *LysM cre**	conditional knockdown	excision of the *Socs3* gene in myeloid cells
Socs3$^{fl/fl}$ *lck cre**	conditional knockdown	excision of the *Socs3* gene in T cells
gp130$^{F/F}$	knockin	mutation of the SOCS3 binding site in gp130 in all cells
gp130$^{F/F}$/*Il-6*$^{-/-}$	knockin/knockout	mutation of the SOCS3 binding site in gp130 in all cells disruption of the *Il-6* gene in all cells
gp130$^{F/F}$/*Stat3*$^{+/-}$	knockin/knockout	mutation of the SOCS3 binding site in gp130 in all cells heterozygous for *Stat3* gene knockout

***Conditional knockdown:**
Mice were generated using the Cre-lox combination system, in which the Cre DNA-recombinase is expressed under a cell-specific promoter. Although the target gene is flanked by *LoxP* sequences in all cells for recognition by the Cre enzyme, it will only be deleted specifically in Cre-expressing cells. The corresponding WT mice contain the floxed gene (*Socs1*$^{fl/fl}$ and *Socs3*$^{fl/fl}$) but are negative for Cre expression.

4 RESULTS AND DISCUSSION

4.1 SOCS1 DURING *M. TUBERCULOSIS* INFECTION (PAPER I)

IFN-γ is essential for protection against *M. tuberculosis* infection. SOCS1 is induced during infection of macrophages with *Mycobacterium avium* and *Mycobacterium bovis* BCG, and may thereby inhibit responses to IFN-γ and play a central role in the outcome of mycobacterial infections[195,196]. Thus, we hypothesized that stimulation of SOCS1 expression could constitute an immune-escape mechanism for the establishment and chronicity of *M. tuberculosis* infection.

How is SOCS1 expression regulated in mycobacteria-infected macrophages?

We found that *Socs1* mRNA was stimulated during BCG and *M. tuberculosis* infections *in vitro* in human and murine macrophages as well as in a mouse model in lung tissue during infections. Using macrophages from genetically manipulated mice, we found that the expression of *Socs1* mRNA during BCG infection required phagocytosis and the presence of NOD2 and IFN-α/β receptors, the adaptor protein MyD88 and the common transcription factor NF-κB. This shows that TLR and non-TLR signals cooperate in optimal *Socs1* mRNA expression in mycobacteria-infected macrophages. Furthermore, our data was confirmed by a recent publication, in which *M. tuberculosis*-induced *Socs1* mRNA expression was dependent on TLR and DC-SIGN receptor 1 in human DCs[198]. Moreover, our finding of SOCS1 induction in lungs tissue of BCG and *M. tuberculosis*-infected mice is further supported by clinical data that demonstrated increased SOCS1 expression in induced sputum of tuberculosis patients compared to healthy individuals and patients with other lung diseases[277]. Mechanisms of SOCS1 action in the mouse model may therefore provide insights into the role of the molecule in determining the onset and course of active tuberculosis in patients.

What is the role of SOCS1 in the regulation of intracellular bacterial levels and cytokine expression in mycobacteria-infected macrophages in vitro?

To study the function of SOCS1 *in vitro*, we took advantage of cells derived from *Socs1* knockout and conditional knockdown mice as well as SOCS1 mimetic peptides. We found that SOCS1 inhibited growth control of *M. tuberculosis* and BCG

in macrophages. Infected *Socs1*$^{-/-}$ macrophages showed lower mycobacterial numbers and expressed higher levels of IFN-γ-responsive genes, but interestingly, also higher levels of IFN-γ itself than infected control cells. In agreement, *Socs1*$^{-/-}$ macrophages secreted higher amounts of IFN-γ in response to IL-12 that was associated with increased IL-12Rβ1 but not IL-12 expression in infected *Socs1*$^{-/-}$ macrophages. Neutralization of IL-12 reduced the elevated IFN-γ expression in infected *Socs1*$^{-/-}$-macrophages. Of importance, the improved control of *M. tuberculosis* and BCG by *Socs1*$^{-/-}$ macrophages was lost in *Ifn-γ*$^{-/-}$/*Socs1*$^{-/-}$ macrophages, demonstrating that IFN-γ secretion mediated the improved mycobacterial control of *Socs1*$^{-/-}$ macrophages. Moreover, *M. tuberculosis*-infected WT macrophages were unimpaired in their response to addition of exogenous IFN-γ despite SOCS1 expression. Altogether, our results suggest that SOCS1 regulates the secretion rather than the response to IFN-γ, via controlling responses to IL-12 causing the increased mycobacterial resistance of *Socs1*$^{-/-}$ macrophages (Fig. 8). This mechanism is unexpected, as to our knowledge, all described effects of SOCS1 on infected host cells were explained by the inhibition of STAT1 signaling leading to impaired type I IFN-signaling for viral infections or IFN-γ-signaling for intracellular bacteria and parasites [166,170,189,195,196]. Since SOCS1 binds to JAK2 that is also associated with the IL-12 receptor, this interaction probably influences IL-12 signaling. Furthermore, *Socs1*$^{-/-}$/*Stat4*$^{-/-}$ mice, deficient for the IL-12 signal-transmitting molecule STAT4, live longer than *Socs1*$^{-/-}$ mice[175]. This suggests an inhibitory role of SOCS1 on STAT4 activation and increased responses to IL-12 in absence of SOCS1 have been found in T cells and DCs[175,278]. Thus, our findings broaden the inhibitory spectrum of SOCS1 from IFN responses in macrophages to IL-12 responses during infections (Fig. 8).

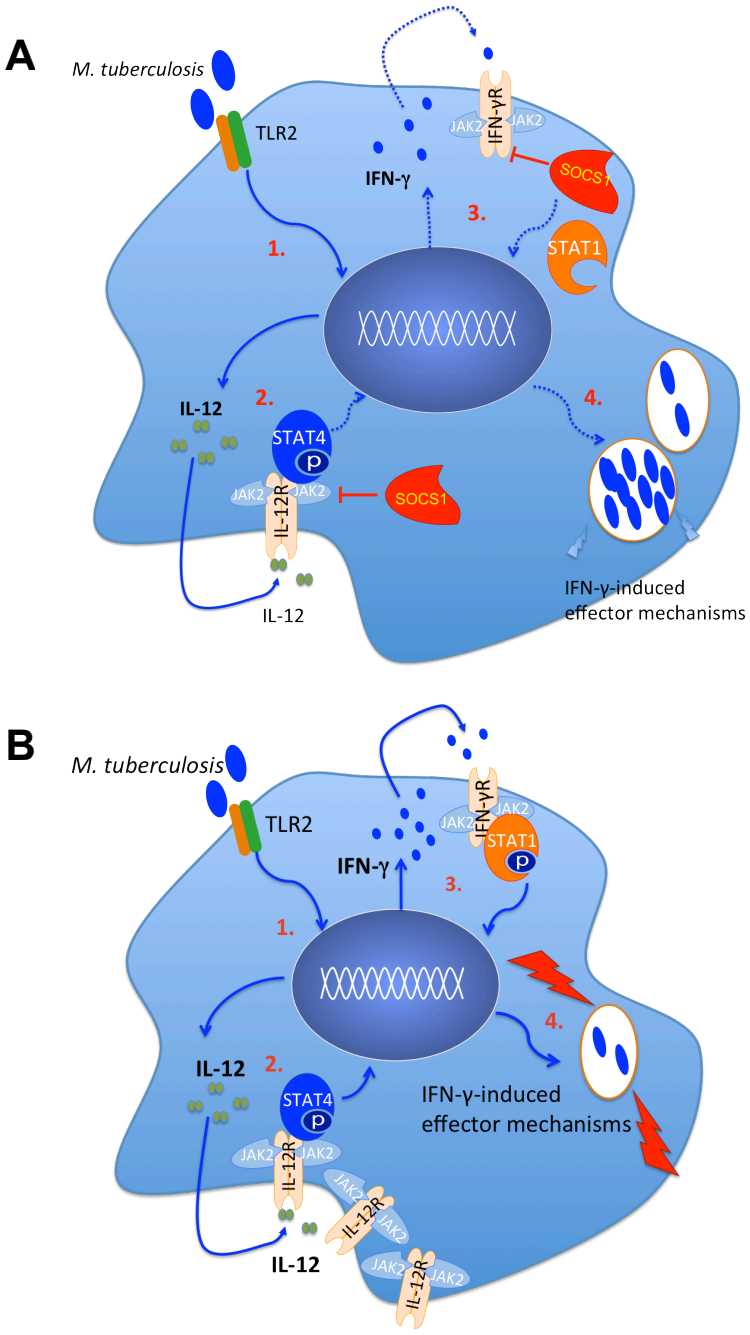

Figure 8: *M. tuberculosis*-infected macrophages in presence (A) and absence (B) of SOCS1.
Infection of macrophages with *M. tuberculosis* stimulates innate immune responses (1) such as the production of IL-12. Responses to IL-12 are inhibited in the presence of SOCS1 (2) leading to reduced IFN-γ induction and IFN-γ–signaling (3). Therefore, presence of SOCS1 results in higher bacterial numbers (4) and reduced IFN-γ–induced effector mechanisms.

To obtain a detailed overview about SOCS1-affected genes during *M. tuberculosis* infection in macrophages, we performed a genome wide expression microarray. In agreement with previous results, a significant fraction of the macrophage genome was altered after *M. tuberculosis* infection[116,279]. Unexpectedly,

the majority of infection-regulated genes was even stronger regulated in the absence of SOCS1, suggesting a major role of SOCS1 in the control of macrophage activity during *M. tuberculosis* infection. The majority of genes involved in the defense and immune responses and almost all IFN-γ–regulated genes showed higher levels in infected *Socs1*$^{-/-}$ macrophages, whereas genes that were decreased in infected control cells were even further downregulated in infected *Socs1*$^{-/-}$ macrophages. Altogether, our data implicates that the presence of SOCS1 attenuates both the negative and positive regulation of the majority of genes that change their expression after infection.

What is the role of SOCS1 during in vivo M. tuberculosis or BCG infection regarding bacterial loads, cytokine responses and immunopathology?

Importantly, the improved bacterial control in *Socs1*$^{-/-}$ macrophages *in vitro* was reflected in the role of SOCS1 during *M. tuberculosis* infection *in vivo*. Aerosol infection with *M. tuberculosis* of *Rag1*$^{-/-}$*/Socs1*$^{-/-}$ as well as *Socs1*$^{fl/fl}$ *LysM cre* mice showed lower bacterial loads in the lungs 7 days after infection compared to control mice (Fig. 9). Moreover, *Ifn-γ* and *iNo*s mRNA levels in the lungs of *Rag1*$^{-/-}$*/Socs1*$^{-/-}$ and *Socs1*$^{fl/fl}$ *LysM cre* mice were elevated compared to control mice (Fig. 9). At this early stage of infection, mainly innate immune mechanisms are present, and no increase of IFN-γ in lungs of infected control mice was detected. Consistent with our *in vitro* results, lungs from infected *Rag1*$^{-/-}$*/Socs1*$^{-/-}$ and *Socs1*$^{fl/fl}$ *LysM cre* mice showed higher levels of IFN-γ that are sufficient to impair bacterial growth *in vivo*, since no improved bacterial control was observed in *Ifn-γ*$^{-/-}$*/Socs1*$^{-/-}$ mice compared to *Ifn-γ*$^{-/-}$ mice.

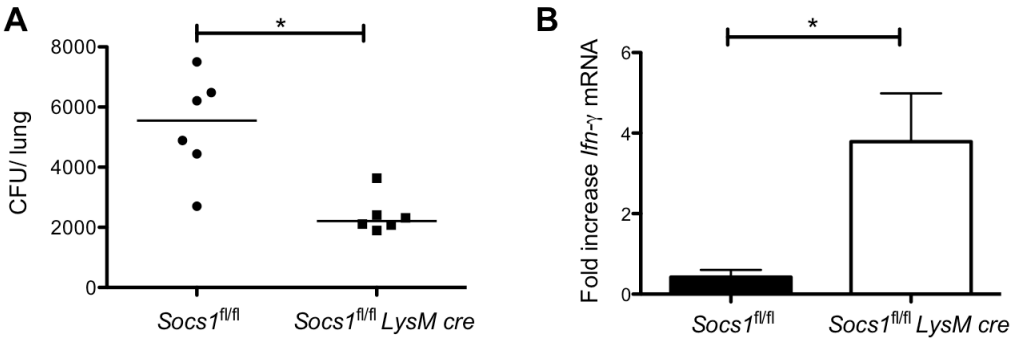

Figure 9: SOCS1 in myeloid cells 7 days after *M. tuberculosis* infection.
7 days after *M. tuberculosis* infection, bacterial loads in the lungs of individual *Socs1*$^{fl/fl}$ and *Socs1*$^{fl/fl}$ *LysM cre* mice and median were determined (A). Expression of *Ifn-γ* mRNA in lungs is shown in (B) as mean ± SEM.

Three weeks after infection, in conjunction with the appearance of IFN-γ producing T cells in lungs of aerosol infected mice, similar bacterial counts were registered in the lungs of *Rag1*$^{-/-}$*/Socs1*$^{-/-}$, *Socs1*$^{fl/fl}$ *LysM-cre* and their respective control mice. We suggest that, at this later time point, the advantage of the SOCS-deficient host is lost when IFN-γ secretion by T cells is prominent and macrophages even in the presence of SOCS1 are able to respond to IFN-γ. Relevant to our observation, SOCS1 expression is elevated in the blood of patients with pulmonary tuberculosis that at the same time express an IFN-induced gene signature[280]. As we found no differences in IFN-γ and IFN-γ-regulated genes in control and *Rag1*$^{-/-}$*/Socs1*$^{-/-}$ mice at these later stage, it is possible that the inhibitory effects of SOCS1 can be shadowed by a potent Th1-mediated immunity.

Additionally, both *Rag1*$^{-/-}$*/Socs1*$^{-/-}$ and also *Ifn-γ*$^{-/-}$*/Socs1*$^{-/-}$ mice showed a very severe pulmonary inflammation 3 weeks after *M. tuberculosis* infection. This inflammatory response was mediated by SOCS1 deficiency in non-macrophage cells, because it was not observed in *M. tuberculosis*-infected *Socs*$^{fl/fl}$ *LysM-cre* mice, which are lacking SOCS1 only in myeloid cells. We conclude that SOCS1-expressing non-macrophage cells are responsible for preventing a detrimental inflammation that is elicited at least partially in an IFN-γ-independent manner. This is an interesting finding as in the absence of SOCS1 inflammation was reported to be mediated to a great extent by increased IFN-γ secretion by T cells and elevated responsiveness of macrophages to IFN-γ[170,171,173]. SOCS1 has also been shown to play a role in type I IFN, IL-4, IL-13 and IL-12 signaling, but apart from IL-12, these cytokines are not classically associated to resistance against *M. tuberculosis* infections[281,282]. However, whether these cytokines can contribute to mycobacterial infection-mediated inflammation remains to be examined.

Overall, we demonstrated that *M. tuberculosis*-induced SOCS1 by diminishing IL-12 responses impaired IFN-γ secretion by macrophages. This resulted in lower levels of IFN-γ-regulated genes and promoted *M. tuberculosis* infection leading to increased pulmonary bacterial levels in the lungs at early time points. Later during infection, mainly T cells produced IFN-γ and SOCS1 in non-macrophages cells protected mice from severe inflammation thereby allowing the establishment of chronic infection (Fig.10).

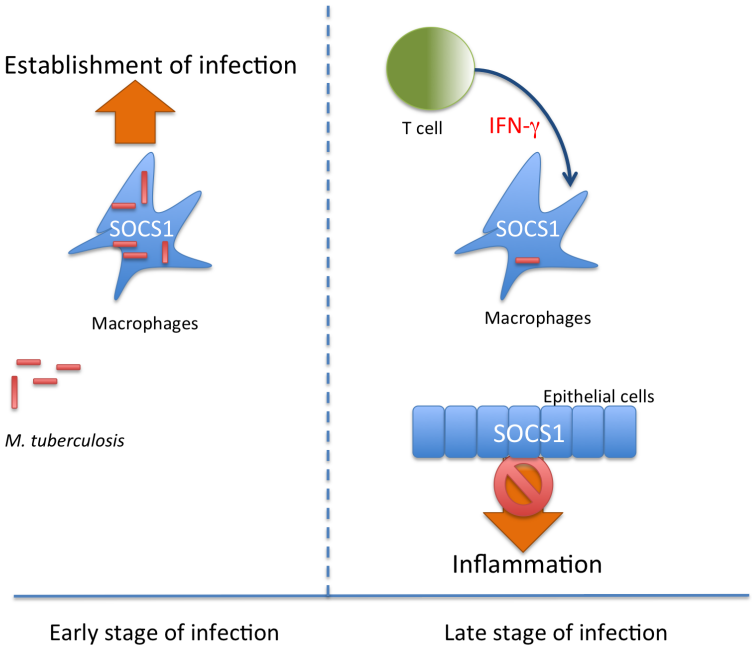

Figure 10: Role of SOCS1 at different stages of *M. tuberculosis* infection.
SOCS1 in macrophages increases bacterial numbers at the early stage of infection, whereas it has no effect on bacterial control at later time points. SOCS1 in non-macrophage cells (e. g. epithelial cells) hampers inflammation at later stages.

4.2 SOCS2 (PAPER II)

Little is known about the regulation of SOCS2 expression in immune cells. In paper II we investigated the regulation of SOCS2 expression in response to TLR4 agonists and to *Mycobacterium bovis* BCG in macrophages.

How is SOCS2 expression regulated in response to TLR4 stimulation?

We demonstrated that LPS-induced SOCS2 expression in human monocyte-derived DCs was dependent on the transcription factors IRF1 and IRF3 as shown by silencing IRFs. The expression and activation of IRF1 and IRF3 is known to be stimulated by type I IFNs, and neutralization of type I IFNs abolished *Socs2* mRNA expression after TLR4 stimulation. Silencing of different STAT transcription factors revealed the requirement of STAT3 and STAT5 in LPS-induced *Socs2* mRNA expression. TLR-signaling has been reported to mediate a direct induction of SOCS2 expression[283] but we clearly could show that inhibition of IRF1, IRF3 and IFN-α/β receptors severely impaired LPS-stimulated SOCS2 induction.

How is SOCS2 expression regulated in mycobacteria-infected macrophages?

Socs2 mRNA levels increased in BMM after infection with *M. bovis* BCG. The reduction of *Socs2* transcript induction in macrophages derived from *Irf3*$^{-/-}$ and *MyD88*$^{-/-}$ mice suggested the involvement of signaling via TLR or other innate immune receptors.

Is there a role for SOCS2 during M. tuberculosis infection in vivo?

Since *Socs2* mRNA expression was stimulated by *M. bovis* BCG, we studied whether SOCS2 played a role in the outcome of infection with *M. tuberculosis*. *Socs2*$^{-/-}$ and control mice were infected via aerosol with *M. tuberculosis* and bacterial burdens in lungs and spleens were determined 6 weeks after infection (Fig. 11).

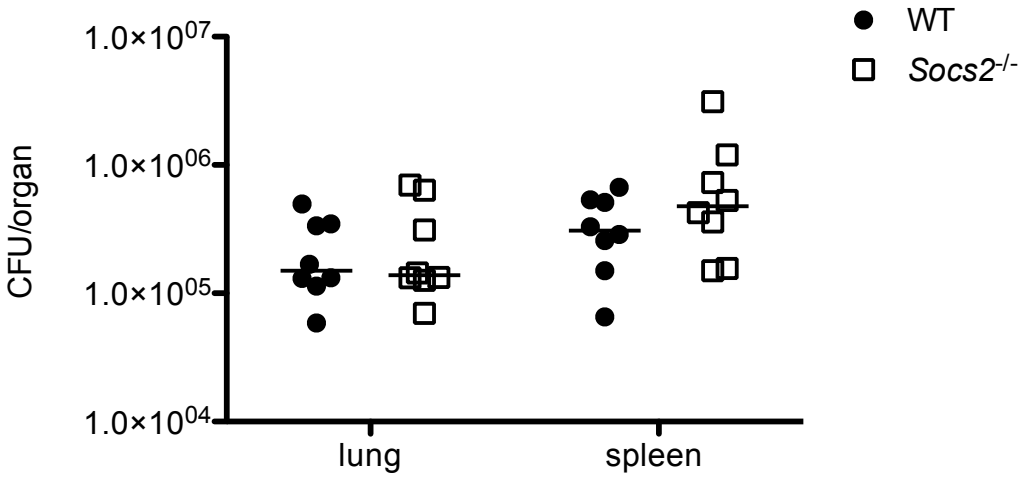

Fig. 11: SOCS2 expression does not change *M. tuberculosis* infection.
WT and *Socs2*$^{-/-}$ mice were infected via aerosol with *M. tuberculosis* Harlingen. Mice were sacrificed 6 weeks after infection and bacterial colony forming units (CFU) in lungs and spleens determined. Individual counts and median of each group is shown.

No differences in bacterial titers were detected, indicating that SOCS2 is mot involved in the control of *M. tuberculosis*. This result contrasts with the role of SOCS2 during infection with *Toxoplasma gondii* that showed increased cumulative mortality in *Socs2*$^{-/-}$ mice[202]. The heightened morbidity and mortality of *T. gondii*-infected *Socs2*$^{-/-}$ mice was explained by the finding that *Socs2*$^{-/-}$ DCs were refractory to the anti-inflammatory properties of the lipoxin LXA4[202]. Both *M. tuberculosis* and *T. gondii* induce LXA4, an eicosanoid mediator with potent anti-inflammatory properties, that e.g. reduces IL-12 secretion by DC[284,285]. However, only *T. gondii*-infected 5-lipoygenase-deficient mice (that are deficient for LXA4) succumbed earlier to infection

than control mice due to lethal encephalitis, whereas *M. tuberculosis*-infected 5-lipoxygenase-deficient mice displayed an improved control of infection without concominant immunopathology[285,286]. This indicates a vital role for LXA4 and its regulation by SOCS2 during *T. gondii* infection in prevention of fatal immune activation. However, our results suggest that SOCS2-mediated lipoxin LXA4 regulation is redundant for the control of infection with *M. tuberculosis*.

Regarding a possible role for SOCS2 in controlling T cells responses, enhanced generation of Th2 cells was described in helminth-infected *Socs2*$^{-/-}$ mice[201]. For *M. tuberculosis* control, specific Th1 cell-mediated immunity is fundamental 6 weeks after infection. Thus, we conclude that the absence of SOCS2 does not hamper Th1 responses during *M. tuberculosis* infection, confirming previous findings, in which SOCS2 was redundant for the control of *Leishmania major* infection [193].

These results suggest that although SOCS2 is induced during mycobacterial infections, its role is minor or redundant for disease control.

4.3 SOCS3 DURING *M. TUBERCULOSIS* INFECTION (PAPER III)

SOCS3 was shown to be induced in macrophages in response to mycobacterial infection with *M. avium* and *M. bovis* and in T cells of patients with active tuberculosis[195,196,272]. Since SOCS3 has multiple functions in the regulation of different cytokine signaling pathways with some of them unapparent in their function of tuberculosis control, we investigated the role of SOCS3 in the regulation of *M. tuberculosis* infection.

How is SOCS3 expression regulated in mycobacteria-infected macrophages?

In a first set of experiments, we found that *Socs3* mRNA expression was increased in lung tissue of *M. tuberculosis* and BCG-infected mice with around 10-fold higher levels during *M. tuberculosis* than in BCG infection. *Socs3* transcript induction in infected BMM was dependent on MyD88 and NF-κB but not on IRF3. To study whether this SOCS3 induction is important during *M. tuberculosis* infection, we examined the outcome of infection in different mouse models.

Which function has SOCS3 in macrophages/DCs during mycobacterial infections regarding the regulation of bacterial loads, cytokine responses and immunopathology?

Mice deficient for SOCS3 in myeloid cells (*Socs3*$^{fl/fl}$ *LysM cre* mice) showed increased bacterial burdens in lungs and spleens from 16 days after infection with *M. tuberculosis*. *Socs3*$^{fl/fl}$ *LysM cre* mice also displayed enhanced immunopathology and succumbed 82 days after infection (median survival), whereas control mice survived for 150 days until the experiment was terminated. Moreover, increased bacterial loads in lungs and spleen were observed after BCG infection of *Socs3*$^{fl/fl}$ *LysM cre* mice, although differences to bacterial loads in control mice were smaller compared to those registered during *M. tuberculosis* infection.

Next, we studied whether SOCS3-deficient macrophages were hampered in their control of mycobacterial growth. Surprisingly, *Socs3*$^{fl/fl}$ *LysM cre* BMM displayed unimpaired control of intracellular of *M. tuberculosis* replication and reduced bacterial loads in response to exogenous IFN-γ. Thus, defects of the bactericidal/ bacteriostatic capacity of SOCS3-deficient macrophages are unlikely to cause the increased *in vivo* susceptibility to *M. tuberculosis* of *Socs3*$^{fl/fl}$ *LysM cre* mice. In line with our data, previous work revealed that SOCS3-deficient macrophages could control *T. gondii* infection even though *Socs3*$^{fl/fl}$ *LysM cre* mice were highly susceptible to the infection with the parasite[276].

Thereafter, we analyzed the presence of cytokines and inflammatory mediators in supernatants of mycobacteria-infected macrophages. We found lower levels of TNF and IL-12p40 and higher levels of nitric oxide (NO) in supernatants of SOCS3-deficient macrophages after infection with *M. tuberculosis* or BCG and after stimulation with TLR-agonists in comparison to control cells. All three molecules – TNF, IL-12 and NO- have been demonstrated to be critical for control of clinical and experimental mycobacterial infections[82,83,96,116,117]. The elevated NO levels could explain the slightly improved bacterial growth control *in vitro,* whereas reduced IL-12p40 levels mainly would be important *in vivo* due to its crucial role in the generation of Th1 responses[97,99,101]. Previous studies showed reduced secretion of TNF and IL-12p40 in *Socs3*$^{fl/fl}$ *LysM cre* BMM in response to LPS, but only in co-culture with IL-6[126]. Interestingly, differences in TNF and IL-12p40 levels between mycobacteria-infected *Socs3*$^{fl/fl}$ *LysM cre* and control BMM were found even in absence of exogenously added IL-6. Addition of recombinant IL-6 to the cultures further diminished IL-12 and TNF concentrations in supernatants of mycobacteria-infected *Socs3*$^{fl/fl}$ *LysM cre* BMM. Furthermore, it has previously been shown that neutralization

of IL-6 in IFN-γ/LPS stimulated cultures of *Socs3*$^{fl/fl}$ *LysM cre* BMM enhanced IL-12p40 production[276]. The increased NO and iNOS levels in BCG-infected *Socs3*$^{fl/fl}$ *LysM cre* BMM could reflect the described IFN-γ-like response to IL-6 in *Socs3*$^{fl/fl}$ *LysM cre* BMM[125,241].

Moreover, we found lower IL-12p40 expression levels in *M. tuberculosis* and BCG-infected SOCS3-deficient BMDC. Thus, we studied whether IFN-γ levels and secretion were impaired in lungs of *M. tuberculosis*-infected *Socs3*$^{fl/fl}$ *LysM cre* mice. Sixteen days after infection, lungs from *Socs3*$^{fl/fl}$ *LysM cre* mice contained significantly lower levels of *Ifn-γ* mRNA compared to control mice. At this time point, *Ifn-γ* mRNA accumulation in lungs from *M. tuberculosis*-infected control mice was mediated by T cells because depletion of CD4+ cells in control mice reduced IFN-γ levels down to levels found in *Socs3*$^{fl/fl}$ *LysM cre* mice. Moreover, the differences in susceptibility to infection of SOCS3$^{fl/fl}$ *LysM cre* and control mice were abrogated under CD4+ cell depletion.

Surprisingly, *Ifn-γ* mRNA levels were only transiently reduced in *Socs3*$^{fl/fl}$ *LysM cre* mice, and no differences were detected 4 weeks after infection, although differences in bacterial levels at this time point were remarkable. However, a delayed recruitment of antigen-specific T cells to the lungs was shown to underlie increased bacterial burdens in susceptible mouse strains[25]. These results suggest that a delay of T cell-derived IFN-γ secretion due to decreased IL-12p40 production in SOCS3-deficient DCs accounts for the increased susceptibility to *M. tuberculosis* infection of *Socs3*$^{fl/fl}$ *LysM cre* mice (Fig. 12).

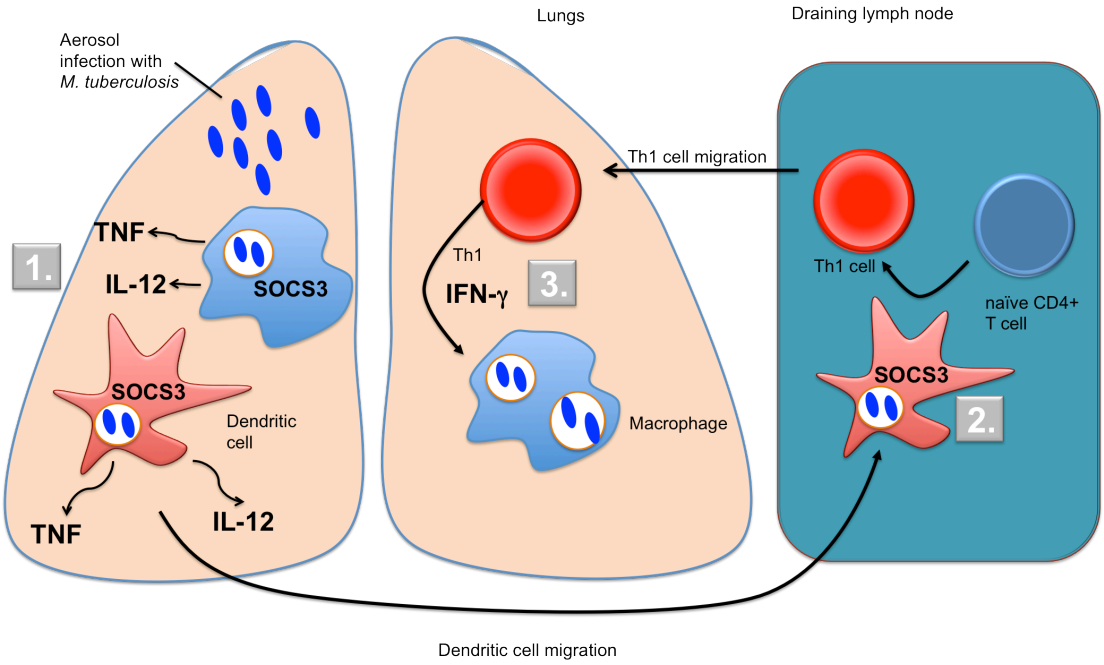

Figure 12: Role of SOCS3 in myeloid cells during *M. tuberculosis* infection.
SOCS3 increases the secretion of IL-12 and TNF in *M. tuberculosis*-infected macrophages and DCs (1). Infected dendritic cells prime Th1 cells in the draining lymph node (2) and in the presence of SOCS3 we found earlier high levels of IFN-γ in the lungs (3) activating infected cells.

In line with our results, the increased susceptibility of *Socs3*$^{fl/fl}$ *LysM cre* mice to *T. gondii* was due to reduced IL-12 responses that could be restored either by IL-6 neutralization or by administration of recombinant IL-12[276]. Since immune defense mechanisms to the intracellular *T. gondii* and intracellular *M. tuberculosis* resemble each other, it is not surprising that we found a similar setting during *M. tuberculosis* infection. Accordingly, in *T. gondii*–infected *Socs3*$^{fl/fl}$ *LysM cre* mice impaired IFN-γ secretion by T and NK cells was found[276], but unlike *M. tuberculosis* defense mechanisms that mainly rely on T cells, IFN-γ produced by NK cells plays a crucial role in protection to *T. gondii*[114,287,288].

Together with our data, this demonstrates a so far unappreciated role of IL-6 that, in the absence of SOCS3 in myeloid cells, becomes a potent antagonist of IFN-γ–mediated protection.

What is the role of SOCS3 in T cells during M. tuberculosis infection in vivo regarding the regulation of bacterial loads, cytokine responses and immunopathology?

Mice with SOCS3-deficient T cells (*Socs3*$^{fl/fl}$ *lck cre* mice) displayed a dramatically increased susceptibility to *M. tuberculosis* infection. In comparison to *Socs3*$^{fl/fl}$ *LysM cre* mice, SOCS3 deficiency in T cells led to higher bacterial loads in the lungs and spleen, increased pathology, and earlier mortality. Transfer of SOCS3-deficient T cells in *Rag1*$^{-/-}$ mice did not convey resistance and suppressed protection by co-inoculated control T cells. Moreover, absence of SOCS3 in T cells in *Leishmania major*-infected mice increased parasite counts confirming a SOCS3-dependent protection mechanism[258].

However, the observed susceptibility to *M. tuberculosis* seemed to be a pathogen-specific mechanism because detected bacterial numbers after BCG infection in *Socs3*$^{fl/fl}$ *lck cre* and control mice were similar. Surprisingly, BCG-vaccinated *Socs3*$^{fl/fl}$ *lck cre* mice showed the same protection to challenge with *M. tuberculosis* as control mice. This suggests that SOCS3 in T cells is not necessarily required for the resistance to *M. tuberculosis* if T cells are primed by BCG-immunization. In contrast to our results, *Socs3*$^{fl/fl}$ *lck cre* mice showed faster LCMV clearance than infected control mice demonstrating a very divergent function of SOCS3 depending on the pathogen[270].

Since we detected higher levels of *Socs3* mRNA expression in *M. tuberculosis* than in BCG-infected lungs, we speculated that *Socs3* expression levels in T cells might be elevated during *M. tuberculosis* in contrast to BCG infection. Unexpectedly, we did not detect any upregulation of *Socs3* mRNA in pulmonary T cells neither after *M. tuberculosis* nor after BCG infection. Our data obtained from mycobacteria-infected mice is consistent with earlier observations that describe a down-regulation of SOCS3 in antigen-stimulated T cells in comparison to naïve T cells[250]. However, since the regulation of SOCS3 expression in T cells during BCG and *M. tuberculosis* infection is comparable, it does not explain the increased susceptibility to *M. tuberculosis* of *Socs3*$^{fl/fl}$ *lck cre* mice.

The immune responses of *Socs3*$^{fl/fl}$ *lck cre* mice were then investigated. We chose 2.5 weeks after *M. tuberculosis* infection as first examined time point as antigen-specific T cells were already present in the lungs, but bacterial loads in control and *Socs3*$^{fl/fl}$ *lck cre* mice were similar. We found that higher levels of IL-17 were secreted in response to antigen-specific stimulation of lung homogenates by *Socs3*$^{fl/fl}$ *lck cre* mice matching previously reported SOCS3-mediated inhibition of Th17 generation[249,254,259]. However, no differences in IL-17 secretion were detected after re-

stimulation of splenocytes from BCG-immunized *Socs3*$^{fl/fl}$ *lck cre* and control mice. Therefore, the increased IL-17 secretion and susceptibility to *M. tuberculosis* in *Socs3*$^{fl/fl}$ *lck cre* mice could be causally associated. The role of IL-17 in resistance to *M. tuberculosis* is controversial and the lack of IL-17 or IL-17R in a low dose model that is also applied in our experiments did not impair the ability of mice to control *M. tuberculosis* infection[144,145]. In contrast, high IL-17 levels may contribute to an increased bacterial dissemination and a neutrophil-mediated inflammation that may account for the early death and immunopathology of *M. tuberculosis*-infected *Socs3*$^{fl/fl}$ *lck cre* mice as described for other susceptible mouse strains[55,289,290]. In fact, increased neutrophil-derived molecules and necrosis could be observed in *M. tuberculosis*-infected *Socs3*$^{fl/fl}$ *lck cre* mice.

Moreover, IFN-γ levels were elevated in *Socs3*$^{fl/fl}$ *lck cre* mice already 2.5 weeks after *M. tuberculosis* infection and might be explained by the described binding of SOCS3 to IL-12Rβ2[257]. However, increased IFN-γ levels cannot justify the dramatic susceptibility to *M. tuberculosis* of *Socs3*$^{fl/fl}$ *lck cre* mice. Kinjyo *et al.* reported that SOCS3-deficient T cells show enhanced development of Th3 cells that produce anti-inflammatory cytokines such as IL-10 and TGF-β[258]. Nevertheless, IL-10 levels were not increased in *Socs3*$^{fl/fl}$ *lck cre* mice.

Altogether, additional studies on IL-17 that is increased during *M. tuberculosis* but not during BCG infection in *Socs3*$^{fl/fl}$ *lck cre* mice may provide promising insights into its role during *M. tuberculosis* infection.

Which function has gp130 signaling in SOCS3-deficient models in vivo and in vitro during mycobacterial infections?

SOCS3 inhibits different signaling receptors. We investigated whether SOCS3 promoted resistance against *M. tuberculosis* via the regulation of gp130-dependent or independent receptor signals. We found that *gp130*$^{F/F}$ mice were highly susceptible to *M. tuberculosis* infection, which was at least in part caused by aberrant IL-6 and STAT3 responses since *gp130*$^{F/F}$*Il-6*$^{-/-}$ and *gp130*$^{F/F}$*Stat3*$^{+/-}$ mice displayed lower bacterial loads than *gp130*$^{F/F}$ mice. Cytokine responses of mycobacteria-infected *gp130*$^{F/F}$ BMM resembled *Socs3*$^{fl/fl}$ *LysM cre* BMM with decreased IL-12 and TNF levels compared to control cells. Previously, it had been demonstrated that *gp130*$^{F/F}$ BMM alike *Socs3*$^{fl/fl}$ *LysM cre* BMM induce reduced TNF and IL-12 levels in response to LPS and IL-6 compared to control BMM[242]. Moreover, we could demonstrate that

the reduction of cytokine concentration during mycobacterial infections was IL-6 dependent since IL-12 and TNF levels in *gp130*$^{F/F}$*/Il-6*$^{-/-}$ BMM were restored to control levels. Accordingly, lower IL-12p40 expression levels were found in the lungs of *gp130*$^{F/F}$ compared to control mice, which were restored in *gp130*$^{F/F}$*Il-6*$^{-/-}$ mice, implying that changed IL-6 signaling in the absence of SOCS3 inhibition of gp130 signaling may account for the increased susceptibility.

Taken together, we propose that, as in *Socs3*$^{fl/fl}$ *LysM cre* mice, delayed T cell priming due to impaired IL-12 induction contributes to the susceptibility to *M. tuberculosis* in *gp130*$^{F/F}$ mice. Similar results were obtained during *T. gondii* infection of *gp130*$^{F/F}$ mice that showed a transient reduction of IFN-γ serum levels[275]. Treatment of *gp130*$^{F/F}$ mice with either neutralizing IL-6 antibodies or exogenous IL-12 improved parasite control demonstrating that SOCS3 provides similar protection mechanisms by modulation of gp130 signaling during *T. gondii* and *M. tuberculosis* infection[275].

Interestingly, T cells from *gp130*$^{F/F}$ mice could transfer protection to *Rag1*$^{-/-}$ to the same extent as transferred control T cells, indicating that the protective SOCS3 expression in T cells is gp130-independent. Since the susceptibility of *gp130*$^{F/F}$ to *M. tuberculosis* was significantly higher than of *Socs3*$^{fl/fl}$ *LysM cre* and T cells were not involved, we suggest that non-hematopoietic cells may also contribute to SOCS3-mediated control to *M. tuberculosis* infection, a suggestion that remains to be tested.

Altogether, SOCS3 plays a critical and non-redundant role in myeloid and T cells during *M. tuberculosis* infection. Notably, mechanisms of resistance conveyed by SOCS3 are different in myeloid and T cells but in both cases necessary to enable a proper T cell differentiation.

5 SUMMARY

In summary, we define the following roles for SOCS proteins during *M. tuberculosis* infection:

SOCS1

… reduces IFN-γ induction in response to IL-12 in macrophages.

… inhibits IFN-γ-mediated growth control during early infection.

… is a major controller of non-macrophage-mediated inflammation.

SOCS2

… has no influence on mycobacterial growth control.

SOCS3

… in myeloid and T cells reduces susceptibility to infection.

… in myeloid cells impairs IL-6 dependent reduction of IL-12 and TNF secretion and leads to early induction of IFN-γ by CD4+ cells in the lungs.

… in T cells hampers IL-17 and IFN-γ secretion during *M. tuberculosis* infection.

… is redundant in T cells for *M. tuberculosis* protection if mice are BCG-vaccinated.

… mediates inhibition of gp130-transmitted signals and thereby reduces susceptibility that is dependent on IL-6 and STAT3.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

Summarizing the studies on SOCS proteins presented in this thesis, I would like to give an evaluation of the role of the different SOCS proteins during mycobacterial infections and discuss under which circumstances a modulation of SOCS levels may be of importance in controlling the infection.

SOCS1 plays a two-faced role during mycobacterial infections. During the innate phase, when bacteria replicate in macrophages without any adaptive immunity present, SOCS1 suppresses IFN-γ induction by macrophages and therefore enables mycobacterial growth. Deletion of SOCS1 could slow down this mycobacterial growth but did not lead to complete eradication of the bacteria. Since it has been proposed that a fraction of the *M. tuberculosis*-exposed individuals remain uninfected, it would be interesting to determine whether SOCS1 is involved in such innate control of infection. In contrast, SOCS1 expression protected from severe inflammation in the lungs without impairing immunity to infection during later stages of *M. tuberculosis* infection. Thus, SOCS1 mimetic peptides may be useful to limit immune-mediated pathology in tuberculosis as it was shown in a vaccinia virus infection model[192].

Regarding our data on SOCS2, its role can be addressed as minor or redundant during *M. tuberculosis* infection. Although enhanced Th2 responses were reported in the absence of SOCS2, we observed similar bacterial burdens in presence and absence of SOCS2 suggesting a well functioning Th1 response. However, it is possible that under Th2-stimulating conditions, such as a common co-infection with helminths, absence of SOCS2 may further enhance Th2 responses and thereby impair *M. tuberculosis* control.

In contrast to SOCS1 and SOCS2, SOCS3 expression by either myeloid or lymphoid cells had a central role in controlling *M. tuberculosis* infection,. Absence of SOCS3 in BMM has been described to prolong STAT3 activation in response to IL-6 that in turn decreased IL-12 and TNF transcription[126]. The knockdown of STAT3 in DCs has been shown to increase cytokine production, antigen-specific T cell activation and resistance to IL-10-mediated suppression[291]. Moreover, increased STAT3 activation prevents recognition of tumor cells by the immune system in different kinds of cancer and inhibition of STAT3 activation by administration of small inhibitory molecules has been shown to impair the growth of cancer cells *in vitro* and *in vivo*[292]. Therefore, it would be a promising approach to limit STAT3

activation to improve resistance to *M. tuberculosis* infection by increasing IL-12 levels and antigen-specific Th1 responses. However, since STAT3 is unique in its capacity to direct anti-inflammatory activities of IL-10 and mice with a knockdown for STAT3 in myeloid cells showed a pro-inflammatory phenotype with spontaneous development of colitis[243], it will be a challenge to keep the balance between pro- and anti-inflammatory signals. Additionally, it has to be taken in consideration that a knockdown of *gp130* in myeloid cells did not improve control of *M. tuberculosis* infection in mice, demonstrating that ablation of all gp130-mediated signals may be contraindicated[293]. Even considering these aspects, our data convincingly argue for further explorations in targeting STAT3 to improve immune control during tuberculosis.

Since SOCS3 expression in T cells was not required to mount protective immunity after BCG-vaccination, it would be of high interest to understand why SOCS3 in turn is required for resistance to primary *M. tuberculosis* infection. Furthermore, the high IL-17 levels in the absence of SOCS3 in T cells may have contributed to the observed susceptibility since we did not detect any elevated anti-inflammatory immune responses that could account for increased bacterial growth. Understanding the role of these two factors, SOCS3 expression and elevated IL-17 secretion by T cells during *M. tuberculosis* infection, may provide important information how to achieve protection against *M. tuberculosis* and thereby how to improve vaccine design. Summarizing, we suggest that modulation of SOCS3-regulated pathways is a promising approach to improve vaccination and the control of mycobacterial infections.

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8 REFERENCES

1 Koch, R. Classics in infectious diseases. The etiology of tuberculosis: Robert Koch. Berlin, Germany 1882. *Rev Infect Dis* **4**, 1270-1274 (1982).
2 Alcais, A., Fieschi, C., Abel, L. & Casanova, J. L. Tuberculosis in children and adults: two distinct genetic diseases. *J Exp Med* **202**, 1617-1621, doi:10.1084/jem.20052302 (2005).
3 Parida, S. K. & Kaufmann, S. H. The quest for biomarkers in tuberculosis. *Drug Discov Today* **15**, 148-157, doi:10.1016/j.drudis.2009.10.005 (2010).
4 Corbett, E. L. *et al.* The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* **163**, 1009-1021, doi:10.1001/archinte.163.9.1009 (2003).
5 WHO. Global tuberculosis control. *WHO, Geneva, Switzerland* (2011).
6 Gustafson, P. *et al.* Tuberculosis in Bissau: incidence and risk factors in an urban community in sub-Saharan Africa. *Int J Epidemiol* **33**, 163-172, doi:10.1093/ije/dyh026 (2004).
7 Lonnroth, K., Jaramillo, E., Williams, B. G., Dye, C. & Raviglione, M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sci Med* **68**, 2240-2246, doi:10.1016/j.socscimed.2009.03.041 (2009).
8 Raviglione, M. C. & Smith, I. M. XDR tuberculosis--implications for global public health. *N Engl J Med* **356**, 656-659, doi:10.1056/NEJMp068273 (2007).
9 Colditz, G. A. *et al.* Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *Jama* **271**, 698-702 (1994).
10 Fine, P. E. Variation in protection by BCG: implications of and for heterologous immunity. *Lancet* **346**, 1339-1345 (1995).
11 Ritz, N. & Curtis, N. Mapping the global use of different BCG vaccine strains. *Tuberculosis (Edinb)* **89**, 248-251, doi:10.1016/j.tube.2009.03.002 (2009).
12 Andersen, P. Tuberculosis vaccines - an update. *Nat Rev Microbiol* **5**, 484-487, doi:10.1038/nrmicro1703 (2007).
13 Okamoto, Y. *et al.* Mycobacterial sulfolipid shows a virulence by inhibiting cord factor induced granuloma formation and TNF-alpha release. *Microb Pathog* **40**, 245-253, doi:10.1016/j.micpath.2006.02.002 (2006).
14 Brosch, R. *et al.* A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proc Natl Acad Sci U S A* **99**, 3684-3689, doi:10.1073/pnas.052548299 (2002).
15 de Chastellier, C. The many niches and strategies used by pathogenic mycobacteria for survival within host macrophages. *Immunobiology* **214**, 526-542, doi:10.1016/j.imbio.2008.12.005 (2009).
16 Smith, D. W., McMurray, D. N., Wiegeshaus, E. H., Grover, A. A. & Harding, G. E. Host-parasite relationships in experimental airborne tuberculosis. IV. Early events in the course of infection in vaccinated and nonvaccinated guinea pigs. *Am Rev Respir Dis* **102**, 937-949 (1970).
17 Turner, O. C., Basaraba, R. J. & Orme, I. M. Immunopathogenesis of pulmonary granulomas in the guinea pig after infection with Mycobacterium tuberculosis. *Infect Immun* **71**, 864-871 (2003).
18 Gupta, U. D. & Katoch, V. M. Animal models of tuberculosis. *Tuberculosis (Edinb)* **85**, 277-293, doi:10.1016/j.tube.2005.08.008 (2005).
19 Smith, D. W. & Harding, G. E. Animal model of human disease. Pulmonary tuberculosis. Animal model: Experimental airborne tuberculosis in the guinea pig. *Am J Pathol* **89**, 273-276 (1977).
20 Ahmad, Z. *et al.* Biphasic kill curve of isoniazid reveals the presence of drug-tolerant, not drug-resistant, Mycobacterium tuberculosis in the guinea pig. *J Infect Dis* **200**, 1136-1143, doi:10.1086/605605 (2009).
21 Gupta, U. D. & Katoch, V. M. Animal models of tuberculosis for vaccine development. *Indian J Med Res* **129**, 11-18 (2009).
22 Young, D. Animal models of tuberculosis. *Eur J Immunol* **39**, 2011-2014, doi:10.1002/eji.200939542 (2009).

23 Kelly, B. P., Furney, S. K., Jessen, M. T. & Orme, I. M. Low-dose aerosol infection model for testing drugs for efficacy against Mycobacterium tuberculosis. *Antimicrob Agents Chemother* **40**, 2809-2812 (1996).
24 Wolf, A. J. *et al.* Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. *J Exp Med* **205**, 105-115, doi:10.1084/jem.20071367 (2008).
25 Chackerian, A. A., Alt, J. M., Perera, T. V., Dascher, C. C. & Behar, S. M. Dissemination of Mycobacterium tuberculosis is influenced by host factors and precedes the initiation of T-cell immunity. *Infect Immun* **70**, 4501-4509 (2002).
26 Pan, H. *et al.* Ipr1 gene mediates innate immunity to tuberculosis. *Nature* **434**, 767-772, doi:10.1038/nature03419 (2005).
27 McCune, R. M., Feldmann, F. M., Lambert, H. P. & McDermott, W. Microbial persistence. I. The capacity of tubercle bacilli to survive sterilization in mouse tissues. *J Exp Med* **123**, 445-468 (1966).
28 McCune, R. M., Feldmann, F. M. & McDermott, W. Microbial persistence. II. Characteristics of the sterile state of tubercle bacilli. *J Exp Med* **123**, 469-486 (1966).
29 Urdahl, K. B., Shafiani, S. & Ernst, J. D. Initiation and regulation of T-cell responses in tuberculosis. *Mucosal Immunol* **4**, 288-293, doi:10.1038/mi.2011.10 (2011).
30 Flynn, J. L. *et al.* Non-human primates: a model for tuberculosis research. *Tuberculosis (Edinb)* **83**, 116-118 (2003).
31 Riley, R. L., Wells, W. F., Mills, C. C., Nyka, W. & McLean, R. L. Air hygiene in tuberculosis: quantitative studies of infectivity and control in a pilot ward. *Am Rev Tuberc* **75**, 420-431 (1957).
32 Armstrong, J. A. & Hart, P. D. Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. *J Exp Med* **134**, 713-740 (1971).
33 Frehel, C., de Chastellier, C., Lang, T. & Rastogi, N. Evidence for inhibition of fusion of lysosomal and prelysosomal compartments with phagosomes in macrophages infected with pathogenic Mycobacterium avium. *Infect Immun* **52**, 252-262 (1986).
34 van der Wel, N. *et al.* M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* **129**, 1287-1298, doi:10.1016/j.cell.2007.05.059 (2007).
35 Cywes, C., Hoppe, H. C., Daffe, M. & Ehlers, M. R. Nonopsonic binding of Mycobacterium tuberculosis to complement receptor type 3 is mediated by capsular polysaccharides and is strain dependent. *Infect Immun* **65**, 4258-4266 (1997).
36 Schlesinger, L. S. Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. *Journal of immunology* **150**, 2920-2930 (1993).
37 Zimmerli, S., Edwards, S. & Ernst, J. D. Selective receptor blockade during phagocytosis does not alter the survival and growth of Mycobacterium tuberculosis in human macrophages. *Am J Respir Cell Mol Biol* **15**, 760-770 (1996).
38 Bafica, A. *et al.* TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. *J Exp Med* **202**, 1715-1724, doi:10.1084/jem.20051782 (2005).
39 Gilleron, M., Quesniaux, V. F. & Puzo, G. Acylation state of the phosphatidylinositol hexamannosides from Mycobacterium bovis bacillus Calmette Guerin and mycobacterium tuberculosis H37Rv and its implication in Toll-like receptor response. *J Biol Chem* **278**, 29880-29889, doi:10.1074/jbc.M303446200 (2003).
40 Jones, B. W. *et al.* Different Toll-like receptor agonists induce distinct macrophage responses. *J Leukoc Biol* **69**, 1036-1044 (2001).
41 Means, T. K. *et al.* Human toll-like receptors mediate cellular activation by Mycobacterium tuberculosis. *Journal of immunology* **163**, 3920-3927 (1999).

42 Kawai, T. & Akira, S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* **34**, 637-650, doi:10.1016/j.immuni.2011.05.006 (2011).
43 Wolf, A. J. *et al.* Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo. *Journal of immunology* **179**, 2509-2519 (2007).
44 Gallegos, A. M., Pamer, E. G. & Glickman, M. S. Delayed protection by ESAT-6-specific effector CD4+ T cells after airborne M. tuberculosis infection. *J Exp Med* **205**, 2359-2368, doi:10.1084/jem.20080353 (2008).
45 Hoang, T. T. *et al.* Distinct differences in the expansion and phenotype of TB10.4 specific CD8 and CD4 T cells after infection with Mycobacterium tuberculosis. *PLoS One* **4**, e5928, doi:10.1371/journal.pone.0005928 (2009).
46 Mogues, T., Goodrich, M. E., Ryan, L., LaCourse, R. & North, R. J. The relative importance of T cell subsets in immunity and immunopathology of airborne Mycobacterium tuberculosis infection in mice. *J Exp Med* **193**, 271-280 (2001).
47 Selwyn, P. A. *et al.* A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med* **320**, 545-550, doi:10.1056/NEJM198903023200901 (1989).
48 Caruso, A. M. *et al.* Mice deficient in CD4 T cells have only transiently diminished levels of IFN-gamma, yet succumb to tuberculosis. *Journal of immunology* **162**, 5407-5416 (1999).
49 Scanga, C. A. *et al.* Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon gamma and nitric oxide synthase 2. *J Exp Med* **192**, 347-358 (2000).
50 Cooper, A. M. *et al.* Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J Exp Med* **178**, 2243-2247 (1993).
51 North, R. J. & Jung, Y. J. Immunity to tuberculosis. *Annu Rev Immunol* **22**, 599-623, doi:10.1146/annurev.immunol.22.012703.104635 (2004).
52 Pearl, J. E., Saunders, B., Ehlers, S., Orme, I. M. & Cooper, A. M. Inflammation and lymphocyte activation during mycobacterial infection in the interferon-gamma-deficient mouse. *Cell Immunol* **211**, 43-50, doi:10.1006/cimm.2001.1819 (2001).
53 Tufariello, J. M., Chan, J. & Flynn, J. L. Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infect Dis* **3**, 578-590 (2003).
54 Sullivan, B. M. *et al.* Increased susceptibility of mice lacking T-bet to infection with Mycobacterium tuberculosis correlates with increased IL-10 and decreased IFN-gamma production. *Journal of immunology* **175**, 4593-4602 (2005).
55 Desvignes, L. & Ernst, J. D. Interferon-gamma-responsive nonhematopoietic cells regulate the immune response to Mycobacterium tuberculosis. *Immunity* **31**, 974-985, doi:10.1016/j.immuni.2009.10.007 (2009).
56 Ivanov, II *et al.* The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* **126**, 1121-1133, doi:10.1016/j.cell.2006.07.035 (2006).
57 Scriba, T. J. *et al.* Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. *Journal of immunology* **180**, 1962-1970 (2008).
58 Guyot-Revol, V., Innes, J. A., Hackforth, S., Hinks, T. & Lalvani, A. Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. *Am J Respir Crit Care Med* **173**, 803-810, doi:10.1164/rccm.200508-1294OC (2006).
59 Kursar, M. *et al.* Cutting Edge: Regulatory T cells prevent efficient clearance of Mycobacterium tuberculosis. *Journal of immunology* **178**, 2661-2665 (2007).
60 Ribeiro-Rodrigues, R. *et al.* A role for CD4+CD25+ T cells in regulation of the immune response during human tuberculosis. *Clin Exp Immunol* **144**, 25-34, doi:10.1111/j.1365-2249.2006.03027.x (2006).
61 Scott-Browne, J. P. *et al.* Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. *J Exp Med* **204**, 2159-2169, doi:10.1084/jem.20062105 (2007).

62 Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nature immunology* **4**, 330-336, doi:10.1038/ni904 (2003).
63 Shafiani, S., Tucker-Heard, G., Kariyone, A., Takatsu, K. & Urdahl, K. B. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J Exp Med* **207**, 1409-1420, doi:10.1084/jem.20091885 (2010).
64 Ozeki, Y. *et al.* Transient role of CD4+CD25+ regulatory T cells in mycobacterial infection in mice. *International immunology* **22**, 179-189, doi:10.1093/intimm/dxp126 (2010).
65 Fenhalls, G. *et al.* Distribution of IFN-gamma, IL-4 and TNF-alpha protein and CD8 T cells producing IL-12p40 mRNA in human lung tuberculous granulomas. *Immunology* **105**, 325-335 (2002).
66 Ulrichs, T. *et al.* Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. *J Infect Dis* **192**, 89-97, doi:10.1086/430621 (2005).
67 Davis, J. M. & Ramakrishnan, L. The role of the granuloma in expansion and dissemination of early tuberculous infection. *Cell* **136**, 37-49, doi:10.1016/j.cell.2008.11.014 (2009).
68 Volkman, H. E. *et al.* Tuberculous granuloma formation is enhanced by a mycobacterium virulence determinant. *PLoS Biol* **2**, e367, doi:10.1371/journal.pbio.0020367 (2004).
69 Dheda, K. *et al.* Lung remodeling in pulmonary tuberculosis. *J Infect Dis* **192**, 1201-1209, doi:10.1086/444545 (2005).
70 Li, Y. J., Petrofsky, M. & Bermudez, L. E. Mycobacterium tuberculosis uptake by recipient host macrophages is influenced by environmental conditions in the granuloma of the infectious individual and is associated with impaired production of interleukin-12 and tumor necrosis factor alpha. *Infect Immun* **70**, 6223-6230 (2002).
71 Hopewell, P. *Overview of Clinical Tuberculosis*. (1994).
72 Nicola, N. A. *Guidebook to Cytokines and Their Receptors*. (Oxford University Press, 1994).
73 Abbas. *Cellular and Molecular Immunology*. 6th edn, (Elsevier, 2007).
74 Hanada, T. & Yoshimura, A. Regulation of cytokine signaling and inflammation. *Cytokine Growth Factor Rev* **13**, 413-421 (2002).
75 Shuai, K. & Liu, B. Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol* **3**, 900-911, doi:10.1038/nri1226 (2003).
76 Krebs, D. L. & Hilton, D. J. SOCS proteins: negative regulators of cytokine signaling. *Stem cells* **19**, 378-387, doi:10.1634/stemcells.19-5-378 (2001).
77 Shuai, K. Regulation of cytokine signaling pathways by PIAS proteins. *Cell research* **16**, 196-202, doi:10.1038/sj.cr.7310027 (2006).
78 Hebenstreit, D., Luft, P., Schmiedlechner, A., Duschl, A. & Horejs-Hoeck, J. SOCS-1 and SOCS-3 inhibit IL-4 and IL-13 induced activation of Eotaxin-3/CCL26 gene expression in HEK293 cells. *Mol Immunol* **42**, 295-303, doi:10.1016/j.molimm.2004.09.004 (2005).
79 Leonard, W. J. & O'Shea, J. J. Jaks and STATs: biological implications. *Annu Rev Immunol* **16**, 293-322, doi:10.1146/annurev.immunol.16.1.293 (1998).
80 Wajant, H., Pfizenmaier, K. & Scheurich, P. Tumor necrosis factor signaling. *Cell Death Differ* **10**, 45-65, doi:10.1038/sj.cdd.4401189 (2003).
81 Mannel, D. N. & Echtenacher, B. TNF in the inflammatory response. *Chem Immunol* **74**, 141-161 (2000).
82 Bean, A. G. *et al.* Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol Mycobacterium tuberculosis infection, which is not compensated for by lymphotoxin. *Journal of immunology* **162**, 3504-3511 (1999).
83 Flynn, J. L. *et al.* Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity* **2**, 561-572 (1995).

84 Gardam, M. A. *et al.* Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. *Lancet Infect Dis* **3**, 148-155 (2003).
85 Botha, T. & Ryffel, B. Reactivation of latent tuberculosis infection in TNF-deficient mice. *Journal of immunology* **171**, 3110-3118 (2003).
86 Chakravarty, S. D. *et al.* Tumor necrosis factor blockade in chronic murine tuberculosis enhances granulomatous inflammation and disorganizes granulomas in the lungs. *Infect Immun* **76**, 916-926, doi:10.1128/IAI.01011-07 (2008).
87 Mohan, V. P. *et al.* Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. *Infect Immun* **69**, 1847-1855, doi:10.1128/IAI.69.3.1847-1855.2001 (2001).
88 Algood, H. M., Lin, P. L. & Flynn, J. L. Tumor necrosis factor and chemokine interactions in the formation and maintenance of granulomas in tuberculosis. *Clin Infect Dis* **41 Suppl 3**, S189-193, doi:10.1086/429994 (2005).
89 Flynn, J. L. & Chan, J. Tuberculosis: latency and reactivation. *Infect Immun* **69**, 4195-4201, doi:10.1128/IAI.69.7.4195-4201.2001 (2001).
90 Kindler, V., Sappino, A. P., Grau, G. E., Piguet, P. F. & Vassalli, P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell* **56**, 731-740 (1989).
91 Roach, D. R. *et al.* TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. *Journal of immunology* **168**, 4620-4627 (2002).
92 Legler, D. F. *et al.* B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* **187**, 655-660 (1998).
93 Tessier, P. A. *et al.* Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-alpha. *Journal of immunology* **159**, 3595-3602 (1997).
94 Clay, H., Volkman, H. E. & Ramakrishnan, L. Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity* **29**, 283-294, doi:10.1016/j.immuni.2008.06.011 (2008).
95 Watford, W. T. *et al.* Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* **202**, 139-156, doi:10.1111/j.0105-2896.2004.00211.x (2004).
96 Filipe-Santos, O. *et al.* Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. *Semin Immunol* **18**, 347-361, doi:10.1016/j.smim.2006.07.010 (2006).
97 Cooper, A. M. *et al.* Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. *Journal of immunology* **168**, 1322-1327 (2002).
98 Flynn, J. L. *et al.* IL-12 increases resistance of BALB/c mice to Mycobacterium tuberculosis infection. *Journal of immunology* **155**, 2515-2524 (1995).
99 Khader, S. A. *et al.* Interleukin 12p40 is required for dendritic cell migration and T cell priming after Mycobacterium tuberculosis infection. *J Exp Med* **203**, 1805-1815, doi:10.1084/jem.20052545 (2006).
100 Holscher, C. *et al.* A protective and agonistic function of IL-12p40 in mycobacterial infection. *Journal of immunology* **167**, 6957-6966 (2001).
101 Feng, C. G. *et al.* Maintenance of pulmonary Th1 effector function in chronic tuberculosis requires persistent IL-12 production. *Journal of immunology* **174**, 4185-4192 (2005).
102 Stobie, L. *et al.* The role of antigen and IL-12 in sustaining Th1 memory cells in vivo: IL-12 is required to maintain memory/effector Th1 cells sufficient to mediate protection to an infectious parasite challenge. *Proc Natl Acad Sci U S A* **97**, 8427-8432, doi:10.1073/pnas.160197797 (2000).
103 Billiau, A. *et al.* Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-gamma. *Journal of immunology* **140**, 1506-1510 (1988).

104 Chu, C. Q., Wittmer, S. & Dalton, D. K. Failure to suppress the expansion of the activated CD4 T cell population in interferon gamma-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. *J Exp Med* **192**, 123-128 (2000).
105 Debray-Sachs, M. *et al.* Prevention of diabetes in NOD mice treated with antibody to murine IFN gamma. *J Autoimmun* **4**, 237-248 (1991).
106 Denis, M. Interferon-gamma-treated murine macrophages inhibit growth of tubercle bacilli via the generation of reactive nitrogen intermediates. *Cell Immunol* **132**, 150-157 (1991).
107 Shankaran, V. *et al.* IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* **410**, 1107-1111, doi:10.1038/35074122 (2001).
108 Darnell, J. E., Jr., Kerr, I. M. & Stark, G. R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**, 1415-1421 (1994).
109 Flynn, J. L. *et al.* An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. *J Exp Med* **178**, 2249-2254 (1993).
110 Jouanguy, E. *et al.* Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. *N Engl J Med* **335**, 1956-1961, doi:10.1056/NEJM199612263352604 (1996).
111 Altare, F. *et al.* Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* **280**, 1432-1435 (1998).
112 Newport, M. J. *et al.* A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. *N Engl J Med* **335**, 1941-1949, doi:10.1056/NEJM199612263352602 (1996).
113 Feng, C. G. *et al.* NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with Mycobacterium tuberculosis. *Journal of immunology* **177**, 7086-7093 (2006).
114 Junqueira-Kipnis, A. P. *et al.* NK cells respond to pulmonary infection with Mycobacterium tuberculosis, but play a minimal role in protection. *Journal of immunology* **171**, 6039-6045 (2003).
115 Jung, Y. J., Ryan, L., LaCourse, R. & North, R. J. Properties and protective value of the secondary versus primary T helper type 1 response to airborne Mycobacterium tuberculosis infection in mice. *J Exp Med* **201**, 1915-1924, doi:10.1084/jem.20050265 (2005).
116 Ehrt, S. *et al.* Reprogramming of the macrophage transcriptome in response to interferon-gamma and Mycobacterium tuberculosis: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. *J Exp Med* **194**, 1123-1140 (2001).
117 Sato, K., Akaki, T. & Tomioka, H. Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha). *Clin Exp Immunol* **112**, 63-68 (1998).
118 Chan, J., Xing, Y., Magliozzo, R. S. & Bloom, B. R. Killing of virulent Mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. *J Exp Med* **175**, 1111-1122 (1992).
119 Flesch, I. E. & Kaufmann, S. H. Mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages: role of reactive nitrogen intermediates. *Infect Immun* **59**, 3213-3218 (1991).
120 MacMicking, J. D. *et al.* Identification of nitric oxide synthase as a protective locus against tuberculosis. *Proc Natl Acad Sci U S A* **94**, 5243-5248 (1997).
121 Nicholson, S. *et al.* Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. *J Exp Med* **183**, 2293-2302 (1996).
122 Rich, E. A. *et al.* Mycobacterium tuberculosis (MTB)-stimulated production of nitric oxide by human alveolar macrophages and relationship of nitric oxide production to growth inhibition of MTB. *Tuber Lung Dis* **78**, 247-255 (1997).
123 Leal, I. S., Smedegard, B., Andersen, P. & Appelberg, R. Failure to induce enhanced protection against tuberculosis by increasing T-cell-dependent interferon-gamma generation. *Immunology* **104**, 157-161 (2001).

124 Majlessi, L. *et al.* An increase in antimycobacterial Th1-cell responses by prime-boost protocols of immunization does not enhance protection against tuberculosis. *Infect Immun* **74**, 2128-2137, doi:10.1128/IAI.74.4.2128-2137.2006 (2006).
125 Lang, R. *et al.* SOCS3 regulates the plasticity of gp130 signaling. *Nature immunology* **4**, 546-550, doi:10.1038/ni932 (2003).
126 Yasukawa, H. *et al.* IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. *Nature immunology* **4**, 551-556, doi:10.1038/ni938 (2003).
127 el-Ahmady, O., Mansour, M., Zoeir, H. & Mansour, O. Elevated concentrations of interleukins and leukotriene in response to Mycobacterium tuberculosis infection. *Ann Clin Biochem* **34 (Pt 2)**, 160-164 (1997).
128 Giacomini, E. *et al.* Infection of human macrophages and dendritic cells with Mycobacterium tuberculosis induces a differential cytokine gene expression that modulates T cell response. *Journal of immunology* **166**, 7033-7041 (2001).
129 Indrigo, J., Hunter, R. L., Jr. & Actor, J. K. Influence of trehalose 6,6'-dimycolate (TDM) during mycobacterial infection of bone marrow macrophages. *Microbiology* **148**, 1991-1998 (2002).
130 Ladel, C. H. *et al.* Lethal tuberculosis in interleukin-6-deficient mutant mice. *Infect Immun* **65**, 4843-4849 (1997).
131 Saunders, B. M., Frank, A. A., Orme, I. M. & Cooper, A. M. Interleukin-6 induces early gamma interferon production in the infected lung but is not required for generation of specific immunity to Mycobacterium tuberculosis infection. *Infect Immun* **68**, 3322-3326 (2000).
132 Nagabhushanam, V. *et al.* Innate inhibition of adaptive immunity: Mycobacterium tuberculosis-induced IL-6 inhibits macrophage responses to IFN-gamma. *Journal of immunology* **171**, 4750-4757 (2003).
133 VanHeyningen, T. K., Collins, H. L. & Russell, D. G. IL-6 produced by macrophages infected with Mycobacterium species suppresses T cell responses. *Journal of immunology* **158**, 330-337 (1997).
134 Bermudez, L. E., Wu, M., Petrofsky, M. & Young, L. S. Interleukin-6 antagonizes tumor necrosis factor-mediated mycobacteriostatic and mycobactericidal activities in macrophages. *Infect Immun* **60**, 4245-4252 (1992).
135 Harrington, L. E. *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nature immunology* **6**, 1123-1132, doi:10.1038/ni1254 (2005).
136 Martin, B., Hirota, K., Cua, D. J., Stockinger, B. & Veldhoen, M. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* **31**, 321-330, doi:10.1016/j.immuni.2009.06.020 (2009).
137 Park, H. *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nature immunology* **6**, 1133-1141, doi:10.1038/ni1261 (2005).
138 Sutton, C. E. *et al.* Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* **31**, 331-341, doi:10.1016/j.immuni.2009.08.001 (2009).
139 Korn, T. *et al.* IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* **448**, 484-487, doi:10.1038/nature05970 (2007).
140 Zhou, L. *et al.* IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nature immunology* **8**, 967-974, doi:10.1038/ni1488 (2007).
141 Lockhart, E., Green, A. M. & Flynn, J. L. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during Mycobacterium tuberculosis infection. *Journal of immunology* **177**, 4662-4669 (2006).
142 Umemura, M. *et al.* IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. *Journal of immunology* **178**, 3786-3796 (2007).

143 Peng, M. Y. *et al.* Interleukin 17-producing gamma delta T cells increased in patients with active pulmonary tuberculosis. *Cell Mol Immunol* **5**, 203-208, doi:10.1038/cmi.2008.25 (2008).
144 Aujla, S. J., Dubin, P. J. & Kolls, J. K. Th17 cells and mucosal host defense. *Semin Immunol* **19**, 377-382, doi:10.1016/j.smim.2007.10.009 (2007).
145 Khader, S. A. *et al.* IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. *Journal of immunology* **175**, 788-795 (2005).
146 Cruz, A. *et al.* Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with Mycobacterium tuberculosis. *J Exp Med* **207**, 1609-1616, doi:10.1084/jem.20100265 (2010).
147 Okamoto Yoshida, Y. *et al.* Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. *Journal of immunology* **184**, 4414-4422, doi:10.4049/jimmunol.0903332 (2010).
148 Holscher, C. *et al.* The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *Journal of immunology* **174**, 3534-3544 (2005).
149 Kamura, T. *et al.* VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. *Genes Dev* **18**, 3055-3065, doi:10.1101/gad.1252404 (2004).
150 Verdier, F. *et al.* Proteasomes regulate erythropoietin receptor and signal transducer and activator of transcription 5 (STAT5) activation. Possible involvement of the ubiquitinated Cis protein. *J Biol Chem* **273**, 28185-28190 (1998).
151 Piessevaux, J., Lavens, D., Peelman, F. & Tavernier, J. The many faces of the SOCS box. *Cytokine Growth Factor Rev* **19**, 371-381, doi:10.1016/j.cytogfr.2008.08.006 (2008).
152 Endo, T. A. *et al.* A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* **387**, 921-924, doi:10.1038/43213 (1997).
153 Nicholson, S. E. *et al.* Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. *Proc Natl Acad Sci U S A* **97**, 6493-6498, doi:10.1073/pnas.100135197 (2000).
154 Kubo, M., Hanada, T. & Yoshimura, A. Suppressors of cytokine signaling and immunity. *Nature immunology* **4**, 1169-1176, doi:10.1038/ni1012 (2003).
155 Yasukawa, H. *et al.* The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. *Embo J* **18**, 1309-1320, doi:10.1093/emboj/18.5.1309 (1999).
156 Zhang, J. G. *et al.* The SOCS box of suppressor of cytokine signaling-1 is important for inhibition of cytokine action in vivo. *Proc Natl Acad Sci U S A* **98**, 13261-13265, doi:10.1073/pnas.231486498 (2001).
157 Naka, T. *et al.* Structure and function of a new STAT-induced STAT inhibitor. *Nature* **387**, 924-929, doi:10.1038/43219 (1997).
158 Starr, R. *et al.* A family of cytokine-inducible inhibitors of signalling. *Nature* **387**, 917-921, doi:10.1038/43206 (1997).
159 Fujimoto, M. & Naka, T. Regulation of cytokine signaling by SOCS family molecules. *Trends Immunol* **24**, 659-666 (2003).
160 Alexander, W. S. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* **2**, 410-416, doi:10.1038/nri818 (2002).
161 Dennis, V. A., Jefferson, A., Singh, S. R., Ganapamo, F. & Philipp, M. T. Interleukin-10 anti-inflammatory response to Borrelia burgdorferi, the agent of Lyme disease: a possible role for suppressors of cytokine signaling 1 and 3. *Infect Immun* **74**, 5780-5789, doi:10.1128/IAI.00678-06 (2006).
162 Dalpke, A. H., Opper, S., Zimmermann, S. & Heeg, K. Suppressors of cytokine signaling (SOCS)-1 and SOCS-3 are induced by CpG-DNA and modulate cytokine responses in APCs. *Journal of immunology* **166**, 7082-7089 (2001).
163 Emanuelli, B. *et al.* SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J Biol Chem* **275**, 15985-15991 (2000).

164 Hamanaka, I. *et al.* Induction of JAB/SOCS-1/SSI-1 and CIS3/SOCS-3/SSI-3 is involved in gp130 resistance in cardiovascular system in rat treated with cardiotrophin-1 in vivo. *Circ Res* **88**, 727-732 (2001).
165 Bhattacharyya, S., Zhao, Y., Kay, T. W. & Muglia, L. J. Glucocorticoids target suppressor of cytokine signaling 1 (SOCS1) and type 1 interferons to regulate Toll-like receptor-induced STAT1 activation. *Proc Natl Acad Sci U S A* **108**, 9554-9559, doi:10.1073/pnas.1017296108 (2011).
166 Fenner, J. E. *et al.* Suppressor of cytokine signaling 1 regulates the immune response to infection by a unique inhibition of type I interferon activity. *Nature immunology* **7**, 33-39, doi:10.1038/ni1287 (2006).
167 Qing, Y., Costa-Pereira, A. P., Watling, D. & Stark, G. R. Role of tyrosine 441 of interferon-gamma receptor subunit 1 in SOCS-1-mediated attenuation of STAT1 activation. *J Biol Chem* **280**, 1849-1853, doi:10.1074/jbc.M409863200 (2005).
168 Naka, T. *et al.* Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice. *Proc Natl Acad Sci U S A* **95**, 15577-15582 (1998).
169 Starr, R. *et al.* Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. *Proc Natl Acad Sci U S A* **95**, 14395-14399 (1998).
170 Alexander, W. S. *et al.* SOCS1 is a critical inhibitor of interferon gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell* **98**, 597-608 (1999).
171 Marine, J. C. *et al.* SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell* **98**, 609-616 (1999).
172 Bullen, D. V., Darwiche, R., Metcalf, D., Handman, E. & Alexander, W. S. Neutralization of interferon-gamma in neonatal SOCS1-/- mice prevents fatty degeneration of the liver but not subsequent fatal inflammatory disease. *Immunology* **104**, 92-98 (2001).
173 Chong, M. M., Metcalf, D., Jamieson, E., Alexander, W. S. & Kay, T. W. Suppressor of cytokine signaling-1 in T cells and macrophages is critical for preventing lethal inflammation. *Blood* **106**, 1668-1675, doi:10.1182/blood-2004-08-3049 (2005).
174 Metcalf, D., Mifsud, S., Di Rago, L. & Alexander, W. S. The lethal effects of transplantation of Socs1-/- bone marrow cells into irradiated adult syngeneic recipients. *Proc Natl Acad Sci U S A* **100**, 8436-8441, doi:10.1073/pnas.1032925100 (2003).
175 Eyles, J. L., Metcalf, D., Grusby, M. J., Hilton, D. J. & Starr, R. Negative regulation of interleukin-12 signaling by suppressor of cytokine signaling-1. *J Biol Chem* **277**, 43735-43740, doi:10.1074/jbc.M208586200 (2002).
176 Metcalf, D., Di Rago, L., Mifsud, S., Hartley, L. & Alexander, W. S. The development of fatal myocarditis and polymyositis in mice heterozygous for IFN-gamma and lacking the SOCS-1 gene. *Proc Natl Acad Sci U S A* **97**, 9174-9179, doi:10.1073/pnas.160255197 (2000).
177 Naka, T. *et al.* SOCS-1/SSI-1-deficient NKT cells participate in severe hepatitis through dysregulated cross-talk inhibition of IFN-gamma and IL-4 signaling in vivo. *Immunity* **14**, 535-545 (2001).
178 Kinjyo, I. *et al.* SOCS1/JAB is a negative regulator of LPS-induced macrophage activation. *Immunity* **17**, 583-591 (2002).
179 Nakagawa, R. *et al.* SOCS-1 participates in negative regulation of LPS responses. *Immunity* **17**, 677-687 (2002).
180 Car, B. D. *et al.* Interferon gamma receptor deficient mice are resistant to endotoxic shock. *J Exp Med* **179**, 1437-1444 (1994).
181 Hashimoto, M. *et al.* Silencing of SOCS1 in macrophages suppresses tumor development by enhancing antitumor inflammation. *Cancer Sci* **100**, 730-736 (2009).
182 Ryo, A. *et al.* Regulation of NF-kappaB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol Cell* **12**, 1413-1426 (2003).

183 Maine, G. N., Mao, X., Komarck, C. M. & Burstein, E. COMMD1 promotes the ubiquitination of NF-kappaB subunits through a cullin-containing ubiquitin ligase. *Embo J* **26**, 436-447, doi:10.1038/sj.emboj.7601489 (2007).
184 He, Y., Zhang, W., Zhang, R., Zhang, H. & Min, W. SOCS1 inhibits tumor necrosis factor-induced activation of ASK1-JNK inflammatory signaling by mediating ASK1 degradation. *J Biol Chem* **281**, 5559-5566, doi:10.1074/jbc.M512338200 (2006).
185 Mansell, A. *et al.* Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nature immunology* **7**, 148-155, doi:10.1038/ni1299 (2006).
186 Baetz, A., Frey, M., Heeg, K. & Dalpke, A. H. Suppressor of cytokine signaling (SOCS) proteins indirectly regulate toll-like receptor signaling in innate immune cells. *J Biol Chem* **279**, 54708-54715, doi:10.1074/jbc.M410992200 (2004).
187 Gingras, S., Parganas, E., de Pauw, A., Ihle, J. N. & Murray, P. J. Re-examination of the role of suppressor of cytokine signaling 1 (SOCS1) in the regulation of toll-like receptor signaling. *J Biol Chem* **279**, 54702-54707, doi:10.1074/jbc.M411043200 (2004).
188 Frey, K. G. *et al.* HSV-1-induced SOCS-1 expression in keratinocytes: use of a SOCS-1 antagonist to block a novel mechanism of viral immune evasion. *Journal of immunology* **183**, 1253-1262, doi:10.4049/jimmunol.0900570 (2009).
189 Hashimoto, K. *et al.* RSV replication is attenuated by counteracting expression of the suppressor of cytokine signaling (SOCS) molecules. *Virology* **391**, 162-170, doi:10.1016/j.virol.2009.06.026 (2009).
190 Yadav, A. *et al.* Increased expression of suppressor of cytokine signaling-1 (SOCS-1): A mechanism for dysregulated T helper-1 responses in HIV-1 disease. *Virology* **385**, 126-133, doi:10.1016/j.virol.2008.11.039 (2009).
191 Yao, Z. Q. *et al.* Differential regulation of T and B lymphocytes by PD-1 and SOCS-1 signaling in hepatitis C virus-associated non-Hodgkin's lymphoma. *Immunol Invest* **40**, 243-264, doi:10.3109/08820139.2010.534218 (2011).
192 Ahmed, C. M. *et al.* SOCS-1 mimetics protect mice against lethal poxvirus infection: identification of a novel endogenous antiviral system. *J Virol* **83**, 1402-1415, doi:10.1128/JVI.01138-08 (2009).
193 Bullen, D. V., Baldwin, T. M., Curtis, J. M., Alexander, W. S. & Handman, E. Persistence of lesions in suppressor of cytokine signaling-1-deficient mice infected with Leishmania major. *Journal of immunology* **170**, 4267-4272 (2003).
194 Yang, T., Stark, P., Janik, K., Wigzell, H. & Rottenberg, M. E. SOCS-1 protects against Chlamydia pneumoniae-induced lethal inflammation but hampers effective bacterial clearance. *Journal of immunology* **180**, 4040-4049 (2008).
195 Imai, K., Kurita-Ochiai, T. & Ochiai, K. Mycobacterium bovis bacillus Calmette-Guerin infection promotes SOCS induction and inhibits IFN-gamma-stimulated JAK/STAT signaling in J774 macrophages. *FEMS Immunol Med Microbiol* **39**, 173-180 (2003).
196 Vazquez, N., Greenwell-Wild, T., Rekka, S., Orenstein, J. M. & Wahl, S. M. Mycobacterium avium-induced SOCS contributes to resistance to IFN-gamma-mediated mycobactericidal activity in human macrophages. *J Leukoc Biol* **80**, 1136-1144, doi:10.1189/jlb.0306206 (2006).
197 Carow, B. *et al.* Silencing suppressor of cytokine signaling-1 (SOCS1) in macrophages improves Mycobacterium tuberculosis control in an IFN-{gamma}-dependent manner. *J Biol Chem*, doi:10.1074/jbc.M111.238287 (2011).
198 Srivastava, V. *et al.* Toll-like receptor 2 and DC-SIGNR1 differentially regulate suppressors of cytokine signaling 1 in dendritic cells during Mycobacterium tuberculosis infection. *J Biol Chem* **284**, 25532-25541, doi:10.1074/jbc.M109.006221 (2009).
199 Hu, J., Winqvist, O., Flores-Morales, A., Wikstrom, A. C. & Norstedt, G. SOCS2 influences LPS induced human monocyte-derived dendritic cell maturation. *PLoS One* **4**, e7178, doi:10.1371/journal.pone.0007178 (2009).

200 Rico-Bautista, E., Flores-Morales, A. & Fernandez-Perez, L. Suppressor of cytokine signaling (SOCS) 2, a protein with multiple functions. *Cytokine Growth Factor Rev* **17**, 431-439, doi:10.1016/j.cytogfr.2006.09.008 (2006).
201 Knosp, C. A. *et al.* SOCS2 regulates T helper type 2 differentiation and the generation of type 2 allergic responses. *J Exp Med* **208**, 1523-1531, doi:10.1084/jem.20101167 (2011).
202 Machado, F. S. *et al.* Anti-inflammatory actions of lipoxin A4 and aspirin-triggered lipoxin are SOCS-2 dependent. *Nat Med* **12**, 330-334, doi:10.1038/nm1355 (2006).
203 Greenhalgh, C. J. *et al.* SOCS2 negatively regulates growth hormone action in vitro and in vivo. *J Clin Invest* **115**, 397-406, doi:10.1172/JCI22710 (2005).
204 Metcalf, D. *et al.* Gigantism in mice lacking suppressor of cytokine signalling-2. *Nature* **405**, 1069-1073, doi:10.1038/35016611 (2000).
205 Auernhammer, C. J. & Melmed, S. Interleukin-11 stimulates proopiomelanocortin gene expression and adrenocorticotropin secretion in corticotroph cells: evidence for a redundant cytokine network in the hypothalamo-pituitary-adrenal axis. *Endocrinology* **140**, 1559-1566 (1999).
206 Bousquet, C., Susini, C. & Melmed, S. Inhibitory roles for SHP-1 and SOCS-3 following pituitary proopiomelanocortin induction by leukemia inhibitory factor. *J Clin Invest* **104**, 1277-1285, doi:10.1172/JCI7924 (1999).
207 Cassatella, M. A. *et al.* Interleukin-10 (IL-10) selectively enhances CIS3/SOCS3 mRNA expression in human neutrophils: evidence for an IL-10-induced pathway that is independent of STAT protein activation. *Blood* **94**, 2880-2889 (1999).
208 Cohney, S. J. *et al.* SOCS-3 is tyrosine phosphorylated in response to interleukin-2 and suppresses STAT5 phosphorylation and lymphocyte proliferation. *Mol Cell Biol* **19**, 4980-4988 (1999).
209 Adams, T. E. *et al.* Growth hormone preferentially induces the rapid, transient expression of SOCS-3, a novel inhibitor of cytokine receptor signaling. *J Biol Chem* **273**, 1285-1287 (1998).
210 Bjorbaek, C. *et al.* Activation of SOCS-3 messenger ribonucleic acid in the hypothalamus by ciliary neurotrophic factor. *Endocrinology* **140**, 2035-2043 (1999).
211 Bjorbaek, C., Elmquist, J. K., Frantz, J. D., Shoelson, S. E. & Flier, J. S. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* **1**, 619-625 (1998).
212 Pezet, A., Favre, H., Kelly, P. A. & Edery, M. Inhibition and restoration of prolactin signal transduction by suppressors of cytokine signaling. *J Biol Chem* **274**, 24497-24502 (1999).
213 Stoiber, D. *et al.* Lipopolysaccharide induces in macrophages the synthesis of the suppressor of cytokine signaling 3 and suppresses signal transduction in response to the activating factor IFN-gamma. *Journal of immunology* **163**, 2640-2647 (1999).
214 Kimura, A. *et al.* SOCS3 is a physiological negative regulator for granulopoiesis and granulocyte colony-stimulating factor receptor signaling. *J Biol Chem* **279**, 6905-6910, doi:10.1074/jbc.C300496200 (2004).
215 Song, M. M. & Shuai, K. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *J Biol Chem* **273**, 35056-35062 (1998).
216 Lehmann, U. *et al.* SHP2 and SOCS3 contribute to Tyr-759-dependent attenuation of interleukin-6 signaling through gp130. *J Biol Chem* **278**, 661-671, doi:10.1074/jbc.M210552200 (2003).
217 Bjorbak, C. *et al.* SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. *J Biol Chem* **275**, 40649-40657, doi:10.1074/jbc.M007577200 (2000).
218 Hortner, M. *et al.* Suppressor of cytokine signaling-3 is recruited to the activated granulocyte-colony stimulating factor receptor and modulates its signal transduction. *Journal of immunology* **169**, 1219-1227 (2002).
219 Shen, X., Hong, F., Nguyen, V. A. & Gao, B. IL-10 attenuates IFN-alpha-activated STAT1 in the liver: involvement of SOCS2 and SOCS3. *FEBS Lett* **480**, 132-136 (2000).

220 Hermans, M. H. *et al.* Signaling mechanisms coupled to tyrosines in the granulocyte colony-stimulating factor receptor orchestrate G-CSF-induced expansion of myeloid progenitor cells. *Blood* **101**, 2584-2590, doi:10.1182/blood-2002-07-2062 (2003).
221 Hirano, T., Nakajima, K. & Hibi, M. Signaling mechanisms through gp130: a model of the cytokine system. *Cytokine Growth Factor Rev* **8**, 241-252 (1997).
222 Boulanger, M. J., Chow, D. C., Brevnova, E. E. & Garcia, K. C. Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex. *Science* **300**, 2101-2104, doi:10.1126/science.1083901 (2003).
223 Ward, L. D. *et al.* High affinity interleukin-6 receptor is a hexameric complex consisting of two molecules each of interleukin-6, interleukin-6 receptor, and gp-130. *J Biol Chem* **269**, 23286-23289 (1994).
224 Andersson, L. C., Gahmberg, C. G., Kimura, A. K. & Wigzell, H. Activated human T lymphocytes display new surface glycoproteins. *Proc Natl Acad Sci U S A* **75**, 3455-3458 (1978).
225 Sawa, S. *et al.* Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of CD4+ T cells. *J Exp Med* **203**, 1459-1470, doi:10.1084/jem.20052187 (2006).
226 Wang, X. J. *et al.* gp130, the cytokine common signal-transducer of interleukin-6 cytokine family, is downregulated in T cells in vivo by interleukin-6. *Blood* **91**, 3308-3314 (1998).
227 Fukada, T. *et al.* Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. *Immunity* **5**, 449-460 (1996).
228 Heinrich, P. C., Behrmann, I., Muller-Newen, G., Schaper, F. & Graeve, L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* **334 (Pt 2)**, 297-314 (1998).
229 Stahl, N. *et al.* Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* **267**, 1349-1353 (1995).
230 Yamanaka, Y., Nakajima, K., Fukada, T., Hibi, M. & Hirano, T. Differentiation and growth arrest signals are generated through the cytoplasmic region of gp130 that is essential for Stat3 activation. *Embo J* **15**, 1557-1565 (1996).
231 Schmitz, J., Weissenbach, M., Haan, S., Heinrich, P. C. & Schaper, F. SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130. *J Biol Chem* **275**, 12848-12856 (2000).
232 Marine, J. C. *et al.* SOCS3 is essential in the regulation of fetal liver erythropoiesis. *Cell* **98**, 617-627 (1999).
233 Roberts, A. W. *et al.* Placental defects and embryonic lethality in mice lacking suppressor of cytokine signaling 3. *Proc Natl Acad Sci U S A* **98**, 9324-9329, doi:10.1073/pnas.161271798 (2001).
234 Takahashi, Y. *et al.* SOCS3: an essential regulator of LIF receptor signaling in trophoblast giant cell differentiation. *Embo J* **22**, 372-384, doi:10.1093/emboj/cdg057 (2003).
235 Yoshida, K. *et al.* Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proc Natl Acad Sci U S A* **93**, 407-411 (1996).
236 Ohtani, T. *et al.* Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. *Immunity* **12**, 95-105 (2000).
237 Tebbutt, N. C. *et al.* Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. *Nat Med* **8**, 1089-1097, doi:10.1038/nm763 (2002).
238 Jenkins, B. J. *et al.* Imbalanced gp130-dependent signaling in macrophages alters macrophage colony-stimulating factor responsiveness via regulation of c-fms expression. *Mol Cell Biol* **24**, 1453-1463 (2004).
239 Peters, M. *et al.* Extramedullary expansion of hematopoietic progenitor cells in interleukin (IL)-6-sIL-6R double transgenic mice. *J Exp Med* **185**, 755-766 (1997).

240 Atsumi, T. *et al.* A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. *J Exp Med* **196**, 979-990 (2002).
241 Croker, B. A. *et al.* SOCS3 negatively regulates IL-6 signaling in vivo. *Nature immunology* **4**, 540-545, doi:10.1038/ni931 (2003).
242 El Kasmi, K. C. *et al.* General nature of the STAT3-activated anti-inflammatory response. *Journal of immunology* **177**, 7880-7888 (2006).
243 Takeda, K. *et al.* Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* **10**, 39-49 (1999).
244 Li, Y., Chu, N., Rostami, A. & Zhang, G. X. Dendritic cells transduced with SOCS-3 exhibit a tolerogenic/DC2 phenotype that directs type 2 Th cell differentiation in vitro and in vivo. *Journal of immunology* **177**, 1679-1688 (2006).
245 Matsumura, Y. *et al.* Selective expansion of foxp3-positive regulatory T cells and immunosuppression by suppressors of cytokine signaling 3-deficient dendritic cells. *Journal of immunology* **179**, 2170-2179 (2007).
246 Johnston, J. A. & O'Shea, J. J. Matching SOCS with function. *Nature immunology* **4**, 507-509, doi:10.1038/ni0603-507 (2003).
247 Croker, B. A. *et al.* SOCS3 is a critical physiological negative regulator of G-CSF signaling and emergency granulopoiesis. *Immunity* **20**, 153-165 (2004).
248 Croker, B. A. *et al.* Socs3 maintains the specificity of biological responses to cytokine signals during granulocyte and macrophage differentiation. *Exp Hematol* **36**, 786-798, doi:10.1016/j.exphem.2008.02.008 (2008).
249 Chen, Z. *et al.* Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. *Proc Natl Acad Sci U S A* **103**, 8137-8142, doi:10.1073/pnas.0600666103 (2006).
250 Yu, C. R. *et al.* Suppressor of cytokine signaling 3 regulates proliferation and activation of T-helper cells. *J Biol Chem* **278**, 29752-29759, doi:10.1074/jbc.M300489200 (2003).
251 Banerjee, A., Banks, A. S., Nawijn, M. C., Chen, X. P. & Rothman, P. B. Cutting edge: Suppressor of cytokine signaling 3 inhibits activation of NFATp. *Journal of immunology* **168**, 4277-4281 (2002).
252 Matsumoto, A. *et al.* A role of suppressor of cytokine signaling 3 (SOCS3/CIS3/SSI3) in CD28-mediated interleukin 2 production. *J Exp Med* **197**, 425-436 (2003).
253 Brender, C. *et al.* Suppressor of cytokine signaling 3 regulates CD8 T-cell proliferation by inhibition of interleukins 6 and 27. *Blood* **110**, 2528-2536, doi:10.1182/blood-2006-08-041541 (2007).
254 Hill, G. R. *et al.* SOCS3 regulates graft-versus-host disease. *Blood* **116**, 287-296, doi:10.1182/blood-2009-12-259598 (2010).
255 Egwuagu, C. E. *et al.* Suppressors of cytokine signaling proteins are differentially expressed in Th1 and Th2 cells: implications for Th cell lineage commitment and maintenance. *Journal of immunology* **168**, 3181-3187 (2002).
256 Seki, Y. *et al.* SOCS-3 regulates onset and maintenance of T(H)2-mediated allergic responses. *Nat Med* **9**, 1047-1054, doi:10.1038/nm896 (2003).
257 Yamamoto, K., Yamaguchi, M., Miyasaka, N. & Miura, O. SOCS-3 inhibits IL-12-induced STAT4 activation by binding through its SH2 domain to the STAT4 docking site in the IL-12 receptor beta2 subunit. *Biochem Biophys Res Commun* **310**, 1188-1193 (2003).
258 Kinjyo, I. *et al.* Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-beta 1. *J Exp Med* **203**, 1021-1031, doi:10.1084/jem.20052333 (2006).
259 Taleb, S. *et al.* Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis. *J Exp Med* **206**, 2067-2077, doi:10.1084/jem.20090545 (2009).
260 Wong, P. K. *et al.* SOCS-3 negatively regulates innate and adaptive immune mechanisms in acute IL-1-dependent inflammatory arthritis. *J Clin Invest* **116**, 1571-1581, doi:10.1172/JCI25660 (2006).

261 Qin, H. *et al.* TGF-beta promotes Th17 cell development through inhibition of SOCS3. *Journal of immunology* **183**, 97-105, doi:10.4049/jimmunol.0801986 (2009).
262 Pillemer, B. B., Xu, H., Oriss, T. B., Qi, Z. & Ray, A. Deficient SOCS3 expression in CD4+CD25+FoxP3+ regulatory T cells and SOCS3-mediated suppression of Treg function. *Eur J Immunol* **37**, 2082-2089, doi:10.1002/eji.200737193 (2007).
263 Cui, W., Liu, Y., Weinstein, J. S., Craft, J. & Kaech, S. M. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. *Immunity* **35**, 792-805, doi:10.1016/j.immuni.2011.09.017 (2011).
264 Siegel, A. M. *et al.* A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. *Immunity* **35**, 806-818, doi:10.1016/j.immuni.2011.09.016 (2011).
265 Yokota, S., Yokosawa, N., Okabayashi, T., Suzutani, T. & Fujii, N. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 confers efficient viral replication. *Virology* **338**, 173-181, doi:10.1016/j.virol.2005.04.028 (2005).
266 Bode, J. G. *et al.* IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. *Faseb J* **17**, 488-490, doi:10.1096/fj.02-0664fje (2003).
267 Le Goffic, R. *et al.* Cutting Edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. *Journal of immunology* **178**, 3368-3372 (2007).
268 Michaud, F. *et al.* Epstein-Barr virus interferes with the amplification of IFNalpha secretion by activating suppressor of cytokine signaling 3 in primary human monocytes. *PLoS One* **5**, e11908, doi:10.1371/journal.pone.0011908 (2010).
269 Pothlichet, J., Chignard, M. & Si-Tahar, M. Cutting edge: innate immune response triggered by influenza A virus is negatively regulated by SOCS1 and SOCS3 through a RIG-I/IFNAR1-dependent pathway. *Journal of immunology* **180**, 2034-2038 (2008).
270 Pellegrini, M. *et al.* IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. *Cell* **144**, 601-613, doi:10.1016/j.cell.2011.01.011 (2011).
271 Narayana, Y. & Balaji, K. N. NOTCH1 up-regulation and signaling involved in Mycobacterium bovis BCG-induced SOCS3 expression in macrophages. *J Biol Chem* **283**, 12501-12511, doi:10.1074/jbc.M709960200 (2008).
272 Jacobsen, M. *et al.* Suppressor of cytokine signaling-3 is affected in T-cells from tuberculosisTB patients. *Clin Microbiol Infect*, doi:10.1111/j.1469-0691.2010.03326.x (2010).
273 Mistry, R. *et al.* Gene-expression patterns in whole blood identify subjects at risk for recurrent tuberculosis. *J Infect Dis* **195**, 357-365, doi:10.1086/510397 (2007).
274 Kamimura, D. *et al.* Tyrosine 759 of the cytokine receptor gp130 is involved in Listeria monocytogenes susceptibility. *Genes Immun* **3**, 136-143, doi:10.1038/sj.gene.6363825 (2002).
275 Silver, J. S., Stumhofer, J. S., Passos, S., Ernst, M. & Hunter, C. A. IL-6 Mediates the Susceptibility of Glycoprotein 130 Hypermorphs to Toxoplasma gondii. *Journal of immunology*, doi:10.4049/jimmunol.1004144 (2011).
276 Whitmarsh, R. J. *et al.* A critical role for SOCS3 in innate resistance to Toxoplasma gondii. *Cell Host Microbe* **10**, 224-236, doi:10.1016/j.chom.2011.07.009 (2011).
277 Almeida, A. S. *et al.* Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity. *Journal of immunology* **183**, 718-731, doi:10.4049/jimmunol.0801212 (2009).
278 Evel-Kabler, K., Song, X. T., Aldrich, M., Huang, X. F. & Chen, S. Y. SOCS1 restricts dendritic cells' ability to break self tolerance and induce antitumor immunity by regulating IL-12 production and signaling. *J Clin Invest* **116**, 90-100, doi:10.1172/JCI26169 (2006).

279 Shi, S. *et al.* MyD88 primes macrophages for full-scale activation by interferon-gamma yet mediates few responses to Mycobacterium tuberculosis. *J Exp Med* **198**, 987-997, doi:10.1084/jem.20030603 (2003).
280 Berry, M. P. *et al.* An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* **466**, 973-977, doi:10.1038/nature09247 (2010).
281 Cooper, A. M. & Khader, S. A. The role of cytokines in the initiation, expansion, and control of cellular immunity to tuberculosis. *Immunol Rev* **226**, 191-204, doi:10.1111/j.1600-065X.2008.00702.x (2008).
282 Cooper, A. M., Mayer-Barber, K. D. & Sher, A. Role of innate cytokines in mycobacterial infection. *Mucosal Immunol* **4**, 252-260, doi:10.1038/mi.2011.13 (2011).
283 Posselt, G., Schwarz, H., Duschl, A. & Horejs-Hoeck, J. Suppressor of cytokine signaling 2 is a feedback inhibitor of TLR-induced activation in human monocyte-derived dendritic cells. *Journal of immunology* **187**, 2875-2884, doi:10.4049/jimmunol.1003348 (2011).
284 Aliberti, J., Hieny, S., Reis e Sousa, C., Serhan, C. N. & Sher, A. Lipoxin-mediated inhibition of IL-12 production by DCs: a mechanism for regulation of microbial immunity. *Nature immunology* **3**, 76-82, doi:10.1038/ni745 (2002).
285 Bafica, A. *et al.* Host control of Mycobacterium tuberculosis is regulated by 5-lipoxygenase-dependent lipoxin production. *J Clin Invest* **115**, 1601-1606, doi:10.1172/JCI23949 (2005).
286 Aliberti, J., Serhan, C. & Sher, A. Parasite-induced lipoxin A4 is an endogenous regulator of IL-12 production and immunopathology in Toxoplasma gondii infection. *J Exp Med* **196**, 1253-1262 (2002).
287 Denkers, E. Y., Gazzinelli, R. T., Martin, D. & Sher, A. Emergence of NK1.1+ cells as effectors of IFN-gamma dependent immunity to Toxoplasma gondii in MHC class I-deficient mice. *J Exp Med* **178**, 1465-1472 (1993).
288 Hunter, C. A., Subauste, C. S., Van Cleave, V. H. & Remington, J. S. Production of gamma interferon by natural killer cells from Toxoplasma gondii-infected SCID mice: regulation by interleukin-10, interleukin-12, and tumor necrosis factor alpha. *Infect Immun* **62**, 2818-2824 (1994).
289 Keller, C. *et al.* Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. *Infect Immun* **74**, 4295-4309, doi:10.1128/IAI.00057-06 (2006).
290 Redford, P. S. *et al.* Enhanced protection to Mycobacterium tuberculosis infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung. *Eur J Immunol* **40**, 2200-2210, doi:10.1002/eji.201040433 (2010).
291 Melillo, J. A. *et al.* Dendritic cell (DC)-specific targeting reveals Stat3 as a negative regulator of DC function. *Journal of immunology* **184**, 2638-2645, doi:10.4049/jimmunol.0902960 (2010).
292 Haftchenary, S., Avadisian, M. & Gunning, P. T. Inhibiting aberrant Stat3 function with molecular therapeutics: a progress report. *Anticancer Drugs* **22**, 115-127, doi:10.1097/CAD.0b013e328341185b (2011).
293 Sodenkamp, J. *et al.* gp130 on macrophages/granulocytes modulates inflammation during experimental tuberculosis. *Eur J Cell Biol* **90**, 505-514, doi:10.1016/j.ejcb.2010.10.010 (2011).